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THE FREQUENCY OF LEG AUTOTOMY AND ITS INFLUENCE ON SURVIVAL
IN NATURAL POPULATIONS OF THE WOLF SPIDER *PARDOSA VALENS*

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ABSTRACT

Autotomy occurs when an animal intentionally sacrifices an appendage to escape predation or free a limb. While immediately beneficial, loss of an appendage can lead to a variety of future costs. In many spiders, leg autotomy is common; previous work has sometimes demonstrated autotomy costs in some behaviors, while other times no costs of autotomy occur. We examined frequency of autotomy in two riparian-zone population of the wolf spider *Pardosa valens* Barnes, 1959, then used both mark-recapture work at these sites and laboratory predation trials to determine whether autotomy affected survival. Autotomy occurred in 31% of spiders; males were more likely than females to have a missing leg, but female reproductive status (carrying an egg sac or not) was unrelated to leg-loss status. At both sites, survival over one week in the field was significantly higher for intact spiders than for spiders missing a leg, for both sexes and both female reproductive states. Additionally, when we paired intact and autotomized spiders with a predator (the larger wolf spider *Rabidosa santrita* (Chamberlin & Ivie, 1942)), autotomized spiders were more likely to be attacked and eaten. Our results suggest leg autotomy in *P. valens* leads to a significant future survival cost, and we discuss how this cost may affect males and females differently.

KEY-WORDS: *Pardosa valens*, antipredator behaviors, autotomy, fitness costs, predation risk, survival, wolf spiders, *Rabidosa santrita*

INTRODUCTION

Animals have many ways to avoid being eaten, ranging from predator avoidance behaviors such as hiding to antipredator behaviors such as crypsis, threat displays or aposematic coloration (review in Lima and Dill 1990). Behaviors which eliminate or reduce the risk of physical contact with the predator should be favored, since these lessen the chance of injury or death to the potential prey individual. However, potential prey cannot always avoid contact with a predator, and so there exist a number of behaviors which may allow animals the opportunity to escape once attacked or captured. Of these “last gasp” antipredator behaviors, which range from fighting to fear screams to thanatosis, one of the most studied is autotomy.

Autotomy involves the intentional or voluntary sacrifice of some body part, typically a leg, tail, claw or other appendage, and has arisen numerous times in both vertebrates and invertebrates (reviews in Maginnis 2006; Fleming et al. 2007; Bateman and Fleming 2009). Separation of the body part usually occurs along one or more well-defined breakage planes (e.g., McVean 1975), and a number of adaptations have evolved to rapidly close the wound and prevent internal fluid loss. Generally, autotomy is thought to result from antagonistic encounters with predators or conspecifics (Fleming et al. 2007), although it can also result from reactions to toxins or injuries (Eisner and Camazine 1983; Emberts et al. 2017) or difficulties during emergence from the exoskeleton in the molting process (Maginnis 2008).

Under the threat of death or injury, the ability to autotomize a body part should be favored by natural selection, as it increases the chance of escape and thus the chance of

immediate survival. In many animals escape is helped by the actions of the sacrificed body part, which may continue to move, distracting the attacker while the individual flees (e.g. lizard and salamander tails: Dial and Fitzpatrick 1983; Ducey et al. 1993). However, despite the immediate fitness benefits of autotomy, numerous studies have demonstrated that individuals that sacrifice a body part can suffer short- or long-term decreases in fitness compared to intact individuals (reviews in Maginnis 2006; Fleming et al. 2007). These fitness costs of autotomy include the extra energy that needs to be obtained for regeneration of the lost body part, which may slow overall growth; decreases in performance variables such as sprint speed or prey capture; reductions in mating success, territory-holding ability, or social status; and reduction in the ability to escape predatory attacks in the future. Thus, autotomy may negatively affect future growth, reproduction and survival.

Studies which have examined the relationship between autotomy and survival have found mixed results. Although the data are limited, field studies of lizards indicate that tail autotomy leads to decreased survival in some but not all cases, with the results dependent on age, sex, or geographic location (reviewed in Bateman and Fleming 2009). In invertebrates, autotomy has been shown to have no effect on survival in crickets and firebrats, while in larval odonates, crustaceans, and stick insects there is evidence both for and against a survival cost of autotomy (reviewed in Fleming et al. 2007). However, many of these invertebrate studies have been conducted in the laboratory or in semi-natural field enclosures, and thus may not fully reflect natural effects of autotomy. We therefore currently lack strong support either for or against a survival cost of autotomy,

and field studies of the association between survival and autotomy are lacking for most taxa.

