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The Effect of Limb Loss on the Courtship and Mating Behavior of the Wolf Spider *Pardosa Milvina* (Araneae: Lycosidae)

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*Limb loss is common in the wolf spider *Pardosa milvina*, appearing in nearly one third of adult males but occurring less frequently among adult females and juveniles. Since males wave their first pair of legs during courtship displays, the reproductive consequences of limb loss may be significant. We measured the courtship and mating effects of the loss of one, two, or four legs among adult male *P. milvina*. Missing one or two legs did not significantly reduce a male's ability to mate, but missing four legs was detrimental to mating success, reduced both courtship intensity and copulation duration, and increased cannibalism frequency. Results suggest behavioral flexibility in compensating for limited leg loss and a defensive function of the anteriormost legs to thwart female cannibalism attempts.*

KEY WORDS: autotomy; limb loss; mate choice; *Pardosa milvina*; wolf spider; cannibalism.

INTRODUCTION

A variety of arthropods is able to autotomize limbs to escape predation or localize the effect of injury (Wood and Wood, 1932; Roth and Roth, 1984; Formanowicz, 1990; Foelix, 1996; Punzo, 1997). Although there is the obvious benefit of immediate survival, voluntary self-amputation of a

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limb may incur substantial long-term costs such as lowered locomotor efficiency (Klawinski and Formanowicz, 1995; Amaya *et al.*, 2001), impaired prey capture (Smith and Hines, 1991) and predator avoidance (Smith, 1995), reduced growth rates (Smith, 1990; Johnson and Jakob, 1999), compromised outcomes of intraspecific contests (Johnson and Jakob, 1999; Berzins and Caldwell, 1983), and limited mating success (Uetz *et al.*, 1996; Smith, 1992).

Leg autotomy is particularly common among most species of spiders (Roth and Roth, 1984) with limb loss typically occurring among 5–20% of the population (Foelix, 1996) but ranging as high as 30–40% depending on the species, age, sex, and time of year (Brueseke *et al.*, 2001, Dodson and Beck, 1993). Such a high frequency of limb loss implies that autotomy-related mortality may be low and that considerable selection pressure exists for behavioral compensation for the loss of a leg.

Spiders and other arachnids may have considerable redundancy in limb function such that the loss of a single limb may incur little or no measurable fitness costs. Johnson and Jakob (1999) showed that the loss of a leg had only small effects on growth rates and competitive ability in the pholcid *Holocnemus pluchei*. Orb-weaving spiders missing legs were shown to make smaller, more irregular webs than intact spiders, but web prey capture was found to be similar (Weissmann and Vollrath, 1998). Brueseke *et al.* (2001) found that wolf spiders missing one limb incurred no significant reduction in locomotor behavior, prey capture success, or predator avoidance. Similarly, Amaya *et al.* (2001) found no difference in foraging ability among wolf spiders before and after autotomizing a limb. These studies tended to focus on the loss of single limbs despite the fact that multiple limb loss among arthropods may be common and contribute to incremental fitness costs (Juanes and Smith, 1995; Brueseke, 2001; Guffey, 1999). Also, the fitness consequences of autotomy may not be equivalent for all appendages or be the same for both sexes (Juanes and Smith, 1995; Dixon, 1989).

Many wolf spider species produce conspicuous signals to conspecifics by waving their first pair of legs (Scheffer *et al.*, 1996; Kaston, 1936; Montgomery, 1903; Hebets and Uetz, 2000). These signals are usually used by males in courtship displays and may be an indicator to females of male quality, condition, or species identification (McClintock and Uetz, 1996; Hebets and Uetz, 1999, 2000). For this reason, the loss of an anterior leg may have a disproportionate effect on the reproductive success of male wolf spiders. Uetz *et al.* (1996) showed that asymmetry of the size and ornamentation of the first pair of legs caused by limb regeneration affects both the outcome of intrasexual agonistic encounters and female mate choice in the wolf spider

Schizocosa ocreata. Uetz *et al.* (1996) also found that the first pair of legs was lost disproportionately more often than other limbs.

Natural populations of the ground-dwelling wolf spider, *Pardosa milvina*, exhibit frequent limb loss. Brueseke *et al.* (2001) found that of 635 *P. milvina*, 16.4% were missing one or more legs, and as the breeding season progressed, the incidence of missing limbs increased to almost 33%. Spiders missing two or more legs were also quite common, occurring in over 9% of the population. Male *Pardosa milvina*, like most wolf spiders, use the first pair of legs extensively during courtship displays (Montgomery, 1903; Kaston, 1936; Koomans *et al.*, 1974). Both leg-waving courtship displays and limb loss are prevalent among lycosids, yet studies quantifying the mating success of limb-autotomized, as opposed to limb-regenerated, males are lacking.

