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# Female remating and sperm competition patterns in a terrestrial crustacean

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In most species, both sexes may mate with more than one partner during their life. In terrestrial isopods (woodlice) female remating can occur within a reproductive season (immediate remating) or after a period of sexual rest and sperm storage, that is in a subsequent reproductive season (delayed remating). The pattern of sperm precedence is unknown in both cases. These two female remating patterns may shape male–male competition in different ways. To elucidate both patterns of female remating and sperm precedence, we used an albinism mutation in *Armadillidium vulgare* to track paternity in laboratory experiments. Males had low remating success after immediate remating attempts, mainly because of the female's refractory behaviour. However, refractory behaviour seemed to be lost after female sexual rest: delayed remating attempts were as successful as first mating attempts with virgin females. In both immediate and delayed remating, competing males had similar fertilization success, but varied in sperm precedence. We hypothesize that males might induce the refractory mating behaviour in females to ensure their paternity. This could be a strategy that evolved in woodlice after the loss of precopulatory mate guarding during adaptation to the terrestrial environment. We discuss the consequences of these findings for woodlice optimal mating strategies.



Males and females in many species mate with more than one partner during their reproductive cycle. The benefits of multiple mating for females are not fully understood, since a single mating is generally enough for females to ensure their reproduction (Eberhard 1996; Birkhead & Parker 1997; Birkhead & Møller 1998; Brooks & Jennions 1999; Arnqvist & Nilsson 2000). Genetic benefits, that is benefits that increase offspring fitness and not directly the mother's fitness, have been invoked to explain the evolution of female multiple mating (e.g. Tregenza & Wedell 1998), but are often insufficiently known (Yasui 1998). A meta-analysis suggested that multiple mating is directly advantageous for females in insects since it increases lifetime offspring production (Arnqvist & Nilsson 2000). This advantage appears to be a balance between increasing fecundity and fertility and decreasing longevity, at least in species without nuptial gifts. Studies on female remating rates also need to take into account the potential gains or losses for males. The benefit that males obtain from multiple mating is obvious, since it allows them to increase their reproductive success by fathering more offspring (Bateman 1948). However, female multiple mating can drive the evolution of sperm competition, and/or the evolution of male–female conflict over mating (Birkhead & Møller 1998). Males are able to drive females away from their optimal mating rate (Arnqvist & Nilsson 2000). For example, males can be selected to entice or coerce already mated females to increase their probability of paternity (Clutton-Brock & Parker 1995; Holland & Rice 1998), or, conversely, to monopolize females and ensure their paternity.

Among the latter strategies, precopulatory mate guarding has frequently been highlighted as a male strategy to monopolize females in crustaceans (Jormalainen 1998). However, Zimmer (2001) noted that mate guarding generally does not occur in terrestrial crustacean species. Mate guarding could have been lost in this group because the cost of this strategy would be higher than the reproductive gain, following the evolution of mating systems during adaptation to the terrestrial environment (Zimmer 2001). For example, the evolution of sperm storage in terrestrial isopod females, following the appearance of internal fertilization, would have meant that a mate-guarding male could not ensure his exclusive paternity (Zimmer 2001). The loss of mate guarding

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would therefore have allowed the evolution of multiple mating in terrestrial crustaceans. Multiple mating has been documented in land isopods (Oniscidea, woodlice; Lueken 1963; Johnson 1976; Sassaman 1978), and sperm stored after a single mating remains functional over many clutches (Schobl 1880; Lueken 1963; Adamkewicz 1969; Johnson 1976). Zimmer (2001) did not propose an alternative strategy for males to increase their probability of paternity in terrestrial crustaceans, but many strategies have been selected in other terrestrial arthropods, for example, the reduction of postmating receptivity in females (Eberhard 1996; Simmons & Siva-Jothy 1998). A better knowledge of female remating in terrestrial species would therefore help us to understand how mating strategies evolved in crustaceans.

Mating patterns in terrestrial isopods are poorly understood. First, nothing is known about the tendency of females to remate after a single copulation. Second, the patterns of sperm precedence, a crucial parameter that can shape the evolution of mating strategies, remain unknown. Third, multiple paternity could be due to multiple mating in a single reproductive season, or multiple mating in two or more reproductive seasons, or both. Female reproduction is generally seasonal with sequential sexually active and resting phases (Mocquard et al. 1989; Warburg 1993). Woodlice females have two sperm storage organs: a bursa copulatrix (the oviduct itself, where fresh ejaculate is stored for a short time before fertilization) and a spermatheca at the base of the oviduct, where remaining sperm is stored after fertilization. Field studies have been unable to distinguish whether sperm mixing occurs between two fresh ejaculates or between fresh and stored sperm (Johnson 1976; Sassaman 1978). Finally, the operational sex ratio (OSR) in woodlice is either approximately balanced or shows an excess of females (Moreau & Rigaud 2000). The latter is due to the frequent infection with feminizing maternally transmitted parasites in populations (Rigaud 1997; Bouchon et al. 1998). When the OSR is female-skewed, it should moderate male–male competition and remating possibilities. Differences in female remating patterns are important to know in the context of female-biased sex ratios, because they will predict optimal sexual strategies of both sexes.