Many spiders can autotomize their legs (Roth and Roth 1984; Foelix 1996), and this behavior appears moderately successfully in allowing escape from predators (Klawinski and Formanowicz 1994; Punzo 1997). A number of studies have demonstrated costs associated with leg autotomy, including decreases in sprint speed (Amaya et al. 2001; Apontes and Brown 2005; Brown and Formanowicz 2012), foraging success (Riechert 1988; Brueseke et al. 2001; Wrinn and Uetz 2008), growth rate (Wrinn and Uetz 2007), total reproductive output (Ramirez et al. 2017), and competitive success against intact individuals (Dodson and Beck 1993; Taylor and Jackson 2003), although there are also cases in which no cost of autotomy occurs (e.g. Johnson and Jakob 1999; Amaya et al. 2001; Brueseke et al. 2001; Brautigam and Persons 2003; Steffenson et al. 2014). As for the invertebrate studies described above, much of the research on spider leg autotomy has been conducted in the laboratory, and there have been no studies examining the effects of autotomy on survival either in the laboratory or the field.

Wolf spiders (Family Lycosidae) are a speciose group whose members do not typically build prey-capture webs; instead, most species are active cursorial hunters or ambush foragers living in burrows. Lycosids use their legs for locomotion, prey capture, burrow construction and courtship, and most have the ability to autotomize these legs; thus, wolf spiders are an excellent choice for studying potential costs of autotomy in spiders. In *Pardosa valens* Barnes, 1959, a small cursorial wolf spider from southeastern Arizona, we have previously shown in a laboratory study that leg loss can decrease sprint

speeds both on land and on water (Brown and Formanowicz 2012). In the current study, we have three goals to further examine the potential costs of autotomy in *P. valens*. First, we use field data to estimate the frequency of autotomy for males, females and females carrying an egg sac in the wild. Second, we use field data from a mark-recapture study to estimate short-term survival of intact and autotomized spiders in all three sex/reproductive categories, to determine if there exists a survival cost of autotomy in the wild. Third, we use laboratory data examining interactions between *P. valens* and the sympatric predatory wolf spider *Rabidosa santrita* (Chamberlin & Ivie, 1942) to determine more directly if survival of *P. valens* depends on whether it has all legs intact.

MATERIALS AND METHODS

Focal Species and Study Sites

Pardosa valens is a small (30-140 mg adult mass) wolf spider found from Arizona and New Mexico south into central Mexico (Barnes 1959). The sexes are dimorphic in size, with females significantly heavier than males (Brown and Formanowicz 2012). In the Chiricahua Mountains of southeastern Arizona, *P. valens* occurs near small mountain streams, where they are the most common lycosid and can be found both in the cobble alongside the stream and moving across the water's surface between rocks. They are rarely encountered more than five meters from the stream border. From late May through July, when our experiments were conducted, individuals are active during both the day and night and the population consists primarily of adults

and recently dispersed spiderlings (C. Brown, C. Amaya and D. Formanowicz, unpublished data).

We collected spiders from two locations in the Chiricahua Mountains, Cochise Co., Arizona. The first of these was a section of Cave Creek running along Forest Road 42A near the Southwestern Research Station (SWRS; 31°53'01" N, 109°12'28" W, altitude = 1620 m). The second site was a section of Turkey Creek near the junction of Forest Road 42 and Forest Road 42B (31°54'33" N, 109°14'56" W, altitude = 1930 m). Both sites are part of the Madrean evergreen woodland biotic community (Brown, 1994). Because the two creeks do not merge and *P. valens* rarely occurs outside the riparian zone, we considered these to be separate populations for the purposes of this study.

