

# Pattern of sperm transfer in redback spiders: implications for sperm competition and male sacrifice

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Many sperm competition studies have identified copulation duration as an important predictor of paternity. This result is often interpreted as a sperm transfer effect—it is assumed that sperm transfer is limited by copulation duration. Here we test the assumption of duration-dependent sperm transfer in the Australian redback spider, *Latrodectus hasselti*, in which a correlation between copulation duration and paternity has been implicated in the evolution of a rare male self-sacrifice behavior. Male redbacks facilitate sexual cannibalism by females during copulation. Sexual cannibalism is apparently adaptive for redback males, in part because it results in longer copulations (25 versus 11 min.), and copulation duration is positively correlated with paternity. We assessed sperm transfer in normal copulations and in copulations that we terminated at 5, 10, or 20 min. Our results show that the paternity advantage of sexual cannibalism is not owing to time-dependent sperm transfer, as redback males transfer the majority of their sperm within the first 5 min of copulation. This suggests that the link between copulation duration and paternity may instead be owing to cryptic female choice or the transfer of nongametic ejaculatory substances. Results further indicate that the act of cannibalism itself might play a role in mediating sperm transfer. This study highlights the importance of understanding mechanisms of sperm transfer when attempting to interpret the outcome of sperm competition studies. *Key words*: copulation duration, *Latrodectus hasselti*, redback spider, sexual cannibalism, sperm competition, sperm transfer. [*Behav Ecol* 15:785–792 (2004)]

Sperm competition, defined by Parker (1970) as competition within a female between the sperm of two or more males for fertilization of the ova, is widely accepted as an important selective force, resulting in the evolution of a wide variety of physiological, morphological, and behavioral adaptations (Arnqvist and Danielsson, 1999a; Birkhead and Møller, 1998a; Elgar, 1998; Simmons, 2002). Theory suggests that where sperm competition occurs, the number of sperm a male transfers to a female will have important consequences for male fitness, and sperm number is therefore predicted to be under strong selection (Birkhead and Møller, 1998b; Parker, 1990, 1998; Parker et al., 1990). In many species, the male who transfers the greatest number of sperm to the female will father the greatest proportion of a female's offspring (Dickinson, 1986; Simmons et al., 1996; Simmons and Siva-Jothy, 1998). But in general, fitness consequences of numerical sperm transfer will depend in part on the mechanism of sperm competition operating in a given system (Cook et al., 1997; Parker, 1998; Parker et al., 1990).

Assessing sperm competition mechanisms can be challenging because of the cryptic nature of most postcopulatory processes. Parker et al. (1990) advocate the use of sperm competition models for investigating underlying mechanisms of sperm competition. Use of these models however, requires knowledge of sperm transfer patterns, in addition to estimates of resulting paternity patterns. Although several techniques exist for reliably estimating paternity patterns (e.g., irradiated male, genetic marker, DNA microsatellite markers; for a review, see Simmons, 2002), measuring sperm transfer in species in which males transfer sperm directly to the female can be problematic, and standard assessment techniques have not

been applied in all studies. It has been suggested that copulation duration may be used to approximate sperm transfer, assuming that sperm transfer occurs continuously throughout copulation (Parker et al., 1990). Although a positive relationship between sperm transfer and copulation duration has been found or inferred in several arthropods (water striders: Arnqvist and Danielsson, 1999b; beetles: Dickinson, 1986; shield bugs: Filippi et al., 2000; flies: Parker and Simmons, 1991; Parker et al., 1990) duration-dependent transfer is not a general rule. Instead, many studies have revealed a nonlinear relationship, in which sperm transfer occurs fairly quickly, either relatively early (millipedes: Barnett and Telford, 1994; beetles: Bloch Qazi et al., 1996; spiders: Bukowski and Christenson, 1997; Eberhard and Huber, 1998; Suter and Parkhill, 1990; bumblebees: Duvoisin et al., 1999; flies: Gilchrist and Partridge, 2000; Yamagishi and Tsubaki, 1990; crickets: Hartmann and Loher, 1999; stink bugs: Hosokawa and Suzuki, 2001; fire bugs: Schofl and Taborsky, 2002) or relatively later in the copulation (water striders: Cambell and Fairbairn, 2001; flies: Lorch et al. 1993; Taylor and Yuval, 1999; true bugs: Rodriguez, 1998; Odonates: Siva-Jothy and Tsubaki, 1989). Clearly, these findings indicate that a simple linear relationship between copulation duration and sperm transfer cannot be assumed.