Our aim in this study was to investigate remating tendencies of females of the species *Armadillidium vulgare*, within a reproductive bout and between two reproductive bouts, and to determine patterns of sperm precedence after remating. We used an albinism mutation to track paternity in laboratory experiments, by crossing albino females with albino and wild-type males. We first tested albino males for their ability to mate in a similar way to their wild-type counterparts. We compared mating rates between virgin females, females mated twice in a single receptive period (to mimic direct male–male competition during a single reproductive season) and females allowed to remate only after having stored their sperm in spermatheca (to mimic male–male competition in two reproductive periods). We examined patterns of sperm precedence, and tested whether remating in females increases the fecundity and fertility of females, as is often found in insects (Arnqvist & Nilsson 2000).

## METHODS

### Reproductive Cycle

The reproductive cycle of *A. vulgare* (Crustacea, Isopoda, Oniscidea) is closely associated with the female moulting cycle. In the sexual rest period, females undergo a normal moult to grow. With the increase of photoperiod and temperature, ovarian maturation begins (Mocquard et al. 1989). Conversely to Zimmer's (2001) assumption, female receptivity in woodlice is limited to the stage where oocyte maturation is nearly over (Lefebvre & Caubet 1999; Moreau & Rigaud 2000; Moreau & Rigaud 2002), whereas males can mate at any time except during moulting (Moreau & Rigaud 2000). Fertilization is internal and isopod females have two genital apertures, each independently linked to one ovary by an oviduct. Males have to perform one insemination in each oviduct to inseminate the female completely. Sperm is deposited within the oviduct in the form of a large white ball. Sperm is not motile in woodlice (Hollande & Fain-Maurel 1965) and is stored in the oviduct until oocyte laying. As far as is known, oocytes are fertilized when they pass through the balls of sperm. After laying, remaining spermatozoon are stored in the spermatheca, at the junction between the oviduct and the ovary, and used to fertilize further broods (Warburg 1993). It is not known whether this storage is under female control, but this is likely since sperm are not motile. Eggs and embryos are incubated in a marsupium (ventral pouch limited by lamellar structures) which differentiates during the special moult called 'parturial moult'. Parental care during embryonic development is solely by females, who are unable to mate during this period. Young emerge from the pouch about 5 weeks after laying. The marsupium remains until the next moult, which generally occurs about 2 weeks later. During this stage, most females cannot mate, owing to the physical barrier of ventral marsupial plates (Moreau & Rigaud 2000). The moult after release of the young can be a second parturial moult (eggs fertilized by the sperm reserve), or a normal moult (growth moult), depending on environmental conditions, such as photoperiod and temperature (Mocquard et al. 1989).

### Strain Maintenance and Breeding Procedures

Subjects came from strains maintained in the same conditions in the laboratory for many years, on moistened soil, at 20°C and at the natural photoperiod of Poitiers (latitude 46°40′N; see Moreau et al. 2001 for routine procedure of strain maintenance). In each generation, males and females from the same brood are sorted by sex before they reach sexual maturity. They are then reared separately, thus ensuring that all females are virgin.

To track paternity in the experiments, we used an albino mutation present in one strain collected at Sète (France). The absence of dark coloration in this strain (body, antennae, legs and eyes) is associated with a recessive allele (classical albinism; Hasegawa et al. 1997). Crosses between albino males and females produce only white young, and crosses between a wild-type individual and an albino produce only wild-type young, following basic Mendelian rules (the genetics of this albinism mutation was verified during the back-crossing experiments described below). To limit the genotypic differences between the two strains prior to the experiments, the albino strain was introgressed with the wild genome. Ten males from a wild-type strain collected in Nice (France) and 10 males from a wild-type strain collected in Heraklion (Crete) were each crossed with an albino female. Among the F1 young (all wild-type, $N$=781) 16 males from the hybrid 'Nice strain' and 16 females from the hybrid 'Heraklion strain' were crossed. Among the F2 young ($N$=582 albinos, 1775 wild-type), five wild-type males were crossed with five albino females, and five albino males were crossed with five wild-type females (sibling mating was avoided). The F2 crosses produced 537 albinos and 507 wild-type F3 offspring. In addition, eight male and female F2 albinos were crossed (sibling mating avoided) and produced 402 F3 offspring, all albinos. Albino F3 progeny was used for the following experiments. In the meantime, crosses were made between wild-type strains from Nice and Heraklion to obtain a hybrid wild-type strain. Only males from this strain were used.