Estimating Frequency of Autotomy

We collected data on the frequency of leg autotomy in adult *P. valens* at both study sites during four years: 29 May-4 June 2003, 13-18 June 2004, 11-13 June 2005, and 15-16 July 2006. Although the methods varied slightly depending on what use was going to be made of the spiders, we typically used the following procedure. A 50-100 m transect that followed the creek channel was laid out, marked at either end with surveyor's flags, and divided roughly into thirds. Each of us then systematically walked through one of these three sections, collecting adult spiders into centrifuge tubes (one spider per tube). Each section of the transect was walked multiple times over the course of 1.5-2 h; we typically stopped when no new spiders had been collected for 10-15 min in each section. In each year except 2004, spiders were collected while visible on the

surface and by turning rocks within 3-5 m of the stream, and searching occurred during daylight only. In 2004, as part of a study on activity patterns we only collected spiders that were visible on the surface, and we performed searches during both the day and night. Once a search had ended, we recorded each spider's sex, reproductive status if female (with or without an egg sac), and number of legs missing (with zero indicating an intact individual; position of missing legs was not recorded). If a transect was searched on two days, as occurred in 2003 and 2004, spiders collected on the first day were marked with a permanent marker on the abdomen or carapace prior to release, and data from recaptures were not included in any analyses.

Field Survival of Intact and Autotomized *P. valens*

During 2005 we performed a field experiment at both Cave and Turkey Creeks to assess short-term survival of adult *P. valens* that were intact or were missing a single leg. At Turkey Creek, on 11 June we established two contiguous 50 m transects that followed the creek channel. One transect was searched in the morning and the other in the early afternoon; for both, searches lasted ~2 h. During this period, each adult spider found active on the surface or by turning rocks was collected into a centrifuge tube and identified by sex, female reproductive status (with or without an egg sac), and number of legs missing. Spiders missing legs were released back into the transect at the end of the search period, while intact spiders were taken to the laboratory at SWRS. Because we obtained relatively small numbers of males and females lacking egg sacs, we returned to Turkey Creek the next day and gathered intact spiders belonging to these two groups (51

males and 33 females without egg sacs) from areas upstream and downstream of our transects.

In the laboratory, intact spiders from each of the sex/reproductive status groups (males, females without egg sacs, and females carrying egg sacs) were randomly divided into two subgroups. In one subgroup, each individual was marked on the abdomen or carapace using a permanent marker. In the other subgroup, we marked each individual using a permanent marker, then induced them to autotomize a randomly selected leg by pinching the leg with a fine forceps at the coxa-trochanter joint. The leg chosen for removal was randomly selected, rather than standardized for all individuals, because we wanted to more closely mimic the natural pattern of leg loss, in which legs from any of the four positions may be missing. We used separate colors to differentiate intact from autotomized individuals (purple and black for females with egg sacs, green and silver for both males and females without egg sacs). After spiders were marked and legs autotomized, we returned all individuals to Turkey Creek, distributing them haphazardly along the full 100 m transect. For females with egg sacs, marking, autotomy, and release all occurred on 11 June; for the other two groups, these occurred on 12 June (thus, some of these individuals were kept in the laboratory for ~24 h). In total, we used 93 males (47 intact, 46 autotomized), 69 females without egg sacs (34 intact, 35 autotomized), and 105 females with egg sacs (53 intact, 52 autotomized)

On 17 June 2005 we returned to Turkey Creek to recapture marked *P. valens*. We divided the complete 100 m transect into thirds, then each searched one section visually and by turning over rocks within 5 m of the creek's edge. All adult spiders, whether

marked or unmarked, were captured individually into centrifuge tubes. We searched the transect twice, once during the morning and once during the early afternoon; both times, searches were stopped only after each of us had gone at least 15 min without finding a spider. Following completion of each search, we recorded the sex, reproductive status (if female), and marking color (if applicable) for each spider.

We followed the same procedures as described above at Cave Creek. Two 50 m transects were laid out on 13 June, one of which was searched in the morning and the other in the afternoon. We then collected up- and downstream of these transects on 14 June to obtain more males (69 additional individuals) and females without egg sacs (59 additional individuals). Females with egg sacs were all marked, autotomized and released on 13 June; males and females without egg sacs were marked, autotomized and released on 14 June. We then returned to Cave Creek on 19 June to search for marked individuals. At this site, we used 114 males (58 intact, 56 autotomized), 103 females without egg sacs (52 intact, 51 autotomized), and 99 females with egg sacs (49 intact, 50 autotomized).