There were two main objectives to our study. The first was a census of the frequency and pattern of single and multiple limb loss by age class and sex in the wolf spider *P. milvina*. We expected that adult male wolf spiders would have a higher incidence of leg loss because of higher activity levels and conspicuousness relative to adult females and juveniles (Cady, 1984; Persons and Uetz, 1999; Persons, 1999; Walker and Rypstra, 2003; Kotiaho *et al.*, 1998). We also expected that adult males would be more likely to lose their first pair of legs since this pattern has been observed in another species of wolf spider (Uetz *et al.*, 1996). The second objective was to measure shifts in male courtship behavior and female response due to male limb loss. We tested if male wolf spiders missing one or more of the first pair of legs were able to utilize the second pair during courtship and if loss of one or more limbs reduced courtship intensity or otherwise compromised courtship displays. Studies of one species of wolf spider, *Hygrolycosa rubrofasciata*, suggest that male courtship intensity may be an honest indicator of male viability and that females may choose males based on this criterion (Mappes *et al.*, 1996; Kotiaho *et al.*, 1996). If limb loss impairs the condition of males, then males with more legs missing should show reduced courtship intensity. We also expected females to reject mating attempts by males missing limbs more often than intact individuals and that females would respond slower to limb-autotomized males, as measured by longer courtship duration and postponed copulation. If leg-waving is an important part of a male wolf spider's courtship display, then males that are unable to perform this behavior should suffer lower mating success. We also expected an increase in cannibalism and aggression toward these males if leg-waving is used as a species recognition cue or if the first pair of legs is defensively used during female cannibalism attempts as has been found in other spider species (Elgar *et al.*, 1990).

METHODS

Limb Loss Census

A total of 1050 *Pardosa milvina* was collected by hand from May through September of 2000 within a 4-ha area of no-till corn and soybean fields (Susquehanna University, Selinsgrove, Snyder County, PA). The number and position of legs missing for each spider were recorded along with maturity status (adult, subadult, juvenile), sex, and reproductive status (females carrying or not carrying an egg sac). Sex of juveniles (preantepenultimate instars) could not be positively ascertained, therefore only subadults (penultimate or antepenultimate instars) and adults were sexed. Position of missing legs were indicated with L or R and numbered according to position from the anterior of the spider (e.g., R1 = right anterior, L4 = left posterior leg). We compared the incidence of limb loss between sex and age classes of spiders using chi-square goodness-of-fit tests. When sample size allowed, for each age and sex category, we also tested whether there were significant differences in the frequency of the loss of a particular limb among the four limb pairs. We used chi-square goodness-of-fit tests in this analysis as described by Guffey (1998). This test compares the distribution of limbs lost among pairs against an a priori null hypothesis assumption that all limbs are equally likely to be lost (i.e., H_0 —of the individuals with one missing limb, there is a 25% chance of it being lost from any particular limb pair).

Limb Loss Mating Trials

General Spider Collection and Maintenance. We tested the effects of male limb loss on courtship and mating behavior. Intact juvenile male and female *P. milvina* were collected in corn and soybean fields (Susquehanna University) from May to August 2001. Spiders were reared until maturity in individual 8-cm-diameter \times 5-cm-high plastic translucent containers lined with moistened peat moss and maintained on a 13L:11D photoperiod at ambient room temperature (22–25°C). Each spider was fed once a week with a mixed diet of four to seven 5-day old house crickets (*Acheta domesticus*) and several fruit flies (Canton-S strain, *Drosophila melanogaster*).

Hunger Standardization and Limb Removal Procedure. To minimize the effects of hunger and body condition variation on male behavior, mate choice, female aggression, and cannibalism, we standardized hunger levels for all test spiders prior to mating. After maturation, unmated male and female spiders were given as many crickets as they could eat within a 24-h period. Excess food was then removed from each container and spiders were

denied additional food for 7 days, approximating natural levels of hunger (Nyffeler and Breene, 1990; Edgar, 1969). On the day of food removal, each male spider was placed in a clean plastic container over ice for 3 min. This served to slow its movement and provide an anesthetic. Leg autotomy was induced by pinching the femur of the designated leg with a pair of entomological forceps, causing the spider to subsequently self-amputate the limb along the coxa–trochanter joint (Johnson and Jakob, 1999). There were five limb-loss treatments of male spiders ($N = 27\text{--}29/\text{treatment}$): (1) intact—no legs removed (control group); (2) R1—anterior right leg removed; (3) L1—anterior left leg removed; (4) R1L1—both anterior legs removed; and (5) R12L12—anterior two pairs of legs removed (total of four legs removed). Spiders in the control group underwent a sham operation by also being put on ice for 3 min, followed by a light pressing of the leg with entomological forceps but not sufficiently to induce autotomization.