At the time of the experiments, females and males were 1 year old. To obtain virgin females receptive to mating, we reared females under an 18:6 h light:dark photoperiod, which stimulates the onset of reproduction (Mocquard et al. 1989). We assessed female receptivity by checking the shape of white plates of calcium carbonate on the ventral surface that differentiate a few days before moulting. Receptive females have incomplete plates and nonreceptive ones complete plates (Moreau & Rigaud 2002). Only females receptive to mating were used in experiments.

In all of the experiments, animals were paired with partners of approximately equal size (asymmetry random) to avoid the possibility of physical incompatibility for reproduction. Pairs or single animals were placed in cylindrical boxes (8 cm diameter), filled with moistened soil, at 20°C under natural photoperiod. We weighed each individual on a Sartorius precision balance.

## Fitness of Albino Males

We compared the fitness of the albino males with that of the wild-type males, to identify any differences that might confound the results.

To compare the sperm density in ejaculates between males, we paired albino females with either an albino male ($N$=15) or a wild-type male ($N$=15) for 12 h. We then anaesthetized the animals with ether and killed them by decapitation. Female oviducts were dissected in a glass watch containing 15 ml of Ringer solution. All females had been inseminated. After gentle homogenization, six drops of 10 µl per sample were deposited on a microscope slide. Sperm were revealed using DAPI (Sigma, concentration of 0.04 mg/ml; Moreau et al. 2001). Observations were made under epifluorescence on a Zeiss Axioplan microscope. Because isopod females have two independent genital apertures, we give results for each oviduct. The estimated density of sperm per oviduct was based on the total count of the six drops.

To compare sperm efficiency, we assessed the proportion of developing embryos. Albino virgin females were paired with either an albino male ($N$=19) or a wild-type male ($N$=22) for 12 h. Females were then isolated. We periodically monitored the developmental stage of their brood by visual inspection under a binocular microscope, through the transparent marsupial plates. A few days before the young emerged, we flushed embryos from the brood pouch with water and counted developed embryos and undeveloped eggs.

## Female Mating And Remating Frequencies

All the following experiments were made in duplicate and counterbalanced to control for the effect of mating order on mating patterns. One series used albino males for mating first, then wild-type males, and one series used wild-type males first, then albino males.

### *Immediate remating*

To determine the mating frequency of virgin females, we paired males with virgin albino females for 3 h. If no copulation was observed during this period, we replaced the male with another one of the same type, for another 3 h. Two attempts of 3 h were made per day, during 5 days. If females did not mate within 5 days, the experiment was stopped, and the female was classified as not mated. Some females made a parturial moult without copulation within the 5 days. In this case, eggs will not develop. Experiments were monitored by visual observation. Two measures were made for each replicate: the time elapsed before a successful mating sequence, and the duration of the copulation sequence per se.

To determine female remating frequency within the same receptive intermoult, we replaced males immediately by a second male (of different phenotype) after a complete copulation sequence had been observed in the preceding experiment. The same monitoring scheme was followed. We obtained 52 replicates with albino males as the first male and 50 replicates with wild-type males as the first male.

### *Delayed remating*

To determine the remating frequency in a second receptivity period (i.e. after a reproductive rest and storage of sperm from the previous mating), we used the following protocol. At the same time, we paired each of 20 albino and 20 wild-type males with one virgin albino female until the female mated. A few females were excluded either because they did not mate ($N$=3), or because they died ($N$=3). The remaining females were

isolated in small boxes immediately after their parturial moult and maintained under a 6:18 h L:D photoperiod until the young were released. This photoperiod favours female sexual rest (Mocquard et al. 1989). The aim of this manipulation was to obtain females performing a normal moult after emergence of the young: that is they produced only one brood with a single ejaculate, and therefore stored sperm in the spermatheca. Only 21 females performed a normal moult following emergence. The others underwent a second parturial moult ($N$=7) or died ($N$=6). After a normal moult, females were maintained under the stimulating photoperiod (18:6 h L:D) again to reinduce female reproduction. During this intermoult, all females reached the stage of receptivity (as assessed by checking calcium carbonate plates). They were then coupled with a male of the other phenotype until they mated, as previously described. No time measure was taken during this experiment.

## Pattern of Sperm Precedence

To determine the sperm precedence between two fresh ejaculates (i.e. after immediate remating), we repeated the design of the preceding experiment, and obtained females that had been fully mated by two males ($N$=12 with wild-type males first, and $N$=15 with albino males first). Females were isolated in a small box filled with moistened soil and food for about 1 month. We then flushed embryos out of the marsupium. We determined the pattern of sperm precedence (P2) as the proportion of offspring sired by the second male. To assess this P2 value, we recorded the number of albinos and wild-type young, by checking their eye pigmentation (body colour of young is white, but eyes are black in wild-type young and white in albino young). The duration of the two successive copulations was measured for 14 females. These females were those for which both the beginning and the end of the two copulations was precisely observed.