Rabidosa santrita* Predation Trials on *P. valens

During July 2006 we conducted an experiment examining the risk faced by intact and autotomized *P. valens* when exposed to *Rabidosa santrita*, a wolf spider that co-occurs with *P. valens* in both creeks. *Rabidosa santrita* are much larger (>300 mg for older juveniles and adults) than *P. valens*, and are known to prey on the smaller species (C. Brown, D. Formanowicz, and C. Amaya, personal observations). All trials were performed in the laboratory at SWRS and began at dusk (~1900 h), when *R. santrita*

typically begin emerging from their diurnal retreats (rocks or leaf packs within or alongside the streams). We used plastic containers ($15 \times 30 \times 11$ cm) as test chambers, with natural soil obtained near Cave Creek as a substrate. All *R. santrita* and most (~80%) *P. valens* were collected from Cave Creek, with the remaining *P. valens* collected from Turkey Creek. Spiders were held in plastic centrifuge tubes stoppered with a wetted cotton ball until used in a trial. All spiders were used within 1-4 days of capture, and were not fed during captivity. The lab experienced ambient lighting, air temperature and humidity during all trials.

In the early afternoon of days on which trials were performed, we selected either 10 or 20 intact female *P. valens* (depending on the number of trials to be run) and weighed them on an electronic balance to the nearest 1 mg. We then grouped them into pairs based on similarity in mass. For each size-matched pair, we randomly selected one female and marked her on the abdomen using a permanent marker. The other female in each pair was induced to autotomize a randomly selected leg by grasping it at the coxa-trochanter joint with a pair of fine forceps. Following autotomy and marking, spiders were set aside and allowed to recover for 4-6 h. Although this recovery time is shorter than typically used (12-24 h), all spiders showed normal behavior and activity at the start of a trial.

Approximately 3 h before the start of trials, *R. santrita* were weighed on an electronic balance to the nearest 1 mg and then a single spider was placed into each test chamber for acclimation. We used either adult female or older juvenile (in their penultimate or antepenultimate instar) *R. santrita* in all trials, and predators were used in

only a single trial, with two exceptions (we reused two spiders once each after they did not feed during their first trial).

To begin a trial, we removed the lid from the chamber and simultaneously introduced both *P. valens* from a single size-matched pair into the test chamber by overturning their tubes. Prey spiders were introduced to the middle of the short side of the chamber farthest from the initial position of the occupant *R. santrita*. We directly observed each chamber until the first attack was made by the predator, recording which *P. valens* was attacked and the outcome of the attack (prey eaten or prey escaped); if 15 min passed with no attack, we terminated the experiment. Ten trials were run on each of two nights, and five trials were run on a third night. Following completion of these trials, we returned all surviving spiders to Cave Creek.

Statistical Analyses

We used log-linear analysis to examine the effects of year, population of origin, sex/reproductive status, and their interactions on frequency of leg loss. Log-linear analysis was also performed on the field survival data to test the effects of population, sex/reproductive status, autotomy treatment, and their interactions on survival. In each case, we selected the best-fitting model using a backwards stepwise approach; in this method, better-fitting models will have larger *P*-values than will poorer-fitting models. For the field survival experiment, we followed the log-linear analysis by performing a series of chi-squared tests comparing survival of intact and autotomized individuals for each sex/reproductive status group separately. Finally, we used a two-tailed binomial test

to compare survival of intact and autotomized individuals in the laboratory predation trials. All data analyses were performed using Statistica version 4.5 (StatSoft 1993) with α set at 0.05.

RESULTS

Over the four years of our study, we captured a total of 1244 *P. valens* from Cave Creek (398 males, 421 females without egg sacs, and 425 females with egg sacs) and 1014 *P. valens* from Turkey Creek (394 males, 328 females without egg sacs, and 292 females with egg sacs). Across all groups (excluding 2006, which had several low sample sizes), between 23% and 41% of spiders were missing a leg. Overall, males were more likely to be missing a leg than were females, while autotomy frequency was similar for females with and without egg sacs (Table 1). Males were also overall more likely to be missing multiple legs than either group of females (Table 1). In our log-linear models we combined both leg loss categories from Table 1 (single leg missing and more than one leg missing) into a single category and omitted the 2006 data because of a low sample size at Turkey Creek. The best-fit model ($\chi^2 = 14.91$, d.f. = 15, $P = 0.46$) indicated a significant effect of sex/reproductive status (confirming that males were more likely to be missing a leg; chi-squared test: $\chi^2 = 17.73$, d.f. = 2, $P < 0.0001$) as well as a significant three-way interaction between year, population, and sex/reproductive status. In general, leg loss was more common for males and females with egg sacs at Turkey Creek than at Cave Creek, while the reverse was true for females without egg sacs. However, these patterns varied in an unpredictable manner across years both within and among populations.