Nevertheless, in species in which copulation duration has been found to correlate with paternity, or in which males are observed to increase copulation duration when mating with nonvirgin females, it is often hypothesized that longer copulations allow for greater volumes of sperm to be transferred, which may then give males an advantage in some form of numerical sperm competition (Andrade, 1996; Elgar et al., 2000; Fahey and Elgar, 1997; Schneider et al., 2000; Zhu and Tanaka, 2002). There are, however, alternative explanations for a correlation between copulation duration and paternity. For example, if females bias sperm use or storage on the basis of copulatory duration or vigor, that is, if cryptic female choice (Eberhard, 1996) determines paternity, this correlation would

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be observed (Andres and Rivera, 2000). This correlation would also be found if the transfer of nongametic ejaculate components that affect a male's share of paternity is duration dependent, in which case paternity patterns may reflect the resolution of intrasexual conflict (Chapman et al., 1995). Thus, assuming a linear relationship between sperm transfer and time, even if paternity is positively correlated with copulation duration, could lead to incorrect interpretation of empirical results. Determining the pattern of sperm transfer in species in which copulation duration predicts paternity (1) can fundamentally change our understanding of the evolutionary processes that shape the system, and (2) may indicate the operation of dynamics that are predicted in theory, but are often difficult to demonstrate empirically, such as intrasexual conflict or cryptic female choice (Eberhard, 1996).

In the present study, we examine sperm transfer patterns in the Australian redback spider, *Latrodectus hasselti*, a species in which paternity is positively correlated with copulation duration. Redback males perform a "somersault" behavior at the onset of copulation, which places their abdomen over the female's mouth parts (Forster, 1992). Most females proceed to cannibalize the male during copulation (for details on the copulatory sequence, see Forster 1995). Male facilitation of sexual cannibalism has been found to be adaptive for redback males, as cannibalized males increase their paternity relative to noncannibalized males (Andrade, 1996). This increase in paternity with cannibalism results, in part, from the fact that cannibalized males copulate for longer (25 versus 11 min), and copulation duration is positively correlated with paternity in doubly mated females (Andrade, 1996). The mechanism by which cannibalized males achieve this paternity advantage, however, is not currently known. Andrade (1996) suggested that cannibalized redback males who copulate for longer gain a paternity advantage by transferring more sperm to the female. This hypothesis makes two major assumptions: (1) sperm transfer is positively related to the duration of copulation across the typical time span of redback copulations (range = 6–31 min; Andrade, 1996), and (2) redback sperm compete in some form of a "raffle" model of sperm competition, in which a male's paternity success is proportional to the relative number of sperm inseminated (Parker et al., 1990).

In the present study, we tested the first assumption of Andrade's (1996) hypothesis by assessing sperm transfer as a function of copulation duration in redbacks. To investigate the mechanism by which cannibalized males might achieve a paternity advantage, staged copulations were interrupted at three points (5, 10, and 20 min), and sperm transfer was assessed. We also allowed copulations to terminate naturally to further explore the relationship among sexual cannibalism, copulation duration, and sperm transfer.

## METHODS

A laboratory population of *L. hasselti* was established from field-collected individuals from Perth, Western Australia (1999 and 2001), and New South Wales, Australia (2002). Spiders were shipped to Toronto and reared in a reversed 12-h dark/12-h light cycle on a standardized diet of fruit flies (*Drosophila* spp.) and house crickets (*Acheta domesticus*). Because redbacks are nocturnal, all mating trials were conducted during the dark cycle under red lights.

### Reproductive morphology and mating trials

Female *L. hasselti* have paired independent sperm storage organs (spermathecae), which are each inseminated by one of a male's paired independent copulatory organs (palps).

Insemination in redbacks occurs while the male is mounted on the female and is handed, with a female's right spermatheca being inseminated by a male's right palp and left spermatheca inseminated by a male's left palp. Palps are inserted one at a time, with each palpal insertion separated by a period of intercopulatory courtship, during which the male dismounts the female. In the present study, all males were allowed to achieve a single palpal insertion with one female, which resulted in each male having one used and one unused palp, and each female having one inseminated and one virgin spermatheca. This procedure allowed two potential assessments of sperm transfer to the female: (1) a comparison of the contents of the inseminated spermatheca and used palp with the contents of the unused palp, and (2) a comparison of the full and empty palp. Male redbacks do not have a direct connection between their palps and gonads (similar to all male spiders, Foelix, 1982) and fill their palps with sperm once at sexual maturity (Andrade and Banta, 2002). Therefore, male sperm complement was constant over the time during which sperm counts took place.