To determine the sperm precedence between a 'fresh' ejaculate and stored sperm (i.e. after delayed remating), we isolated the females mated twice in the 'delayed remating' experiment described above, and assessed the P2 value. This yielded 11 females in the first series (albino males first) and 10 females in the second (wild-type males first).

We estimated the number of sperm stored in the reserve as follows. Wild males were crossed with 33 virgin females, and which were allowed to lay. We anaesthetized the animals with ether and killed them by decapitation. The spermatheca of each was then dissected, and sperm was counted following the procedure described for fresh ejaculates.

## Female Fecundity After Single And Double Mating

To assess the effect of remating on both female fecundity and egg fertilization success, we compared the counts made in previous experiments. The count for females of the 'male fitness' series provided the one-mating series ($N$=41), and the count for the 'sperm precedence' series provided the immediate remating series ($N$=27) and the delayed remating series ($N$=19). Fecundity was measured as the total number of eggs laid, determined by counting both developed embryos and unfertilized eggs in the marsupium, and fertilization success was measured as the ratio of the number of developed embryos to the total number of eggs laid.

## Mating Behaviour

We studied both male and female mating behaviours during 1-h encounters, by direct observation. Experiments were staged in a petri dish lined with moistened paper (diameter 9.5 cm), topped with transparent glass to limit air disturbance (temperature 20°C, light 100 lx, relative humidity 90%). At time $t_0$, one male and one virgin albino female were placed at opposite sides of the dish. We recorded behavioural sequences with a programmable WorkAbout recorder (Psion, U.K.). Behaviours were classified into three types: (1) noninteractive activity: locomotion, immobility; (2) nonsexual interactions: brief (<1 s) reciprocal antennae contact or antennae contact on the body; (3) sexual interactions: long male exploration of female body with antennae (assessment), male mounting on female's dorsal surface (first step of mating sequence), female's response to mounting, copulation sequence (Mead 1973). The female's response to mounting was categorized as acceptance (rolling, immediately followed by a slight opening) or rejection of the male (rolling without opening, immobilization, escape or jerky body movements; Mead 1973; Moreau et al. 2001). The criteria analysed for each item were, respectively, (1) individual showing the behaviour; (2) number of occurrences; and (3) duration. Replicates where no sexual interaction was observed were removed from analysis. We obtained 16 replicates with wild-type males and 15 with albino males. We replaced mated females 24 h later with a second male of the other type to study the copulation sequence of nonvirgin females (eight replicates with albino males second, and nine with wild-type males second).

## Statistical Analysis

All statistical tests were done with JMP software (SAS 3.2.2, SAS Institute 1995). Data were ln or arcsine square-root transformed when required to meet normality. In most standard least square models, the effects of male phenotype (wild-type versus albino), the effect of female virginity status (virgin versus already mated), and the masses of both males and females were taken into account. A stepwise analysis (backward procedure) allowed us to simplify most of the models by removing nonsignificant ($P$>0.05) effects and interactions (SAS Institute 1995). Only the resulting models are presented here. In cases where no factor was significant in the global model, only the factor of interest is presented. Kruskal–Wallis or Wilcoxon two-sample nonparametric tests were used when data did not meet normality or homogeneity of variances. Means are given ± SEs.

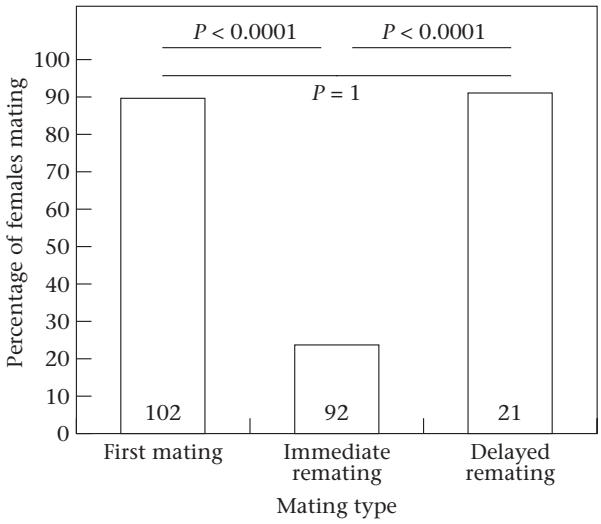


**Figure 1.** Percentage of females that mated in the experiment, according to their mating type. The numbers within the bars are the sample sizes. *P* values are those of Fisher's exact tests (two-tailed).