In the field recapture experiment, our best-fit log-linear model ($\chi^2 = 7.28$, d.f. = 10, $P = 0.70$) included a significant effect of autotomy treatment on recapture, as well as a significant interaction effect between sex and population. Autotomized spiders were significantly less likely to be recaptured than were intact spiders, and we found this to hold for each sex/reproductive status group in both populations (Turkey Creek: males: $\chi^2 = 5.69$, $P = 0.02$; females without an egg sac: $\chi^2 = 5.83$, $P = 0.02$; females with an egg sac: $\chi^2 = 9.27$, $P = 0.002$; Cave Creek: males: $\chi^2 = 4.85$, $P = 0.03$; females without an egg sac: $\chi^2 = 16.32$, $P < 0.0001$; females with an egg sac: $\chi^2 = 15.74$, $P < 0.0001$; d.f. = 1 for all tests; Fig. 1). Recapture success of both intact and autotomized spiders was greater at Turkey Creek than at Cave Creek for males and females without egg sacs, while the reverse was true for females with egg sacs.

While recapturing marked spiders, we also collected 159 unmarked individuals from Turkey Creek (43 males, 43 females without egg sacs, and 73 females with egg sacs) and 154 unmarked individuals from Cave Creek (28 males, 59 females without egg sacs, and 67 females with egg sacs). Unfortunately, we did not differentiate intact individuals from those missing a leg for these unmarked spiders; however, we expect that the frequency of individuals missing a leg was similar to the values described above.

We ran 25 laboratory trials with *R. santrita* (mass range 335-701 mg) as a predator; average ($\pm$ SE) masses of intact (63.76 ± 3.28 mg) and autotomized (65.24 ± 3.25 mg) *P. valens* used in these trials were not significantly different (paired $t_{24} = 1.27$, $P = 0.22$). In eight of these trials no *P. valens* was attacked within 15 min. In the remaining 17 trials, the autotomized individual was attacked first in 14 trials and the

intact individual was attacked first in 3 trials (two-tailed binomial test: $P = 0.01$). In all cases, the *P. valens* that was attacked was captured and consumed by the predator.

DISCUSSION

Leg loss was a common occurrence in our two populations of *Pardosa valens*, with nearly one-third (31.4%) of all individuals missing at least one leg, and 9% missing multiple legs. The frequency of leg loss in *P. valens* was similar to that of the congener *Pardosa milvina* (Hentz, 1844) from agricultural fields in the eastern United States (Brautigam and Persons 2003) and to male *Misumena vatia* (Clerck, 1757) crab spiders (Lutzy and Morse 2008), but was generally higher than previously reported in spiders (e.g. Dodson and Beck 1993; Johnson and Jakob 1999; Brueseke et al. 2001; Apontes and Brown 2005; Wrinn and Uetz 2007; Chen et al. 2008; Pasquet et al. 2011; Martinez et al. 2017).

Autotomy frequency was not related to reproductive status in females, as the proportion of individuals missing a leg was similar in females with or without an egg sac. However, the sex of a spider did influence autotomy frequencies, with males more likely to have a leg missing than females. Sex differences have been documented for limb autotomy in several invertebrates (Fleming et al. 2007) and for tail autotomy in several species of lizard (Bateman and Fleming 2009, although most lizard studies show no effects of sex on autotomy). Either males or females may have higher autotomy frequencies; in invertebrates, it appears that higher frequencies most often occur in the sex that experiences higher rates of potentially risky interactions (such as intrasexual

combat, resource defense, or predator encounters) with con- or heterospecifics (Fleming et al. 2007; see Emberts et al. 2016 for a counterexample in coreid bugs). In *P. valens*, and wolf spiders generally, males are more likely than females to wander in search of mates, and thus are probably exposed to greater risks of predation (e.g. Kotiaho et al. 1998), which could explain their higher frequencies of leg loss. Additionally, males may be involved in intrasexual competition for mates or be at risk of sexual cannibalism from females (e.g., Wilder and Rypstra 2008), either of which could elevate their risk of autotomy relative to females.