### Treatments

All mating trials were video-recorded by using cameras with macrozoom lenses to allow detailed analysis of the mating sequence. Virgin males and females were randomly chosen, weighed, and assigned either to a 5-min ( $n = 20$ ), 10-min ( $n = 20$ ), or 20-min ( $n = 15$ ) manipulated duration treatment, or to an unmanipulated duration treatment ( $n = 27$ ). In all treatments females were introduced to mating arenas 48 h before the mating trial and allowed to construct webs on wooden frames. A male was then introduced to a female's web and was allowed to proceed through courtship. If a male failed to achieve an insertion after 9 h of courtship, the male and female were separated and not included in the present study ( $n = 16$ , normal redback courtship duration is  $5.03 \pm 0.38$  h; Forster, 1992). In successful matings, the time of the first palpal insertion and which palp was inserted were noted. We artificially terminated the first palpal insertion after the preassigned copulation duration had passed, except in the *ad lib* treatment, in which copulations were allowed to terminate naturally and total copulatory duration was recorded.

We artificially terminated copulations by teasing the male off the female with a paintbrush. In most cases the male and female separated immediately or shortly after the initial contact of the paintbrush. In all trials the time of final separation was noted. If the pair failed to separate within 90 s of the assigned duration, the trial was not included in the final analysis ( $n = 5$ ). If the copulation terminated naturally, before the preassigned duration, total copulation duration was noted and the trial was included in the unmanipulated treatment ( $n = 9$ ). Thus, final sample size was 41 for the manipulated duration treatments (5 min,  $n = 18$ ; 10 min,  $n = 17$ ; 20 min,  $n = 6$ ) and 36 for the unmanipulated treatment.

We assessed cannibalism post hoc by examining the degree of abdomen damage sustained by the male. We classified males in two groups: (1) having no evidence of cannibalism or minimal cannibalism (only slight surface damage from digestive enzymes, which males can typically survive) or (2) having substantial cannibalism (abdomen punctured by the female's fangs or severely damaged and abdominal contents partially or completely consumed).

Spiders were weighed before trials (Ohaus balance accurate to 0.01 mg). Immediately after copulation, males and females were separated and killed via hypothermia. We estimated the size of males by the mean patella-tibia length of both front legs. Measurements were made on digital images taken under a dissecting microscope by using Act 1 (Nikon Corp., 2000) and

Simple PCI (Compix Inc. Imaging Systems, 2002) software programs. Male and female reproductive organs were isolated for sperm quantification immediately after death.

### Sperm quantification

Spider sperm have a tendency to bind and clump together, making quantification a challenge (Bukowski and Christenson, 1997; Cohn, 1990). We modified the sperm quantification technique of Bukowski and Christenson (1997) and Bukowski et al. (2001) to optimize the procedure for *L. hasselti* sperm and to ensure a uniform distribution of sperm within each sample. We rarely saw sperm clumps when using the modified procedure described below. When clumps were encountered, the total number of sperm in the clump was estimated (see Bukowski and Christenson, 1997). No clumps exceeded an estimated 50 sperm, and clumps larger than 15 sperm were rarely encountered.

Male palps were isolated by cutting through the palpal femur under a dissecting microscope. Each palp was placed individually in a 1.5-ml Brinkman polypropylene centrifuge tube with 50 ml sperm counting solution. We obtained the sperm counting solution by adding 150 ml of a solution consisting of 10 ml "spider" saline (Juusola and French, 1998) and 100 ml of 10% triton-X detergent, to 10 ml of spider saline. The palp was ground by using forceps to release sperm into the solution. The sample was vortexed for 30 s and then centrifuged at 1000g for 10 min. The process of vortexing and centrifuging was repeated twice more. The sample was then vortexed for a final 30 s. Two 15 ml samples were drawn and placed on an improved Neubauer double-chamber hemocytometer. Total sperm numbers were estimated by using a subsampling technique, with four randomly chosen quadrats counted per sampling chamber, under a compound microscope at  $\times 400$ . Counts from the four quadrats were highly correlated (Pearson correlation coefficients ranging from 0.812–0.954), supporting the idea that there was an even distribution of sperm across the sampling field. The mean of the two sampling chambers was used to estimate the total number of sperm in each palp.

Similarly, after trials, the female's spermathecae and associated copulatory ducts were removed under a dissecting microscope. The inseminated spermatheca was subjected to the same sperm quantification procedure used for palpal sperm counts. The virgin spermatheca was crushed under a cover slip in spider saline and examined under a light microscope at  $\times 400$  for the presence of sperm. In all cases we confirmed that sperm were found only in the spermatheca observed to be inseminated by the male.