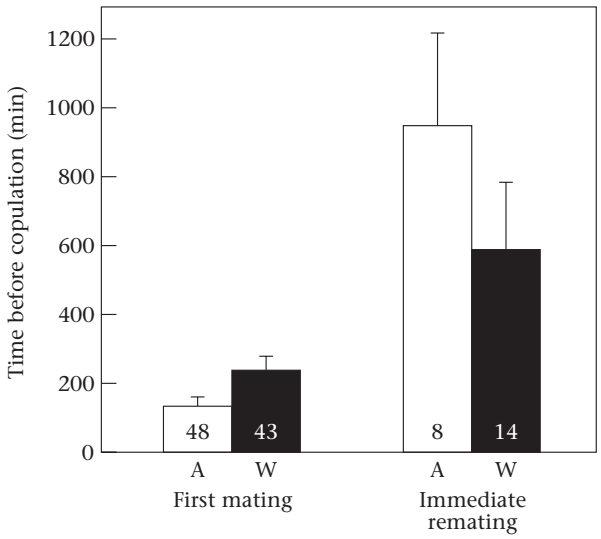


**Figure 2.** Time elapsed before copulation ($\bar{X}$+SE), according to mating type (first mating versus immediate remating), and according to male type (albino, A and wild-type, W).

## RESULTS

### Quantity and Efficiency of Sperm

Albino males supplied 811.68 ± 49.80 sperm/60 µl per oviduct and wild-type males supplied 705.48 ± 54.78 sperm/60 µl per oviduct. There was no significant difference between number of sperm supplied to females by albino and wild-type males (ANOVA: $F_{1,54}$=3.01, NS).

Sperm from albino males fertilized 94.67 ± 2.12% of embryos and sperm from wild-type males fertilized 85.80 ± 4.59% of embryos. Again, ANOVA revealed no significant difference between the proportion of fertilized embryos of the male types ($F_{1,40}$=1.73, NS).

### Female Mating and Remating Frequencies

Mating frequencies did not depend on the male phenotype used in first mating, but differed only by mating type (first mating, immediate remating or delayed remating; logistic regression: whole model: likelihood ratio $\chi^2_5$=105.75, *P*<0.0001; effect of male phenotype: L-R $\chi^2_1$=0.02, NS; effect of mating type: L-R $\chi^2_2$=104.77, *P*<0.0001; interaction: L-R $\chi^2_2$=2.36, NS). In each experiment, we therefore pooled the two series using the different male types for further analysis. The probability of mating was significantly lower during immediate remating attempts than during first mating or delayed remating (Fig. 1). Remating attempts after a period of sexual rest were as successful as first matings (Fig. 1).

Copulation occurred more rapidly with virgin females than during immediate remating attempts (Fig. 2). Albino males mated more rapidly than wild-type males in the first mating, but the reverse pattern occurred in immediate remating (ANOVA on ln transformed data: general model: $F_{3,112}$=18.88, *P*<0.0001; effect of mating type: $F_{1,112}$=47.38, *P*<0.0001; effect of male type: $F_{1,112}$=0.51, NS; interaction: $F_{1,112}$=9.96, *P*<0.003; Fig. 2). For females mated twice, the delay before remating was not dependant on the delay before the first mating ($Y$=5.61+0.1$X$, $r^2$=0.006, $F_{1,21}$=0.13, NS). The female's virginity status did not significantly influence copulation duration (first copulation: 72.17 ± 2.86 min, *N*=90; remating: 84.00 ± 8.12 min, *N*=22; $F_{1,111}$=1.40, NS).

### Patterns of Sperm Precedence

There was strong individual variation of P2 values between females, ranging from 0.15 to 0.73 for the competition between two fresh ejaculates (immediate remating), and from 0.23 to 0.91 for the competition between one fresh ejaculate and stored sperm (delayed remating). These two distributions did not differ from normality (Shapiro–Wilk test: *P*>0.20 and *P*>0.90, respectively).

The average P2 values were different between the two types of experiment ($F_{1,44}$=5.55, *P*=0.02; Fig. 3). Sperm from the second male was therefore at a slight disadvantage when competing with a fresh ejaculate, compared to when competing with stored sperm. On average, values of P2 were around 0.5, and paired *t* tests revealed that the number of young produced by first males did not differ significantly from those sired by second males within each experiment (competition between two fresh ejaculates: $t_{26}$=1.50, NS; competition between fresh ejaculate and stored sperm: $t_{18}$=1.70, NS).

Differences in copulation duration between the two males (duration for male 1 minus duration for male 2) were not related to the P2 values (measurements made on 14 females mated twice successively; $F_{1,13}$=0.009, NS; $r^2$=0.0007). The proportion of offspring fathered by a given male was therefore not proportional to the time he spent copulating.

