Sexual Selection for Male Sacrifice in the Australian Redback Spider

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During copulation, male redback spiders (*Latrodectus hasselti:* Theridiidae) position themselves above the female's jaws. This apparent male complicity in sexual cannibalism is favored by sexual selection because cannibalized spiders receive two paternity advantages. First, cannibalized males copulated longer and fertilized more eggs than those that survived copulation. Second, females were more likely to reject subsequent suitors after consuming their first mate. These results represent empirical evidence for male copulatory suicide as an adaptive behavior.

Darwin (*1*) proposed sexual selection to explain the evolution of traits that decrease the probability of survival but give an advantage in the struggle for reproduction. However, there has been resistance to the idea that sexual cannibalism—in which males are consumed by females during copulation—might be adaptive for the consumed male (*2*). Sexual cannibalism is generally considered to be the result of predatory females overcoming the defenses of weaker males (*2*), but in theory there are circumstances in which males could benefit from being eaten (*3–5*) and might therefore facilitate their own consumption. Recent behavioral observations have documented apparent male complicity in cannibalism (*6, 7*). For example, male redback spiders (*Latrodectus hasselti*) always perform a somersault behavior during sperm transfer, in which the dorsal surface of the abdomen is placed directly over the female's mouthparts and remains there throughout copulation (*6, 8*). This somersault occurs a few seconds after the male intromittant organ is inserted and has not been reported in any other *Latrodectus* species (*6*). Although these reports lend some support to the "male suicide" hypothesis, the adaptive basis of such a sacrifice has never been demonstrated. Here I show that male redback spiders are sexually selected to facilitate sexual cannibalism because it results in two paternity advantages for males that are eaten. These results show that sexual cannibalism need not be the result of a sexual conflict of interest (*5*) but instead can be an adaptive male strategy.

I investigated the occurrence and consequences of the copulatory somersault in field populations of redbacks (Perth, Western Australia, January 1994) and found that males were consumed during copulation in 65% (11/17) of observed matings. In every case, cannibalism began while the male was in the somersault posture; females were never observed to eat males in any other context (that is, never during courtship or web cohabitation). Although males always somersault, their fate is related to the female's hunger level. In a separate field experiment, the relative condition of cannibalistic females was significantly lower than that of noncannibals [(*9*), but see (*6*)]. As the copulatory somersault does not appear to be the result of manipulation by females, it probably represents male complicity in cannibalism (*6, 10*).

If male sacrifice is adaptive, cannibalized males must benefit reproductively from suicide. Theoretically, males may gain by contributing their somatic nutrients to their own offspring ("paternal effort") (*3–5, 11, 12*), by enhancing their fertilization success ("mating effort") (*3, 12*), or through some combination of the two. Because sexual cannibalism involves the consumption of nutrients that could be transferred from the female to the eggs, paternal effort is generally considered to be the most likely source of possible benefits to cannibalized males (*3–5, 12*). However, male redbacks (median field mass ± SE = 4.4 ± 0.3 mg; n = 33) represent only 1 to 2% of the female's mass (256 ± 30 mg; n = 22) (*13*) and approximately 2.5% of the mass of a typical egg sac (Table 1). Moreover, consumption of one male does not result in an increase in egg number or mass (Table 1); therefore, copulatory suicide as paternal effort appears unlikely.

Because copulatory suicide in redbacks occurs during sperm transfer, it might function as male mating effort by increasing paternity. Male-male competition for fertilizations occurs in this species. In nature (Perth, Western Australia, December 1993), webs of individual females contained up to six males at a time (n = 23 webs, median number of males per web per day = 2, range = 0 to 6), and dissections of female reproductive organs revealed that a minimum of 17.4% (4/23) of females collected near the end of the mating season had copulated with at least two (but not more than three) males (*14*).

In the laboratory, I used a sterile male technique to determine the paternities of pairs of males mated to single females in cannibalistic and noncannibalistic matings. Laboratory-reared virgin females (n = 22) were mated consecutively to two virgin males [each of which was either normal (N) or irradiated (I); these males were exposed to 11 krad of gamma radiation] in the four possible sequences of these two types (that is, NI, IN, NN, and II). The numbers of hatched and unhatched eggs were counted in the first egg sac produced after both matings. I calculated the proportion of eggs fertilized by the second male (paternity of the second male or P_2) in mixed treatments (IN or NI mating order) by assuming that unhatched eggs had been fertilized by an irradiated male and correcting for variation in fertility using the results of NN and II matings (*15*).