### Control sperm counts

In addition to experimental males, sperm counts were performed on 25 randomly selected adult virgin males. The total number of sperm contained in each palp was quantified to determine the mean and variation in virgin male sperm numbers, as well as the relationship between the number of sperm in a male's right and left palps. The latter was necessary to determine whether the number of sperm transferred to the female could be accurately estimated by comparing the used and unused palp and to allow a test of whether estimates of sperm transfer based on counting only the sperm in the female's spermatheca were accurate (see Results).

### Estimates of sperm transfer

In the present study we assumed that the number of sperm counted in the inseminated spermatheca was equal to the number of sperm transferred by the male. To ensure that we

counted all sperm transferred, we assessed the number of sperm contained in both the inseminated spermatheca and the associated insemination ducts. We also limited the opportunity for postcopulatory sperm manipulation by females (e.g., selective killing, transporting, or ejecting a particular male's sperm) by killing females immediately after copulation.

In addition to the absolute number of sperm transferred, we calculated the percentage of sperm transferred to the spermatheca, as an estimate of sperm transfer standardized for the total number of sperm which a given male initially had in his palp. The percentage of sperm transferred to the spermatheca ( $P$ ) was calculated by dividing the number of sperm counted in the female ( $T$ ) by the total number of sperm originally available to the male in his used palp:

$$P = [T/(U + T)] \times 100\%,$$

where  $U$  is the number of sperm remaining in the used palp. This method of sperm transfer estimation proved to be very reliable (see Results); therefore, it was used over the alternative method of sperm transfer estimation, which compares the percentage of difference between the used and unused palp with the percentage of difference in virgin control males.

### Analyses

All statistical and power analyses were completed by using Systat 10.2 (SPSS 2002) with consultation from Zar (1984). All summary statistics are reported as mean  $\pm$  1 SE, unless otherwise indicated. The percentage of sperm transferred variable was arcsine transformed and male leg length was log transformed for all statistical analyses to satisfy the assumption of normality. If transformed data were not normally distributed ( $p < .05$ , Lilliefors test) then nonparametric statistics were used as reported. The summary statistics for the percentage of sperm transferred variable are reported as mean percentage transferred  $\pm$  95% confidence interval (CI) in the original percentage scale.

## RESULTS

### Control sperm counts

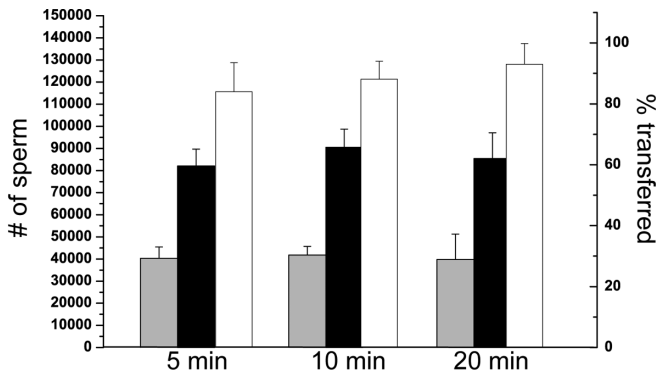
Virgin males have an average of  $105,359 \pm 10,660$  sperm within their two palps, with the number of sperm in the right palp being significantly correlated to the number of sperm contained in the left palp, (Pearson correlation coefficient  $R = .806$ ,  $p < .0001$ ,  $n = 25$ ), and the absolute number of sperm in the two palps did not differ significantly (paired  $t = -1.273$ ,  $p = .215$ ,  $n = 25$ ). The total number of sperm stored in a male's two palps was not found to be significantly correlated with male size ( $R^2 = .001$ ,  $p = .891$ ,  $n = 22$ ) or weight ( $R^2 = .073$ ,  $p = .192$ ,  $n = 25$ ), despite significant variation in total sperm numbers.

### Sperm transfer and copulation duration

#### *Manipulated duration treatments*

Males transfer the majority of their sperm within the first 5 min of copulation. There was no significant increase in the percentage of sperm transferred in the 10-min ( $n = 17$ ) or 20-min ( $n = 6$ ) treatments compared with the 5-min ( $n = 18$ ) treatment (ANOVA:  $F_{2,37} = 0.636$ ,  $p = .535$ ) (Figure 1). This result holds even if the 10- and 20-min treatments are combined to increase the power of the test (ANOVA:  $F_{1,38} = 0.875$ ,  $p = .355$ ). In this analysis, power to detect a difference of 20% was 0.996.