Pairing Procedure. Six days after the limb autotomy procedure, each experimental male was paired with a randomly selected unmated female. Prior to pairing, a set of 20-cm-diameter round plastic containers was rinsed with ethanol to remove any extraneous odors that might modify spider responses. A circular sheet of paper was then placed on the bottom of each container and fixed in place using masking tape. A female spider was then placed inside the container and given access to water via two 2.5-cm-diameter vial lids placed on opposite sides of the sheet of paper. Females were left in the container overnight and allowed to deposit pheromone-laden silk draglines for 20 h. The following day, 1 full week after male autotomization of limbs, the water lids were removed from the female's container and male and female spiders were allowed to interact. The male spider was introduced into the female's container under an inverted clear plastic 40-dram vial and allowed to acclimate for 5 min. The male spider was then released to freely interact with the female for 30 min or until the male mated and dismounted the female. All pairings were videotaped from above and analyzed later to reduce the possible effects of observer presence on spider behavior. Each videotaped trial was observed in its entirety for the duration of the testing period. Testing was conducted from 800 to 1800 h from late May through early September 2001. All treatment testing was randomized throughout the day and over subsequent days and weeks to control for possible time-of-day and sequence effects. All spiders, both male and female, had been mature for no more than 14 days in the laboratory at the time of testing.

Behavioral Measurements. For each mating trial the following information was recorded: leg-loss treatment, number of male leg raises, number of male body shakes, number of pedipalp raises, female lunges toward the male, cannibalism of males by females, female leg tapping, time of courtship initiation by male (courtship latency), duration of time elapsed prior to when the

male mounted the female (copulation latency), and total time the male was mounted on the female (copulation duration). The leg raises, pedipalp raises, and body shakes are components of the male mating display and have been described in detail elsewhere (see Kaston [1936] and Montgomery [1903] for a complete description of *Pardosa milvina* courtship) but are reiterated here for clarity. The anterior pair of legs is used in a visual display where the legs are simultaneous raised and lowered. Male spiders will usually raise their pedipalps in unison with leg raises but will also raise them independently of leg raises; therefore, pedipalp raises were recorded as another measure of courtship intensity. The third component, body shakes, occurs when the spider's body vibrates rapidly for bouts lasting from 1 to 2 s. Leg raises, pedipalp raises, and body shakes were all converted to rates by dividing total number of occurrences of that courtship element by the total courtship duration. Courtship duration was defined as the time from initiation of leg raises, body shakes, or pedipalp raises until mounting occurred or until the 30-min trial period had expired. Sometimes a female will respond to the male's presence by tapping one or more of her legs rapidly on the ground in a discrete arc about the distal portion of the limb (Kaston, 1936). We also recorded the incidence of this behavior to test if it is positively associated with mating success of males and, therefore, a possible receptivity display.

Statistical Analyses. We used separate one-way ANOVAs and post hoc comparison of means (Tukey test) to analyze courtship latency, body shakes per second, pedipalp raises per second, leg raises per second, total courtship displays per second, copulation latency, and copulation duration by limb loss treatment. Courtship latency, copulation latency, and copulation duration were natural log transformed prior to analysis to conform to assumptions of normality. All other variables approximated normal distributions without transformation. We also used a sequential Bonferroni adjustment to the 0.05 α level to compensate for an increased probability of a tablewide Type I error due to multiple tests (Rice, 1989). We used chi-square goodness-of-fit tests to compare mating success, female lunges, and female tapping responses across treatments. We examined the incidence of sexual cannibalism across treatments. However, sexual cannibalism rates are generally low in this species, causing an expected frequency of less than five. Therefore, we combined categories (see Siegel and Castellan, 1988) and tested the null hypothesis: Males with four limbs missing will have the same probability of being cannibalized as males with fewer than four legs missing. This hypothesis was tested using a Fisher's exact test.

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Table 1. The Natural Occurrence of Limb Loss in *Pardosa mitivina* by Sex and Age^a

Limb status	Age/sex					
	Adult female w/o egg sac (N = 127)	Adult female w/ egg sac (N = 166)	Subadult female (N = 247)	Juveniles (N = 190)	Subadult male (N = 92)	Adult male (N = 228)
No. intact	99 (77.9)	132 (79.5)	210 (85)	171 (90)	78 (84.8)	159 (69.7)
No. missing 1 leg	21 (16.5)	24 (14.5)	30 (12.1)	15 (7.9)	10 (10.9)	47 (20.6)
No. missing 2 legs	6 (4.7)	7 (4.2)	4 (1.6)	2 (1.1)	3 (3.3)	19 (8.3)
No. missing 3 legs	1 (0.8)	3 (1.8)	3 (1.2)	2 (1.1)	1 (1)	3 (1.3)

^aPercentage of individuals within an age/sex category are indicated in parentheses.

Table II. Pairwise Comparisons of Naturally Occurring Limb Loss by Age/Sex Category

Comparison	χ^2	<i>P</i>
Adult female vs. adult female w/eggs	0.106	0.7451
Adult female vs. subadult female	2.918	0.0876
Adult female vs. juvenile	8.75	0.0031*
Adult female vs. subadult male	1.606	0.2051
Adult female vs. adult male	2.773	0.0959
Adult female w/egg vs. juvenile	7.682	0.0056
Adult female w/eggs vs. subadult female	2.111	0.1462
Adult female w/eggs vs. subadult males	1.083	0.2979
Adult male vs. adult female w/eggs	4.760	0.0291
Juvenile vs. subadult female	2.384	0.1226
Subadult female vs. subadult male	0.003	0.9566
Adult male vs. subadult female	15.98	<0.0001*
Juvenile vs. subadult male	1.633	0.2013
Adult male vs. juvenile	25.603	<0.0001*
Adult male vs. subadult male	7.725	0.0054

*Significant difference following a sequential Bonferroni to control for multiple tests.