Regardless of sex or reproductive status, *P. valens* with a missing leg were recaptured at a significantly lower rate than were intact individuals in both of our study streams; when comparing individuals of the same sex or reproductive state, recapture rates were between 21% and 42% lower for autotomized spiders. If recapture rates serve as an accurate proxy for survivorship (see further discussion below), then to our knowledge this represents the first report of a survival cost of autotomy in a field population of spiders, and one of the few such results in any natural population of animals. Previous work on survival after autotomy (reviewed in Fleming et al. 2007; Bateman and Fleming 2009; see also Frisch and Hobbs 2011) has roughly split between finding no effect of appendage loss and finding lower survivorship in autotomized individuals. Many of the studies that demonstrate a cost are based on survival in a laboratory setting, especially for invertebrate studies, and thus may not accurately reflect the same costs in nature. For example, caudal lamellae autotomy in the damselfly *Lestes sponsa* (Hansemann, 1823) has no effect on survivorship in the lab, but in the field larvae

that are missing lamellae have approximately 20% lower survivorship than intact larvae (Stoks et al. 1999; Stoks 2001). Field studies of tail autotomy in lizards are slightly more common, and several have shown a survival cost, with a relative decline in survivorship for autotomized individuals similar to what we found in *P. valens* (e.g. 16% lower for autotomized *Uta stansburiana* Baird & Girard, 1852: Fox and McCoy 2000; up to 30% lower for autotomized *Takydromus viridipunctatus* Lue & Lin, 2008: Lin et al. 2017).

Based on the generally-accepted primary causes of leg loss (Fleming et al. 2007), our results would suggest that survival differences between intact and autotomized spiders were due to variation in either susceptibility to predation or in mortality as a result of intraspecific interactions. The latter seems unlikely to have a strong effect, as we have never observed prolonged combat either within or between sexes of *P. valens*, especially of a kind that would lead to severe injury or death. Thus, we suggest that potential survival differences between intact and autotomized individuals are most likely caused by a differential susceptibility to predation. Our predation experiment supports this conclusion, as spiders missing a leg were much more likely to be killed by the larger wolf spider *Rabidosa santrita* than were intact spiders.

In our paired trials, intact *P. valens* may be attacked at a lower rate than autotomized spiders because they are faster, as has been demonstrated by Brown and Formanowicz (2012). Greater burst speeds could enable these spiders to more readily escape from a variety of potential predators in their riparian habitat, including *R. santrita*, the scorpion *Vaejovis cashi* Graham, 2007, several species of birds (e.g., American robins *Turdus migratorius* Linnaeus, 1766 and Mexican jays *Aphelocoma wollweberi* Kaup,

1855) and several species of lizards (e.g., the mountain spiny lizard *Sceloporus jarrovi* Cope, 1875, the striped plateau lizard *S. virgatus* Smith, 1938, and the tree lizard *Urosaurus ornatus* (Baird & Girard, 1852)). Alternatively, if movement gives away a spider's location (via either visual or vibrational cues), intact spiders may be attacked less if they are able (or willing) to remain stationary longer than spiders missing a leg. Intact spiders may allow predators to come closer to them because they perceive their burst speed is enough to allow them to escape, or because they still have all 8 legs to sacrifice, and thus have a greater "protective radius" of legs that can be grabbed by predators that strike towards the periphery of the spider's body compared to spiders already missing a leg.

Recapture rates of male *P. valens* were substantially higher at Cave Creek than at Turkey Creek; conversely, recapture rates for females with egg sacs were substantially higher in the Turkey Creek population. The reason for these differences is unknown, but could result from between-population differences in several factors: behaviors associated with predation exposure, such as activity levels; levels of intraspecific competition; risk of mortality from factors other than predation; and home range size or movement patterns. Further research will be needed to address this issue.