The number of sperm in the female's spermatheca depended on the total number of sperm in her mate's two



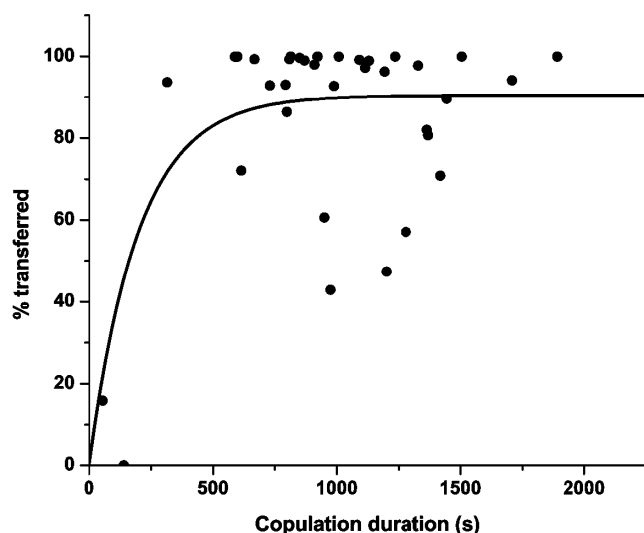
**Figure 1**

Mean and SE for sperm transfer variables (grey bars indicate no. of sperm in spermatheca; white bars, percentage transferred) a total number of sperm in a male's two palps (total palpal sperm = sperm counted in used palp + sperm counted in unused palp + sperm counted in spermathecae, black bars) for males in each of three manipulated copulation duration treatments.

palps (total palpal sperm = sperm counted in used palp + sperm counted in unused palp + sperm counted in spermathecae) across treatments ( $F_{1,35} = 11.528$ ,  $p = .002$ ). Once this covariate was included, there was no difference in the total number of sperm transferred to the female's spermatheca in the three treatments (ANCOVA:  $F_{2,35} = 0.232$ ,  $p = .794$ ) (Figure 1). Combining the 10- and 20-min treatments again revealed no difference in the number of sperm transferred to the female (ANOVA:  $F_{1,38} = 0.027$ ,  $p = .871$ ). In this analysis, power to detect a difference of 15,000 sperm was 0.64.

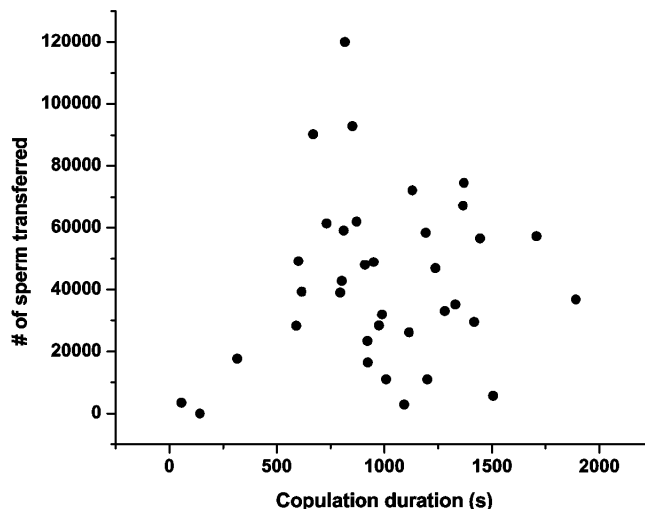
#### Unmanipulated duration treatments

As seen in the manipulated duration treatment, males in the unmanipulated treatment transferred the majority of their sperm complement early in the copulation (Figure 2). Males transferred an average of  $90.4\% \pm 4.0\%$ , of their sperm to the female during copulations which terminated naturally. The



**Figure 2**

Percentage of total sperm complement transferred to female's spermatheca as a function of copulation duration in unmanipulated copulation duration treatment. Relationship follows a nonlinear one-phase exponential association curve  $Y = 90.44(1 - e^{-0.005X})$ ,  $R^2 = .466$ ,  $n = 36$ .



**Figure 3**

The absolute number of sperm transferred to the female's spermatheca in relation to copulation duration in the unmanipulated copulation duration treatment ( $n = 36$ ).

absolute number of sperm transferred to the female's spermatheca was not correlated with copulation duration ( $R^2 = .012$ ,  $p = .449$ ,  $n = 36$ ) (Figure 3).

Male size does not appear to influence sperm transfer. Male size was not correlated with the number of sperm found in the female's spermatheca ( $R^2 = .053$ ,  $p = .198$ ,  $n = 33$ ) or with sperm transfer rate (number of sperm in spermatheca/copulation duration;  $R^2 = .002$ ,  $p = .787$ ,  $n = 33$ ).