The counting of sperm stored in the reserve revealed very few sperm (5.32 ± 0.57 sperm/60 µl per oviduct, *N*=66). Comparison with the measures made in fresh

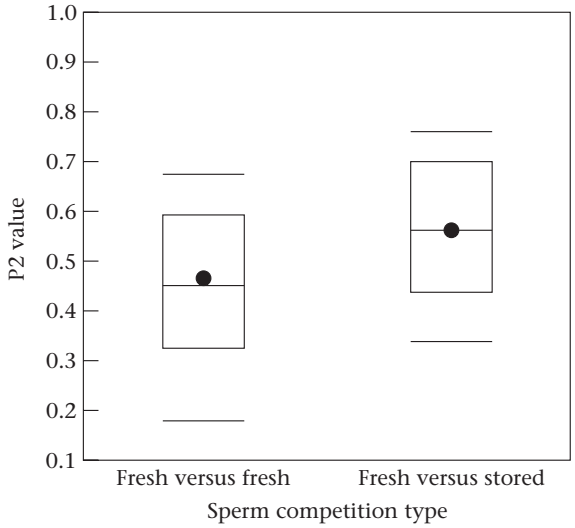


**Figure 3.** Box plot showing the proportion of offspring fathered by the second male (P2) after two copulations, in the sperm competition experiment. The median is the line across the box, boxes are limited by the 75th quantile (up) and 25th quantile (down). Up and down lines are the 90th and 10th quantiles, respectively. Dots are means.

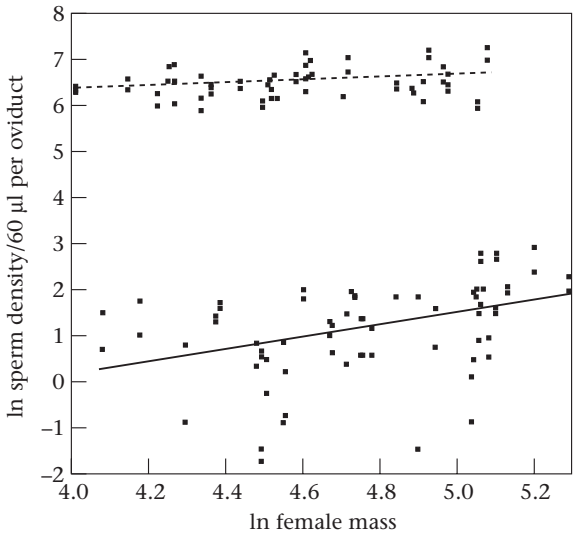


**Figure 4.** Relation between female mass and the density of sperm (data log transformed). ——: Sperm stored in the spermatheca; – – –: sperm in fresh ejaculate.

ejaculates (see above) revealed that sperm density was strongly influenced by both female weight ($F_{1,120}$=13.17, $P$=0.0004) and sperm location (stored versus fresh ejaculate; $F_{1,120}$=23.57, $P$<0.0001), as well as their interaction ($F_{1,120}$=5.31, $P$=0.02; global model: $F_{3,120}$=504.95, $P$<0.0001; Fig. 4). The amount of sperm was therefore much higher in a fresh ejaculate than in the spermatheca. There was also a relation between female weight and the number of stored sperm ($r^2$=0.15, $F_{1,65}$=12.60, $P$=0.0007). This relationship was weaker for fresh ejaculate ($r^2$=0.05, $F_{1,54}$=3.91, $P$=0.06).

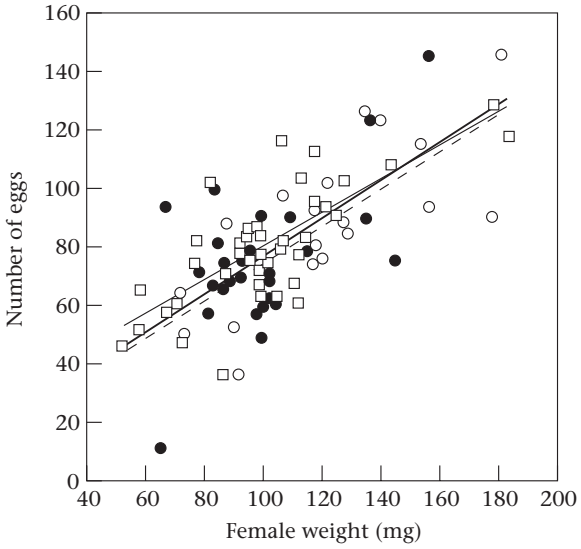


**Figure 5.** Relation between female weight and number of eggs laid in the marsupium. □, ——: Females mated once; ●, – – –: females mated twice with immediate remating; ○, ——: females mated twice with delayed remating.