RESULTS

Limb Loss Census

Limb loss was most common among adult male *Pardosa* (Table I) and least common among juveniles (Table 1). The probability of missing one or more limbs was significantly different by age and sex category ($\chi^2 = 33.04$, $P < 0.0001$, $df = 5$). Both adult males and adult females were significantly more likely to be found with missing limbs than juveniles (Table II) and adult males were significantly more likely to have missing legs than subadult females (Table II). However, we found no difference among other age and sex categories after correcting for multiple comparisons (sequential Bonferroni) (Table II). All spiders surveyed had between zero and three missing legs (Table I). As expected, loss of two or more legs occurred less frequently than loss of one leg. Forty-one of 1050 spiders (3.9%) had two missing legs, with adult males comprising the majority of cases. A smaller number of captured spiders were missing three legs ($N = 13$; 1.2%) (Table I).

Adult females, with or without egg sacs, were significantly more likely to have one of their first pair of legs missing compared to any other pair, whereas adult males were significantly more likely to have one of their fourth pair of legs lost (Table III). Subadult females were equally likely to lose a leg from any of the four leg pairs (Table III). Juveniles and subadult males had too small of a sample size to determine limb loss differences between leg pairs reliably (Zar, 1984).

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Table III. The Pattern of Limb Loss by Age and Sex Among *Pardosa milvina* Missing One or Both Limbs from the Specified Pair of Legs^a

Age/sex class	Limb pair		missing		χ^2	<i>P</i>
	First	Second	Third	Fourth		
Adult female w/o egg sac	12	3	1	5	13.09	<0.001
Adult female w/o egg sac	13	3	5	5	9.07	<0.05
Subadult female	6	5	9	10	2.266	NS
Juveniles	5	4	1	6	Not tested	*
Subadult male	2	2	4	3	Not tested	*
Adult male	9	8	9	23	11.78	<0.01

^aIndividuals missing additional legs from pairs other than those indicated were excluded from the table.

Limb Loss Mating Trials

We found significant differences in mating success across treatments (Table IV). Mating success was similar among intact, seven-legged, and six-legged spiders, but four-legged spiders were much less successful mating with females (Table IV). Only 14% of four-legged male spiders mated, compared to 66% of intact males. Females occasionally showed aggression toward males. Across all treatments, 52% of paired females lunged at the male, however, there was no significant difference among treatments in the number of females that lunged (Table IV). There was a significant increase in premating cannibalism among males missing four legs (21.4% being consumed) compared to males missing fewer limbs (Table IV).

Females that performed a leg-tapping display were significantly more likely to mate with males than those that did not ($\chi^2_{1,138} = 7.480$, $P = 0.0062$),

Table IV. Number of Females Responding with Various Behaviors Toward Courting Males Among Five Limb Loss Treatments^a

Behavior	Intact (<i>N</i> = 29)	L1 (<i>N</i> = 27)	R1 (<i>N</i> = 27)	L1R1 (<i>N</i> = 27)	L12R12 (<i>N</i> = 28)	$\chi^2_{4,138}$	<i>P</i>
Mated w/ male	19 (66)	19 (70)	17 (63)	16 (59)	4 (14)	23.435	0.0001
Cannibalized male	0 (0)	0 (0)	1 (3.7)	2 (7.4)	6 (21.4)	*	0.0023
Lunged at male	13 (45)	12 (44)	13 (48)	16 (59)	18 (64)	3.62	<0.5
Leg tapping toward male	10 (34.5)	10 (37)	12 (44)	3 (11)	5 (17.9)	10.39	0.03

^aPercentage of males showing behavior is indicated in parentheses.

**P* value based on a Fisher exact test of H_0 : Males with four limbs missing will have the same probability of being cannibalized as males with fewer than four limbs missing.

suggesting that this behavior is a receptivity display. Female leg-tapping displays occurred significantly less frequently toward males with two or four legs missing (Table IV).

Males for all treatments showed at least some courtship activity. After correcting the alpha level for the number of statistical tests (sequential bonferroni), there was no significant difference in mean courtship latency across treatments, however, males with four limbs missing took more than twice as long to begin courtship as intact, L1, and L1R1 males. There was also no significant difference in courtship duration across treatments (Table V), however, males missing four limbs tended to court for a longer period of time than males in other treatments.

There were no significant differences in copulation latency between treatments, though there was a nonsignificant trend toward four-legged spiders having longer copulation latency (Table V). Limb loss, however, did significantly affect copulation duration (Table V). Males with one (L1 or R1) or two (L1R1) legs missing did not differ from intact males with respect to copulation duration, whereas males missing four limbs had significantly shorter mean copulation duration than any other treatment.