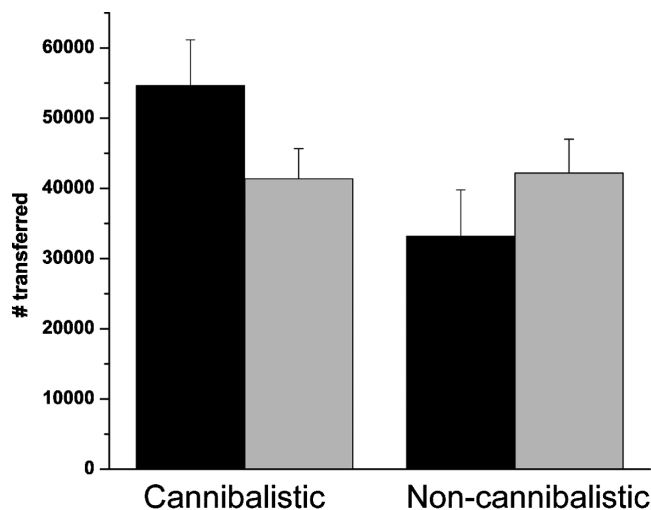
#### Cannibalism and sperm transfer

##### Manipulated duration treatments

There was no difference in the proportion of males cannibalized in the long copulation treatments (10- and 20-min treatments pooled, 13/21 males cannibalized) compared with the short copulation treatment (5-min treatment, 13/18 males cannibalized; Pearson  $\chi^2 = 0.464$ ,  $p = .496$ ). There was no effect of cannibalism on sperm transfer when copulation duration was manipulated. When the 5-, 10-, and 20-min treatments were pooled, no significant difference was found between cannibalized ( $41\,356 \pm 4\,296$ ,  $n = 26$ ) and noncannibalized ( $42\,188 \pm 4\,812$ ,  $n = 13$ ) males in the number of sperm transferred to the spermatheca (ANOVA:  $F_{1,36} = 0.01$ ,  $p = .905$ ) (Figure 4). There was also no difference in the percentage of sperm transferred to the spermatheca by cannibalized ( $88.4\%$ , 95% CI: 80.5–94.5%,  $n = 26$ ) and noncannibalized ( $85.9\%$ , 95% CI: 75.1–94.1%,  $n = 13$ ) males (ANOVA:  $F_{1,37} = 0.178$ ,  $p = .676$ ) (Figure 5).

##### Unmanipulated duration treatment

Similar to results in Andrade (1996), cannibalized males were found to copulate for significantly longer durations than were noncannibalized males ( $19.03\text{ min} \pm 1.13\text{ min}$ ,  $n = 17$  versus  $13.23\text{ min} \pm 1.58\text{ min}$ ,  $n = 13$ ; ANOVA:  $F_{1,28} = 9.409$ ,  $p = .005$ ). In addition, cannibalized males transferred more sperm than did noncannibalized males ( $54\,629 \pm 6\,549$ ,  $n = 17$ , versus  $33,183 \pm 6587$ ,  $n = 13$ ; ANOVA:  $F_{1,28} = 5.518$ ,  $p = .031$ ) (Figure 4). Although cannibalized males also tended to transfer a greater percentage of their sperm than did noncannibalized males ( $90.7\% \pm 2.9\%$ ,  $n = 17$  versus  $78.1\% \pm 7.8\%$ ,  $n = 13$ ), this difference was not statistically significant (Mann-Whitney  $U = 103$ ,  $p = .753$ ) (Figure 5). Cannibalized males also tended



**Figure 4**

Mean number of sperm transferred to the female's spermatheca in cannibalistic and noncannibalistic copulations in unmanipulated ( $n = 30$ , black bars) and manipulated ( $n = 39$ , grey bars) copulation duration treatments.

to possess a greater total number of sperm ( $115,284 \pm 12,280$  versus  $83,205 \pm 11,900$ ), although this difference was again not statistically significant (ANOVA:  $F_{1,28} = 3.364$ ,  $p = .077$ ).