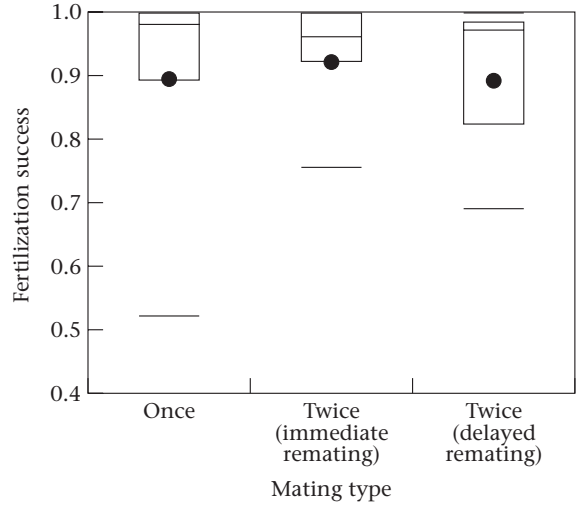


**Figure 6.** Box plot showing the fertilization success according to the mating pattern. Legend as in Fig. 3.

## Female Fecundity After Single And Double Mating

Females mated once and females that were remated immediately laid equivalent numbers of eggs (ANCOVA: global model: $F_{3,67}$=18.58, $P$<0.0001; effect of female mass: $F_{1,67}$=48.10, $P$<0.0001; effect of mating pattern: $F_{1,67}$=0.44, NS; interaction: $F_{1,67}$=0.19, NS; Fig. 5). The same was true for females mated once and females that remated after a period of sexual rest (ANCOVA: global model: $F_{3,59}$=24.55, $P$<0.0001; effect of female mass: $F_{1,59}$=68.03, $P$<0.0001; effect of mating pattern: $F_{1,59}$=0.46, NS; interaction: $F_{1,59}$=0.23, NS; Fig. 4). There was also no significant effect of mating pattern on the proportion of eggs fertilized (Wilcoxon two-sample test: $\chi^2_2$=1.45, NS; Fig. 6).

**Table 1.** Observations recorded over 1 h in mating behaviour experiments, according to female virginity status

| | Female status $\bar{X}$±SE | | |
|---|---|---|---|
| | Virgin (*N*=31) | Mated (*N*=17) | *P** |
| Male activity before first mount* | 0.69±0.04 | 0.74±0.07 | 0.26 |
| Female activity before first mount† | 0.58±0.05 | 0.69±0.07 | 0.15 |
| Duration of male 'assessment' before first mount (s) | 23.72±9.38 | 11.45±3.86 | 0.78 |
| Number of interactions with male before first mount | 4.23±0.56 | 3.35±0.74 | 0.30 |
| Ratio of sexual to total interactions | 0.29±0.05 | 0.31±0.08 | 0.96 |
| Number of mounts | 1.23±0.22 | 2.88±0.49 | 0.002 |

*Wilcoxon two-sample test.
†Proportion of time male or female was active.

## Mating Behaviour

During the 1-h monitoring, no significant difference was found between virgin and nonvirgin females for all behaviours analysed, except the number of mounts (Table 1). More mounts were observed on nonvirgin than virgin females. The nonvirgin females were more likely to show a behaviour that stopped the mating sequence and induced male retreat (Fisher's exact test, two-tailed, $P$=0.02; Table 2). The high number of attempts (mounts) on nonvirgin females might therefore be explained by the low success of each attempt.

## DISCUSSION

*Armadillidium vulgare* females showed reduced receptivity when remating attempts occurred immediately after the first mating. Therefore, a relatively low proportion of females (ca. 20%) did mate twice within the same intermoult. In natural populations, this proportion may be lower, since the small mating area used in this study tended to maximize the encounters between individuals, and therefore optimized mating probabilities. Low immediate remating rate did not appear to be due to male discrimination, since males interacted with the two types of females in the same proportions and showed the same proportion of sexual interactions with them. The main behavioural components involved in this relative mating deficiency were the female's responses to male mating attempts. Recently mated females were more likely to disrupt the continuation of the mating sequence than were virgin females. This behavioural difference in female responses to male attempts was not qualitative, because some already mated females showed appropriate behaviour after several mating attempts. As a consequence of these responses, immediate remating, when observed, took much longer than first mating. Our experimental design (i.e. the use of males of similar size to females) excludes male size effects on female remating. This aspect needs further investigation, to see, for example, whether female mated first to a small male would be more likely to remate with a larger one.

**Table 2.** Female behaviour recorded after each male mounting, according to female virginity status

| Female behaviour | Virgin (*N*=31) | Mated (*N*=17) |
|---|---|---|
| Rolling, opening | 9 | 3 |
| Other* | 28 | 47 |

*Rolling without opening, immobilization, jerky movement, escape, indicating rejection of male.

Remating after the females had stored sperm (i.e. when remating occurred during two different receptive intermoults) was as frequent as first mating. Sperm mixing therefore, seems more likely when *A. vulgare* females already have stored sperm. Under natural conditions, sperm mixing should be more likely between two reproductive seasons than during a single reproductive season.