All behavioral measures of courtship intensity (body shakes/s, leg waves/s, pedipalp waves/s) were significant predictors of mating success based on separate single logistic regression analyses (body shakes/s, $\chi^2 = 5.06$, $P = 0.025$; leg raises/s, $\chi^2 = 68.53$, $P < 0.0001$; pedipalp raises/s, $\chi^2 = 35.98$, $P < 0.0001$). Courtship intensity was also significantly reduced by limb loss; this was true regardless of the courtship behavior measured (leg raises, body shakes, pedipalp raises, or total displays) (Table V); however, the magnitude of the effect of limb loss was not the same for all courtship elements. Leg-waving displays were significantly reduced with the loss of two legs and were, not surprisingly, absent altogether in four-legged males (Table V). Pedipalp raises significantly increased among six-legged males, suggesting compensatory behavior for reduced or absent leg-waving displays, but this could be attributable to the ease of detecting pedipalp raises when the anterior legs are missing rather than an actual increase in the number of raises by these males. Intact males and males missing only one leg generally had lower pedipalp display rates. The rates of body shakes and total displays were not significantly reduced among treatments except among four-legged males (Table V)

DISCUSSION

Frequency and Patterns of Limb Loss by Age and Sex

Limb loss was exceptionally common among field populations of *P. milvina*. We found that adult males and adult females without egg sacs

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Table V. Comparison of Means \pm SE for Courtship and Mating-Related Behaviors Across Five Limb Loss Treatments^a

Behavior	N	Intact	L1	R1	L1R1	L12R12	F	P
Courtship latency (s)	138	245.93 \pm 85.59	112.48 \pm 26.88	450.63 \pm 120.69	258.07 \pm 94.70	754.55 \pm 142.60	2.673	0.0348
Courtship duration (s)	138	650.89 \pm 135.09	675.79 \pm 129.86	428.00 \pm 121.97	580.44 \pm 133.42	805.25 \pm 155.79	1.03	0.3942
Copulation latency (s)	75	896.82 \pm 143.55	791.33 \pm 134.40	878.63 \pm 139.26	838.51 \pm 145.79	1327.57 \pm 138.04	2.402	0.0530
Copulation duration (s)	75	536.31 \pm 83.01 ^A	652.07 \pm 98.07 ^A	401.96 \pm 71.64 ^A	410.70 \pm 103.69 ^A	112.46 \pm 55.40 ^B	5.802	0.0002*
Leg raises/s	138	.206 \pm .033 ^A	.171 \pm .024 ^{AB}	.183 \pm .028 ^{AB}	.099 \pm .021 ^C	0 \pm 0 ^C	12.170	<0.0001*
Pedipalp raises/s	138	.038 \pm .010 ^A	.071 \pm .014 ^A	.068 \pm .012 ^A	.141 \pm .027 ^B	0.088 \pm .028 ^{AB}	3.681	0.0071*
Body shakes/s	138	.145 \pm .027 ^A	.132 \pm .017 ^A	.141 \pm .024 ^A	.145 \pm .025 ^A	0.031 \pm .010 ^B	5.325	0.0005*
Total displays/s	138	.389 \pm .061 ^A	.375 \pm .044 ^A	.392 \pm .052 ^A	.385 \pm .055 ^A	0.118 \pm .037 ^B	5.611	0.0003*
Female lunges	138	2.03 \pm .718	1.33 \pm .427	1.852 \pm .538	3.11 \pm 1.00	2.29 \pm .501	0.933	0.4469

^aDifferent letters indicate significant differences between limb loss treatments based on a Tukey post hoc comparison of means test. *F* values were obtained from one-way ANOVA.

*Significant difference after a sequential Bonferroni test corrected for tablewide alpha level.

were significantly more likely to have missing limbs compared to juveniles (Table II). There are a number of factors acting alone or in concert that may contribute to this phenomenon. First, several studies have shown that there is a significant increase in activity level of both male and female wolf spiders after maturation (Persons and Uetz, 1999; Persons 1999), with males being particularly more active than females (Cady, 1984). Increases in general activity as well as courtship displays of male wolf spiders may increase conspicuousness to predators (Kotiaho *et al.*, 1998) and also contribute to increased agonistic interactions among conspecifics (Aspey, 1977). Leg tapping by *P. milvina* females may also be conspicuous to potential predators and contribute to higher limb loss frequencies among adult females compared to juveniles.

In general, limb loss may be simply due to wolf spider age. Predator encounters and agonistic interactions likely increase with age, allowing more opportunities for autotomy-based defensive behaviors. Also, adult wolf spiders do not continue to go through ecdysis whereas juveniles do. Thus differences in the frequency of limb loss among adults and juveniles may be due to the fact that juveniles may be able to regenerate lost limbs during subsequent molts.