The second male's paternity varied extensively (median P_2 = 0.56, range = 0.0 to 1.0, n = 11), and most of this variation was explained by the duration of his copulation relative to that of the first male: The paternity of the second male (P_2) was positively related to his copulation duration (CD_2) and negatively related to the copulation duration of the first male (CD_1) ($P_2 = 0.251 - 0.019\ CD_1 + 0.045\ CD_2$; correlation coefficient r^2 = 0.77, P = 0.006, n = 10). A male mating with a nonvirgin female (that is, the second mating in my experiment) could increase his paternity by increasing his copulation duration relative to that of the first male ($P_2 = -0.076 + 1.412[CD_2/(CD_1 + CD_2)]$; r^2 = 0.42, P = 0.043, n = 10), which suggests that longer copulations may result in the transfer of more sperm (*16*). Copulation duration ranged from 6 to 31 min, but second males that were cannibalized (n = 5) spent a median of 25 min in copulation as compared with only 11 min for second males that survived copulation (n = 12, P = 0.035, Mann-Whitney test). It is likely that females control total copulation duration and permit longer copulations while they are occupied in consuming the male. In cannibalistic matings, the female began to masticate the male's abdomen a few seconds after it came in contact with her mouthparts. Movement of the female's mouthparts could be seen throughout the copulation, suggesting that females continued to eat as copulation proceeded.

The median increase in copulation duration achieved by cannibalized males mating with nonvirgin females (from 11 to 25 min) predicts a twofold increase in their median paternity, from P_2 = 0.45 (95% confidence interval = 0.23 to 0.67) to P_2 = 0.92 (95% confidence interval = 0.68 to

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Table 1. A comparison of cannibalistic and noncannibalistic matings from a laboratory study of paternity. CL, confidence limits.

Factor	Cannibalistic matings			Noncannibalistic matings			Mann-Whitney (*P*)
	*N**	95% CL	Median	*N**	95% CL	Median	
			Female				
Weight (mg)	9	440 to 562	476	13	375 to 533	433	0.117
Ceph. width (mm)†	8	2.917 to 3.592	3.36	13	2.908 to 3.425	3.18	0.095
Age (days)‡	9	26 to 29	28	13	24 to 30	26	0.361
Time to egg sac deposition (days)	9	8 to 11	9	13	8 to 10	10	0.781
Egg sac mass (mg)	9	170 to 230	198	13	155 to 198	179	0.082
Number of eggs per sac	9	223 to 361	256	13	199 to 321	249	0.526
			First male				
Weight (mg)	3	5 to 7	6	19	3 to 7	5	0.085
Ceph. width (mm)†	3	0.979 to 1.056	1.042	17	0.965 to 1.035	0.986	0.288
Age (days)‡	3	6 to 41	25	19	21 to 39	32	0.598
Courtship duration (min)	3	246 to 326	281	18	244 to 309	277	0.687
			Second male				
Weight (mg)	9	4 to 6	4	13	3 to 5	4	0.216
Ceph. width (mm)†	9	0.903 to 1.035	0.986	12	0.903 to 1.014	0.965	0.336
Age (days)‡	9	17 to 31	22	13	21 to 36	28	0.366
Courtship duration (min)	6	199 to 413	291	11	191 to 379	273	0.841

*Sample sizes vary because some data could not be collected for some matings. †Cephalothorax (ceph.) width, measured at its widest point with an ocular micrometer. ‡Days since final moult.

1.0) (Fig. 1). This would result in a substantial increase in the number of offspring fathered, even after a single copulation. For example, if a twice-mated female produces 256 eggs in one egg sac (Table 1), a cannibalized male would fertilize approximately 235 of those eggs, as compared with only 115 if he survived copulation.

Males mating with virgin females (that is, the first matings in my experiment) also increase their paternity by being eaten, because cannibalism decreases the likelihood that the female will remate. In nine pairings, females cannibalized the first male that was presented to them, and in six of these cases (67%) the female rejected a second suitor. In comparison, in only 1 of 23 pairings (4%) in which the female did not cannibalize the first male did she reject a second male (Fisher exact test, $P = 0.001$) (*17*).