In the case of sperm mixing, the sperm usage pattern departed from random only slightly; the two males had nearly the same fertilization success when sperm competition occurred between stored sperm and fresh ejaculate as between two fresh ejaculates. This result contrasts with strong sperm precedence observed in some other arthropod species, either showing last-male sperm precedence (in many Lepidoptera; Drummond 1984) or first-male sperm precedence (in some spiders; Austad 1984). However, considerable variation existed in paternity patterns between males of *A. vulgare* (Fig. 3), as has been observed in many other species (Cook et al. 1997; Birkhead & Biggins 1998). Patterns of sperm precedence do not seem to be influenced by the duration of copulation, but this prediction needs to be tested with a larger sample size. Also, raffle competition alone (i.e. precedence of the more numerous sperm) cannot explain sperm precedence in woodlice: the P2 value when sperm of a fresh ejaculate competed with sperm stored in the reserve was not proportional to the difference in sperm number. Probably the position of the sperm has some importance in sperm precedence. If the hypothesis is correct that fertilization occurs when oocytes pass through the sperm, the sperm encountered first may gain a slight advantage. This prediction could explain the 'competitiveness' of the stored sperm despite its low number, because stored sperm is encountered first during egg laying. However, we cannot dismiss the possibility that some males could father more offspring than others simply because their sperm is of higher quality (Dziuk 1996).

Since sperm mixing is almost total, males will gain reproductively if they can somehow cause females to

refrain from remating. It is therefore tempting to impute the low remating rate during the same female receptivity period to males. We have no data on how male woodlice could achieve a reduction in female remating receptivity, but many hypotheses can be tested. Since females are still attractive to males after a first mating, we can dismiss the hypothesis that males transfer an antiaphrodisiac substance during mating, as occurs in butterflies (Kingan et al. 1995). Males might transfer substances in their ejaculate that induce the refractory mating behaviour in females (Engelmann 1970; Eberhard 1996). Alternatively, the female nonreceptivity might also be induced by the presence of sperm within the female genital duct, or with the physical stimuli associated with mating (Bergh et al. 1992). Whatever the cause, this refractory behaviour seems to be lost after some time of sexual rest in females. After this time, the male that has mated the female first no longer has any control over future paternity. However, his sperm remains almost as efficient as a fresh ejaculate, and he will sire at least half of the offspring produced if the female remates. The pattern of male–male competition is therefore different when considering immediate or delayed remating attempts. In the first case, direct male–male competition could have selected males to induce nonreceptivity in females. Furthermore, males may gain by 'marking' nonvirgin females, to avoid their being chosen for further mating. Since many females are receptive at the same time (Moreau & Rigaud 2000), and since there is an excess of females in the wild (Juchault et al. 1993), mating with a recently mated female may be costly: males will sire, on average, only half of the putative offspring, while they may lose considerable time and energy detecting, assessing and trying to inseminate these females. In cases of delayed remating, male–male competition is indirect through competition between fresh and stored sperm. The second mating partner could be considered a dupe, since he will share paternity with a former mate. Recent field data show that most 2-year-old females already have stored sperm from an earlier mating (J. Moreau, unpublished data). However, field and simulation data indicate that 2-year-old females make a greater contribution to woodlice population dynamics than 1-year-old females, mainly because of their size-related higher fecundity (Caubet 1998). Any given male may therefore gain an advantage by mating with an already mated 2-year-old female, even sharing paternity, if siring half of a large brood is as efficient as all of a smaller brood.

It is possible that male induction of a female refractory period in crustaceans is a strategy for males to increase the probability of their paternity, as an alternative to mate guarding. To understand the evolution of mating strategies in crustaceans, it will be important to compare the relative costs of these two strategies: for example, for the same reproductive gain, mate guarding could have been replaced by the induction of a refractory period in terrestrial species if the former strategy is more costly than the latter (Zimmer 2001). Because a few 'semi-terrestrial' isopods do guard their mates, isopods provide a good model to study this evolution (Zimmer 2001).

Despite the potential immediate advantage of inducing low female remating frequency for a male, we cannot dismiss the possibility that this low remating frequency is under female control; but to consider that case, one has to understand the relative advantage of remating versus no remating to females (Jormalainen 1998; Arnqvist & Nilsson 2000). Remating does not seem to provide fecundity benefits to woodlice females (we did not measure any effect on survival), since oocyte maturation begins before female receptivity in most woodlice (e.g. Moreau & Rigaud 2002). A direct benefit of avoiding sperm depletion is also unlikely, since the fertility efficiency of one or two ejaculates is the same. A recent study showed that remating tendency has a strong genetic component in a butterfly species (Wedell 2001), as well as the sperm storage pattern and the duration of postmating nonreceptivity. A similar study in woodlice could help us to understand why some females accept immediate remating, while most others are not receptive.

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