Although the probability of missing a leg was not significantly different among adult male and female spiders, the pattern of limb loss varied by sex. Adult females were more likely to have one of their first pair of legs missing, whereas males were more likely to have lost one of the fourth pair. This pattern runs counter to our original predictions and other published studies of wolf spider limb loss patterns. Uetz *et al.* (1996) found that adult males of the wolf spider *Schizocosa ocreata* were more likely to lose their first pair of legs rather than their fourth pair. *Schizocosa ocreata* males have large conspicuous ornaments in the form of heavily pigmented tufts on the first pair of legs but such ornamentation is lacking on all other legs. These ornaments may render the first pair of legs both more conspicuous and easier to grasp than other pairs. *Pardosa milvina*, however, lack tufts and heavy pigmentation on all legs, which may contribute to the observed differences in limb loss patterns among males of these two species. The limb loss patterns found in our study were also different from that found by Brueseke *et al.* (2001). Brueseke *et al.* (2001) found no significant difference in the loss of particular pairs of limbs in *P. milvina*. Notably, however, Brueseke *et al.* (2001) did not compare limb loss patterns between age and sex categories as was done here.

Our results suggest that for our population, males and females may have different selective pressures acting on limb loss. These patterns could arise because (1) the effectiveness of limb autotomy as an escape mechanism differs by sex and leg pair, (2) *Pardosa* directional movement differs by sex,

(3) predator attack is nonrandom and each sex is prone to attack by different predators, (4) the sexes differ in their readiness to autotomize particular limbs, or (5) survival probability due to the loss of particular limbs differs for each sex. We only have weak direct evidence for the fifth possibility. Males with missing anterior legs incurred higher cannibalism by females during courtship attempts. Therefore, males with the first and second pair of legs missing may be selected against in the population and contribute to our observed differences in male and female limb loss patterns. Given the importance of the anterior pair of legs for male courtship displays, males may also be less willing to detach these limbs compared to females.

Although the pattern of limb loss differed substantially from that found by Brueseke *et al.* (2001), the total frequencies of limb loss were similar. A high percentage of spiders were missing a single limb, while between 1 and 9% of spiders had two limbs missing and only about 1 or 2% of the population had three missing limbs. No spiders were seen missing four or more legs. This suggests that four-legged spiders may suffer increased mortality in the field. Alternatively, spiders with more than three missing limbs may be much less conspicuous due to lowered mobility and, therefore, less likely to be detected and collected. Our results indicate that, for males at least, sexual cannibalism by conspecific females is a significant source of mortality among four-legged males. Four-legged males might also have difficulty capturing and subduing prey, contributing to poor survival (Guffey, 1999).

Courtship and Mating Effects of Male Limb Loss

The loss of one or two front legs had no measurable effect on mating success in male *P. milvina*. However, there was a dramatic reduction in mating success with the loss of four legs. Four-legged males appeared unable or unwilling to perform leg-waving displays, which may be an essential species-recognition cue or may be a key signal of male quality. Males with one or two missing legs simply recruited one or both of the second pair to complete the leg-waving display. The display of these males was qualitatively similar to that of intact males, however, males with two missing limbs had an average leg-raising rate about half that of an intact male. Presumably four-legged males were unable to perform leg raises because doing so rendered the spider biomechanically unstable.

Lack of leg-waving displays may not be the only reason for poor mating success among four-legged males. We found that courtship intensity, the rate of display, was a significant predictor of mating success as well. Four-legged males not only failed to exhibit leg raises, but performed fewer body shakes and pedipalp raises per second than spiders missing fewer legs. However, our results suggest that courtship intensity is probably a minor factor in

determining mating success compared to the presence or absence of leg-waving. Six-legged males performed pedipalp raises and leg raises at significantly lower rates yet did not appear to have lower levels of mating success than intact males.

Four-legged males showed significantly shorter copulation duration. This important mating behavior could possibly translate into compromised sperm transfer or reduced copulatory courtship. However, the pattern of sperm transfer or the significance of copulatory courtship in lycosids is largely unknown. Further, insertion rates were not measured in this study, only duration mounted on the female. Nonetheless, we speculate that the loss of four legs may coincide with a substantial loss of hemolymph. Copulation in spiders involves significant exertion in males via a rapid increase in hemolymph pressure and subsequent inflation of a copulatory apparatus (hematodochae and associated emboli). This process occurs multiple times, with each pedipalp corresponding to coupling with each side of the female epigynum (external copulatory organ of the female) (Foelix, 1996; Stratton *et al.*, 1996). Loss of hemolymph volume during multiple autotomy events may be sufficient to prevent or limit expansion of the hematodochae of each pedipalp and thus result in shorter copulation times.

Some females performed a leg-tapping behavior toward males. Female tapping was positively associated with male mating success and was observed more frequently among males missing fewer limbs. Collectively, these results suggest that female leg tapping functions as a probable receptivity display. However, the relationship between leg tapping and mating was not definitive since some females mated in the absence of leg tapping and some males failed to mate despite female leg tapping.