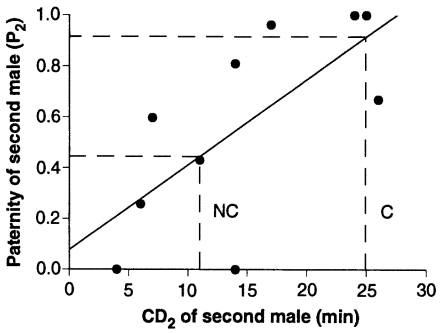

Fig. 1. The proportion of eggs fertilized by a male mating with a nonvirgin female (P_2) as a function of his copulation duration (CD_2). The slope of the line ($P_2 = 0.071 + 0.034\ CD_2$; $r^2 = 0.49$, $P = 0.025$, $n = 10$) was used to estimate P_2 from the median copulation durations of cannibalistic (C) and noncannibalistic (NC) matings (dashed lines). When males were cannibalized, their median copulation duration was 25 min, which predicts that they would fertilize most of the female's eggs (C; $P_2 = 0.92$); whereas males that survived mating had a median copulation duration of 11 min, which would result in less than half as many fertilizations (NC; $P_2 = 0.45$).

I examined other possible explanations for the difference in copulation duration between cannibalistic and noncannibalistic matings in the paternity experiment. A female's age or size or the phenotype of her suitor might affect her likelihood to permit long copulations as well as her tendency to be cannibalistic. For example, females might prefer long copulations with larger, presumably more fit males, but might also be more likely to cannibalize such males because of their higher nutrient content. However, of the phenotypic variables measured, no significant differences between cannibalistic and noncannibalistic matings were detected (Table 1).

Facilitation of sexual cannibalism may evolve as an adaptive male strategy if the benefits of being eaten in a given mating exceed the male's expected future reproductive value (*4*, *5*). The two benefits of sexual cannibalism reported here (increased paternity and a decreased likelihood of female remating) appear to meet this criterion. The cost of cannibalism (that is, death and lost opportunity for future matings) is probably low for male redbacks because they appear to be unlikely to mate more than once, even if they survive copulation (*6*). There are several indications that this is the case. Male spiders typically experience high mortalities when traveling between female webs [for example, 80% mortality or more in an orb-weaver spider (*18*)]. In addition, as with other *Latrodectus*, the tip of the male redback's intromittant organ always breaks off inside the female when used in copulation (*19*). Male redbacks have a short lifespan relative to that of females [2 to 4 months after maturation, as compared with up to 2 years for females under laboratory conditions (*6*, *20*)] and rarely eat after reaching maturity (*21*), and those that survive copulation often do not leave the webs of their mates afterward [see (*4*, *22*) for examples from other *Latrodectus* species]. After preliminary laboratory mating trials in which I provided redback males with a refuge separate from the female, five of seven (71%) undamaged males remained and eventually died on the webs of their mates.

Many male arthropods present food gifts to their mates and thereby ensure complete sperm transfer (*11*, *23*). Such mating gifts apparently differ from the somatic gift of the male redback only in the degree of their effect on male survivorship. Because females may mate multiply, male redbacks are selected to invest heavily in mechanisms that protect their paternity in the single mating they may achieve. Therefore, the two paternity advantages of sexual cannibalism outweigh the low cost of suicide for males. Male facilitation of cannibalism probably evolved through sexual selection as the most extreme mating gift (*23*).

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10. Female redbacks do not behaviorally induce males to somersault onto their chelicerae. Forster (*6*) reports that redback males always somersault in heterospecific pairings with the closely related *Latrodectus katipo* (a species in which the copulatory somersault does not normally occur). In addition, there is no unique structural aspect of redback genitalia that would make a somersault necessary for successful sperm transfer. Redback spider genitalia are indistinguishable from those of the North American black widow, *L. mactans*, which does not somersault [H. W. Levi, *Science* **127**, 1055 (1958); *Trans. Am. Microsc. Soc.* **78**, 7 (1966)].
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13. Spiders were collected on the grounds of the Uni-