#### Sperm number and palp use

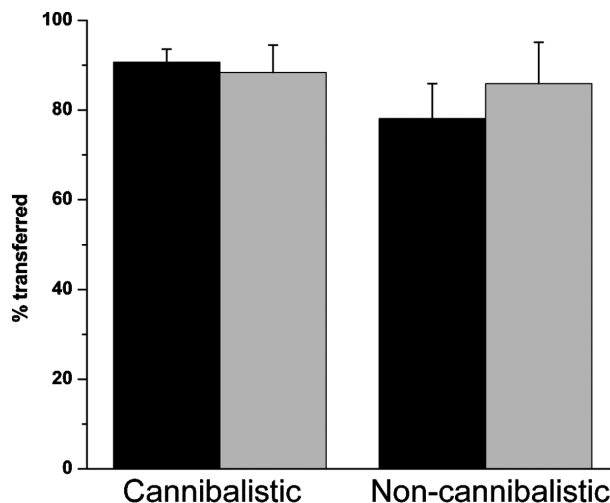
Within the experimental trials, there was a significant tendency for males to copulate with the palp that contained more sperm ( $49,463 \pm 2730$  versus  $41,590 \pm 2902$ ; paired  $t = 2.414$ ,  $p = .018$ ,  $n = 75$ ). To ensure this result was not an artefact of our counting method, we compared the estimated number of sperm contained in a male's two palps in the experimental trials (used palp + sperm in the spermatheca,  $91,508 \pm 4727$ ,  $n = 75$ ) with the total sperm in the two unused palps in the control counts performed on virgin males ( $105,359 \pm 10,660$ ,  $n = 25$ ). There was no significant difference between these groups ( $t = -1.188$ ,  $p = .243$ ), indicating our method of estimating sperm transfer and percentage of transfer did not result in a systematic over- or underestimate of male sperm numbers.

## DISCUSSION

### Pattern of sperm transfer in redbacks

Sperm transfer is not limited by copulation duration in redbacks. Instead, males transfer the majority of the sperm in a single palp within the first 5 min of insertion. This pattern of rapid sperm transfer was seen in both manipulated and unmanipulated duration treatments and is likely adaptive for male redbacks. Females sometimes forcefully terminate copulations early in the copulation (Snow L, personal observations) and males are often found in direct competition with one or more males in the field (Andrade, 1996). The high risk of interruptions or takeovers during copulation would likely favor rapid sperm transfer (Austad, 1982).

This pattern of rapid sperm transfer indicates that the paternity advantage enjoyed by males who copulate for longer durations does not result from the transfer of more sperm as suggested by Andrade (1996). However, longer copulations could still lead to higher paternity if they allow the transfer of other ejaculatory substances important in postcopulatory



**Figure 5**

Mean percentage of sperm transferred to female in cannibalistic and noncannibalistic copulations in unmanipulated ( $n = 30$ , black bars) and manipulated ( $n = 39$ , grey bars) copulation duration treatments.

competition. In many insect species, the male's ejaculate contains nongametic substances that affect female reproductive behaviors, such as receptivity to further matings and oviposition patterns (for a review, see Arnqvist and Nilsson, 2000; Cordero, 1995; Eberhard, 1996; Hartmann and Loher, 1999; Johnstone and Keller, 2000; Parker, 1970; Pitnick et al., 2001). A second advantage of cannibalism for males—decreased receptivity of the cannibalistic female to future mating attempts (Andrade, 1996) is consistent with a role for such manipulative ejaculatory substances. In other species, male ejaculatory substances have been found to play a role in direct male-male sperm competition by removing or destroying the sperm of competing males (Chapman et al., 1995; Harshman and Prout, 1994; Pitnick et al., 2001; Price et al., 1999). Along these lines, redback males who are cannibalized and/or copulate for longer durations may transfer greater volumes of ejaculatory substances important in direct male-male sperm competition and, thus, achieve a greater share of paternity.

Alternatively, copulation duration could serve as copulatory courtship, with females biasing their storage and/or use of sperm in favor of males who are cannibalized and copulate for longer durations (Eberhard, 1996, 1997). Copulation duration potentially influences female storage and/or use of sperm in other insect and spider species (Andres and Rivera, 2000; Bukowski and Christenson, 1997), although the possibility that females are able to cryptically select from among the sperm they receive remains controversial and difficult to demonstrate empirically (Birkhead, 1998; Eberhard, 2000; Pitnick and Brown, 2000; but see Aviles et al. 2000).

### Cannibalism and sperm transfer

In the unmanipulated duration treatment, males who were cannibalized transferred a greater number of sperm and exhibited a nonsignificant tendency to transfer a greater percentage of their sperm to the female than did males who were not cannibalized. This increase in sperm transfer was not owing to the correlated increase in copulation duration enjoyed by cannibalized males (19 versus 13 min), as demonstrated by the manipulated duration treatments. This suggests cannibalism itself may lead to increased sperm transfer.