Cannibalism increased with the number of limbs missing. Based on the number of lunges, there was no difference in female aggression levels across treatments, yet there were significant differences in the incidence of cannibalism among treatments. This suggests that the first pair of legs may have an important defensive function in mitigating female attacks. Elgar *et al.* (1990) found that sexual dimorphism in leg length in orb-weaving spiders may exist because longer limbs in males minimize female attacks. Our data support this general conclusion. Premating cannibalism is thought to occur due to mistaken identity, due to a female's foraging decision, or because the female displays a high degree of mate choice and consumes any male that does not meet the minimum criteria for a mate (Elgar, 1992). Koomans *et al.* (1974) suggested that leg waving in *Pardosa* species may act as a display with an aggression-lowering function. Since lunge responses were similar across treatments, it appears that leg waving does not reduce female aggression or likely serve a species-recognition function. Instead, females appear to attack all males with equal frequency, and variation in cannibalism is due to the

male's ability to fend off female attacks by grappling with the anteriormost legs. Although admittedly weak evidence, our inability to find males with the anteriormost legs missing, yet finding females that do, suggests that these males may be cannibalized under field conditions as well as in the laboratory.

In conclusion, our study supports a "spare legs hypothesis" for *P. milvina* (Brueseke *et al.*, 2001). Males can lose one or two legs with little consequence to their reproductive success. However, multiple limb loss is exceptionally common, and the corresponding costs of losing additional limbs are high including reduced mating success and increased cannibalism risk.

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REFERENCES

- Amaya, C. C., Klawinski, P. D., and Formanowicz D. R. (2001). The effects of leg autotomy on running speed and foraging ability in two species of wolf spider (Lycosidae). *Am. Midl. Nat.* **145**: 201–220.
- Aspey, W. P. (1977). Wolf spider sociobiology: II-Density parameters influencing agonistic behavior in *Schizocosa crassipes*. *Behaviour* **62**: 143–163.
- Berzins, I. K., and Caldwell, R. L. (1983). The effect of injury on the agonistic behavior of the stomatopod, *Gonodactylus bredini* (Manning). *Mar. Behav. Physiol.* **10**: 83–96.
- Brueseke, M. A., Rypstra, A. L., Walker, S. E., and Persons, M. H. (2001). Leg autotomy in the wolf spider *Pardosa milvina*: A common phenomenon with few apparent costs. *Am. Midl. Nat.* **146**: 153–160.
- Cady, A. B. (1984). Microhabitat selection and locomotor activity of *Schizocosa ocreata* (Walckenaer) < Araneae: Lycosidae. *J. Arachnol.* **11**: 297–307.
- Dixon, K. A. (1989). Effect of leg type and sex on autotomy in the Texas bush katydid, *Scudderia texensis*. *Can. J. Zool.* **67**: 1607–1609.
- Dodson, G. N., and Beck, M. W. (1993). Pre-copulatory guarding of penultimate females by male crab spiders, *Misumenoides formosipes*. *Anim. Behav.* **46**: 951–959.
- Edgar, W. D. (1969). Prey and predators of the wolf spider *Lycosa lugubris*. *J. Zool.* **159**: 405–411.
- Elgar, M. A. (1992). Sexual cannibalism in spiders and other invertebrates. In Elgar, M. A., and Crespi, B. I. (eds.), *Cannibalism: Ecology and Evolution Among Diverse Taxa*, Oxford University Press, Oxford, pp. 128–155.
- Elgar, M. A., Ghaffar, N., and Read, A. (1990). Sexual dimorphism in leg length in orbweaving spiders: A possible role for sexual cannibalism. *J. Zool. London* **222**: 455–470.
- Foelix, R. F. (1996). *Biology of Spiders*, Oxford University Press, Oxford.
- Formanowicz, D. R., Jr. (1990). The antipredator efficacy of spider leg autotomy. *Anim. Behav.* **40**: 400–409.
- Guffey, C. (1998). Leg autotomy and its potential fitness costs for two species of harvestment (Arachnida, Opiliones). *J. Arachnol.* **26**: 296–302.
- Guffey, C. (1999). Costs associated with leg autotomy in the harvestment *Leiobunum nigripes* and *Leiobunum vittatum* (Arachnida: Opiliones). *Can. J. Zool.* **77**: 824–830.