versity of Western Australia and the Western Australia Department of Agriculture (Perth, Western Australia, in December 1993 and January 1994) and weighed with a Mettler AE50 balance that was accurate to 0.1 mg.
14. Male spiders copulate with paired structures (emboli) that are inserted through separate coiled ducts into the paired sperm storage organs (spermathecae) of the female. In *Latrodectus*, the tip of the male's embolus always breaks off during insertion and remains inside the spermathecae or coiled ducts (*19*). By counting broken emboli inside 23 nonvirgin females (collected near Perth, Western Australia, in January 1994), I determined that 30.4% (7/23) had received only one palpal insertion, 52.2% (12/23) had received two palpal insertions and so had mated with one or two males, and 17.4% (4/23) had received three palpal insertions and so had mated with at least two and possibly three males.
15. G. A. Parker, *Biol. Rev.* **45**, 525 (1970); B. Sillen-Tullberg, *Behav. Ecol. Sociobiol.* **9**, 283 (1981). The proportion of eggs fertilized by the N male (x) was $x = (a - c)/(b - c)$ where a is the proportion hatched in the NI or IN group, b is the proportion hatched in the NN group, and c is the proportion hatched in the II group. P_2 in the IN group equaled x, and P_2 in the NI group equaled $1 - x$.
16. When the spermathecae of field-collected females (n = 23) were dissected (*14*), a total of 43 emboli were discovered. Of these, the majority (40/43) were located inside the female spermathecae rather than in the coiled ducts, suggesting that most males ejaculate directly into the sperm storage organ. Ejaculates of second males might then mix randomly with sperm already in the spermatheca, which suggests that increased sperm transfer is the mechanism by which longer copulation durations result in increased paternity.
17. Female rejection behavior is distinct and readily observable. In interactions that led to a successful copulation, the female remained quiescent in the web during most of the male's courtship. In comparison, rejection behavior consisted of a female repeatedly hitting at a courting male with her front legs, causing the male to drop from the web on a dragline. The male usually returned to the web and resumed courtship after the first few displacements, but nonreceptive females continued this behavior until the male eventually ceased courtship completely and moved to the substrate below the web.
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Direct Observation of Protein Solvation and Discrete Disorder with Experimental Crystallographic Phases

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A complete and accurate set of experimental crystallographic phases to a resolution of 1.8 angstroms was obtained for a 230-residue dimeric fragment of rat mannose-binding protein A with the use of multiwavelength anomalous dispersion (MAD) phasing. An accurate image of the crystal structure could thus be obtained without resort to phases calculated from a model. Partially reduced disulfide bonds, local disorder, and differences in the mobility of chemically equivalent molecules are apparent in the experimental electron density map. A solvation layer is visible that includes well-ordered sites of hydration around polar and charged protein atoms, as well as diffuse, partially disordered solvent shells around exposed hydrophobic groups. Because the experimental phases and the resulting electron density map are free from the influence of a model, they provide a stringent test of theoretical models of macromolecular solvation, motion, and conformational heterogeneity.

Solvation and motion play key roles in protein folding, stability, and function. Water molecules are integral components of folded proteins, and the differential preference of amino acids for the aqueous environment is the basis of the hydrophobic effect, which is thought to be an important driving force in protein folding (*1*). Hydration of residues that participate in protein-ligand interactions and macromolecular association is important in determining the thermodynamics of binding. Molecular motion and flexibility are also essential aspects of macromolecular function, particularly in the induced fit, flexible-to-rigid transitions and large-scale conformational changes that occur in many proteins (*2*).

X-ray diffraction data result from temporal and spatial averaging over a large number of molecules in the crystal lattice and therefore provide an averaged view of solvation and flexibility. At the resolution limits typical of macromolecular crystals, the structure is usually modeled by a single conformer together with discrete sites of hydration. Thermal fluctuations are approximated by isotropic, harmonic motions. These models provide an incomplete description of the crystal structure. For example, solvent molecules constitute a large volume of macromolecular crystals (*3*), but only a small fraction of the solvent, consisting of fully occupied hydration sites ("bound" or "ordered" water molecules), is modeled. The remaining solvent is disordered but not completely featureless (*4*, *5*). It is particularly difficult to describe regions containing disordered solvent, portions of the molecule that display large thermal fluctuations [likely anisotropic and possibly anharmonic (*6*)], and conformational variability, all of which appear at relatively low electron density levels because of averaging over the copies in the crystal.

The interpretation of low electron density levels is made difficult by the presence of model bias that arises when inaccurate or incomplete experimental phases are substituted or augmented by phases derived from a model. When a feature is included in the model used to calculate phases for electron density maps, it often appears in the maps whether or not it is correct (*7*). In addition to causing model bias, inclusion of incorrect features in refinement can produce a relatively low conventional R value [$R = \Sigma_h||F_{obs}(h)| - |F_{calc}(h)||/\Sigma_h|F_{obs}(h)|$], which measures the agreement between structure factor amplitudes ($|F_{calc}|$) calculated from the refined model and the observed amplitudes ($|F_{obs}|$) over all reflections h. Misinterpretation or overinterpretation of the diffraction data can be reduced by monitoring the cross-validated or free R value (*8*); reduction of the free R value indicates a meaningful improvement of the model, whereas changes in the model that increase the free R value are likely fitting noise in the data.

The final R and free R values of macromolecular models are much larger than would be predicted from the statistical error in the observed amplitudes, presumably because current refinement models do not provide adequate descriptions of solvation

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