Female cannibalistic behavior could have a direct effect on sperm transfer. During cannibalistic copulations, female

redbacks often remain very still (Snow L, personal observations), and it is possible that this behavioral quiescence could facilitate increased sperm transfer (Bloch Qazi, 2003). Conversely, in a subset of the matings in which males are not cannibalized (i.e., sustain no abdomen damage), females move about the web and actively push the male's abdomen away from their jaws throughout insemination (Snow L, personal observations). This female noncannibalistic or "interference" behavior could hinder insemination and result in decreased sperm transfer. The fact that females demonstrate this behavior in only a subset of noncannibalistic copulations may also help to explain why there was such large variability in the percentage of sperm transferred by noncannibalized males in the unmanipulated treatment. It is clear, however, that any mechanism used by the female to hinder sperm transfer would have to occur very early on, as males transfer the majority of their sperm during the first 5 min of the copulation. This is the case with female interference behavior, which usually begins at the onset of copulation and continues until the male is forced to withdraw his palp (Snow L, personal observations).

Because cannibalism was not manipulated in the present study, however, it is also possible that females preferentially cannibalized males with more sperm—such a correlation could produce the results seen here.

In contrast to the unmanipulated treatment, no difference in sperm transfer was observed between cannibalistic and noncannibalistic copulations in the manipulated duration treatments. This difference could be an artifact of our post hoc method of identifying cannibalism based on male abdominal damage. This problem could arise if there was insufficient time in 5-min matings for the males to accumulate sufficient damage to be correctly classified as "cannibalized" when females were behaving in a "cannibalistic" manner (i.e., remaining still and extruding digestive enzymes). However, this is not likely to have been a problem as the proportion of males cannibalized did not differ across manipulated duration treatments.

More detailed observations of the female behaviors that comprise cannibalistic and noncannibalistic matings and an assessment of their relation to insemination are needed to determine the influence of sexual cannibalism on sperm transfer.

### Sperm numbers, male size, and paternity

Body size is predicted to be positively related to the absolute number of sperm possessed by a male, in part because larger males would likely have more resources for sperm production and greater storage capacity (Berrigan and Locke, 1991; Parker and Simmons, 1994; Pitnick and Markow, 1994). However, we found that virgin redback males have highly variable numbers of sperm in their palps, and this variation is not related to variation in male size. Similarly, we found no relationship between male size and the absolute number or rate of sperm transfer. Although in many species male body size is positively correlated with the number of sperm possessed by virgin males (Berrigan and Locke, 1991; Cohn, 1990), the number of sperm transferred during copulation (Berrigan and Locke, 1991; Cohn, 1990; Pitnick and Markow, 1994), and/or the rate of sperm transfer (Simmons and Parker, 1992), a clear relationship with body size is not always found (Arnqvist and Danielsson, 1999b; Cook et al., 1997; Oronen, 1997; Schneider and Elgar, 2001; Watson, 1991; Woodhead, 1984). In redbacks, sperm numbers may not be under as strong selection as is often assumed when some form of numerical sperm competition determines paternity (Cook et al., 1997; Eady, 1995; Gage, 1998). In redbacks, one palp

insertion is often sufficient to fertilize a female's lifetime production of eggs, suggesting female redbacks are not sperm limited (Andrade and Banta, 2002). If variation in sperm numbers across the range exhibited by virgin males in the present study is not crucial to male success in postcopulatory competition, male redbacks may instead invest resources in the production of other ejaculatory substances or behaviors that are important in postcopulatory competition (Alonzo and Warner, 2000). This could help to explain our finding that redbacks copulate for longer than is necessary for the transfer of sperm. However, the possibility that the number of sperm transferred does influence male success in postcopulatory competition cannot be ruled out. We found that male redbacks preferentially insert the palp with the greatest number of sperm during their first copulation, which suggests sperm numbers may be important in determining relative paternity. Determining the relative importance of sperm numbers and ejaculatory substances on paternity will require more detailed investigation into underlying mechanisms of postcopulatory competition operating in this system.

### Conclusion

Copulation duration is a predictor of paternity in several insect and spider species (Elgar, 1998; Simmons and Siva-Jothy, 1998). This has often been taken to indicate that males who copulate for longer transfer more sperm and/or displace more sperm than do competitors and have greater success in some form of a numerical raffle competition for fertilization (Andrade, 1996; Elgar et al., 2000; Zhu and Tanaka, 2002). These studies examine the empirical outcome of the internal processes that occur during fertilization and conclude that variation in paternity is likely mediated by intermale sperm competition. However, such variation, even when correlated with copulation duration, could also arise from female-mediated processes, intersexual conflict, or from some form of intermale competition that is not related to the number of sperm transferred, as we conclude here. These alternative mechanisms suggest a different evolutionary history and differences in current selective pressures than would be expected under numerical sperm competition. Thus, it is important to determine mechanism as well as outcome, particularly when investigating postcopulatory sexual selection.

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