- Hebets, E. A., and Uetz, G. W. (1999). Female responses to isolated signals from multimodal male courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Anim. Behav.* **57**: 865–872.
- Hebets, E. A., and Uetz, G. W. (2000). Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behav. Ecol. Sociobiol.* **47**: 280–286.
- Johnson, S. A., and Jakob, E. M. (1999). Leg autotomy in a spider has minimal costs in competitive ability and development. *Anim. Behav.* **57**: 957–965.
- Juanes, F., and Smith, L. D. (1995). The ecological consequences of limb damage and loss in decapod crustaceans: A review and prospectus. *J. Exp. Mar. Biol.* **193**: 197–223.
- Kaston, B. J. (1936). The senses involved in the courtship of some vagabond spiders. *Entomol. Am.* **16**: 97–167.
- Klawinski, P. D., and Formanowicz, D. R. (1995). Ontogenetic change in survival value of leg autotomy in a wolf spider, *Gladicosa pulchra* (Keyserling) (Araneae: Lycosidae), during scorpion attacks. *Can. J. Zool.* **72**: 2133–2157.
- Koomans, M. J., Van der Poley, S. W. F., and Dijkstra, H. (1974). Leg wave behaviour of wolf spiders of the genus *Pardosa* (Lycosidae, Araneae). *Bull. Br. Arach. Soc.* **3**: 53–61.
- Kotiaho, J., Alatalo, R. V., Mappes, J., and Parri, S. (1996). Sexual selection in a wolf spider: Male drumming activity, body size, and viability. *Evolution* **50**: 1977–1981.
- Kotiaho, J., Alatalo, R. V., Mappes, J., Parri, S., and Rivero, A. (1998). Male mating success and risk of predation in a wolf spider: A balance between sexual and natural selection? *J. Anim. Ecol.* **67**: 287–291.
- Mappes, J., Alatalo, R. V., Kotiaho, J., and Parri, S. (1996). Viability costs of condition-dependent sexual male display in a drumming wolf spider. *Proc. R. Soc. Lond. B* **263**: 785–789.
- McClintock, W. J., and Uetz, G. W. (1996). Female choice and pre-existing bias: Visual cues during courtship in two *Schizocosa* wolf spiders (Araneae: Lycosidae). *Anim. Behav.* **52**: 167–181.
- Montgomery, T. H. (1903). Studies on the habits of spiders particularly those of the mating period. *Proc. Acad. Nat. Sci. Philos.* **55**: 59–149.
- Nyffeler, M., and Breene, R. G. (1990). Evidence of low daily food consumption by wolf spiders in meadowland and comparison with other cursorial hunters. *J. Appl. Entomol.* **110**: 73–81.
- Persons, M. H. (1999). Hunger effects on the foraging responses to perceptual cues in immature and adult wolf spiders (Lycosidae). *Anim. Behav.* **57**: 81–88.
- Persons, M. H., and Uetz, G. W. (1999). Age and sex-based differences in the use of prey sensory cues in wolf spiders (Araneae: Lycosidae). *J. Insect. Behav.* **12**: 723–736.
- Punzo, F. (1997). Leg autotomy and avoidance behavior in response to a predator in the wolf spider, *Schizocosa avida* (Araneae, Lycosidae). *J. Arachnol.* **25**: 202–205.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Roth, V. D., and Roth, B. M. (1984). A review of appendotomy in spiders and other arachnids. *Bull. Br. Arachnol. Soc.* **6**: 137–146.
- Scheffer, S. J., Uetz, G. W., and Stratton, G. E. (1996). Sexual selection, male morphology, and the efficacy of courtship signalling in two spiders (Araneae: Lycosidae). *Behav. Ecol. Sociobiol.* **38**: 17–24.
- Siegel, S., and Castellan, N. J. (1988). *Nonparametric Statistics for the Behavioral Sciences*, 2nd ed., McGraw-Hill, New York.
- Smith, L. D. (1990). Patterns of limb loss in the blue crab, *Callinectes sapidus* Rathbun, and the effects of autotomy on growth. *Bull. Mar. Sci.* **46**: 23–36.
- Smith, L. D. (1992). The impact of limb autotomy on mate competition in blue crabs *Callinectes sapidus* Rathbun. *Oecologia* **89**: 494–501.
- Smith, L. D. (1995). Effects of limb autotomy and tethering on juvenile blue crab survival from cannibalism. *Mar. Ecol. Prog. Ser.* **116**: 65–74.
- Smith, L. D., and Hines, A. H. (1991). The effect of cheliped loss on blue crab *Callinectes sapidus* Rathbun foraging rate on soft-shelled clams *Mya arenaria* L. *J. Exp. Mar. Biol. Ecol.* **151**: 245–256.
- Stratton, G. E., Hebets, E. A., Miller, P. R., and Miller, G. L. (1996). Pattern and duration of copulation in wolf spiders (Araneae, Lycosidae). *J. Arachnol.* **24**: 186–200.

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- Uetz, G. W., McClintock, W. J., Miller, D., Smith, E. I., and Cook, K. K. (1996). Limb regeneration and subsequent asymmetry in a male secondary sexual character influences sexual selection in wolf spiders. *Behav. Ecol. Sociobiol.* **38**: 253–257.
- Weissmann, M., and Vollarath F. (1998). The effect of leg loss on orb-spider growth. *Bull. Br. Arachnol. Soc.* **11**: 92–94.
- Walker, S. E., and Rypstra, A. L. (2003). Sexual dimorphism and the differential mortality model: Is behaviour related to survival? *Biol. J. Linn. Soc.* **78**: 97–103.
- Wood, F. D., and Wood, H. E. (1932). Autotomy in decapod crustacea. *J. Exp. Zool.* **62**: 1–55.
- Zar, J. H. (1984). *Biostatistical Analysis*, 2nd ed., Prentice–Hall, Upper Saddle River, NJ.