Three aspects of our study design could negatively affect survival of autotomized individuals, separate from any expected effects of predation. The first of these relates to the addition of spiders to each study transect; 84 individuals were added to Turkey Creek and 128 individuals were added to Cave Creek. Higher densities might lead to greater competition (e.g., for food or space) or an increase in aggressive interactions (leading to

mortality or migration), and these effects may affect autotomized spiders more strongly than intact ones. However, the increase in density was small at each study site. Cobble habitat typically extends at least 1 m on either side of the stream, so that the area of cobble along each 100 m transect should be a minimum of 200 m². Thus, adding spiders led to an increase in density of at most 0.42 spiders/m² at Turkey Creek and 0.64 spiders/m² at Cave Creek. We think this minor increase in density should have limited impact on spider behavior, especially given the general lack of intraspecific aggressiveness in this species.

The second aspect is the forced removal of the leg itself, which could increase mortality due to hemolymph loss or via a general stress response. However, there was no mortality of spiders from the autotomized group while kept in the lab for up to 24 h during this study, and in previous work on this species (Brown and Formanowicz 2012) we have not observed any differences in mortality between intact and autotomized *P. valens* kept in the lab for periods of 7-10 days (similar to the length of the current study). Finally, the third aspect is related to the marking procedure: colored markers may increase mortality directly (if the ink is toxic to spiders) or, since different colors were used for each leg treatment, some spiders may have been more visible to visually-oriented predators. Although we are not aware of any study that has examined the effects of paint/ink marking on spider survival, no effect on survival has been found in several other groups (e.g., lizards: Jones and Ferguson 1980; wasps: De Souza et al. 2012). In addition, paint marking is a very common field-marking technique used across a wide variety of terrestrial arthropod species, including wolf spiders, and so seems unlikely to

lead to increased mortality. As for color differences, silver was used to mark autotomized males and females without egg sacs, and this was the easiest color to see for at least one type of potential predator (the researchers). In retrospect, we should have switched colors between streams as a control for this (i.e., mark intact individuals with silver at one of the two streams). However, recent mark-recapture work by one of us (C. Brown and B. Baggett, unpublished data) exploring home range size in *P. valens* suggests that color choice may not have a profound effect on survival, as individuals marked with brighter or more reflective colors (white, yellow, silver, or gold) were observed for similar periods of time over a two-week period as were individuals marked in darker colors (green, blue, or purple). Thus, we suggest that neither the leg removal procedure nor the marking process should have differential negative effects on survival of intact versus autotomized spiders.

We have made the assumption that differences in recapture frequencies between intact and autotomized spiders reflect differences in survivorship; however, it is also possible that these reflect differences in behaviors between the two groups that simply lower the recapture success of autotomized individuals relative to intact individuals. If correct, actual survivorship could then be relatively similar between intact and autotomized spiders. One possibility is that, following autotomy, spiders choose to stay hidden under cobble alongside the streams, emerging less frequently, or at different times, than intact individuals. Similar behavioral changes to reduce predation risk following autotomy have been shown in some species of both vertebrates (Bateman and Fleming, 2009; Cooper and Wilson 2010) and invertebrates (Fleming et al. 2007). To address this, we looked for hidden individuals by turning over most rocks near the

stream, and searched at two different times during the day. A second, albeit less likely, possibility is that spiders that have lost a leg were more likely to emigrate from the study site than were intact spiders. Although autotomy generally leads to a decrease, or no change in, activity levels in invertebrates (Fleming et al. 2007), for *P. valens* a higher emigration rate could arise for autotomized individuals if they increased movement rates during the night, when this species is also commonly active. To address this possibility, we searched 10-15 m beyond each end of the transects, both looking for active spiders and turning rocks to find any hidden ones. Of the 282 marked spiders that were recaptured, fewer than 15 were found outside the transect (nearly all within 5 m of the end of the transect), and there was no bias towards finding either intact or autotomized individuals outside a transect. We are therefore confident that we have observed real differences in survival between intact and autotomized *P. valens*, rather than just differences in the ability to relocate marked intact and autotomized individuals.

Finally, we note that there were more unmarked (313 in total) than marked (282 in total) *P. valens* collected during the recapture phase. These individuals were either present but overlooked during the initial capture phase, or entered from outside the transect after this phase; we strongly suspect the former, for the following reasons. First, we did not exhaustively sample the habitat during the initial capture phase, and thus it is not unexpected that there would be spiders in the transects that were missed or uncollected. This is especially true for females with egg sacs, which are the most common category of *P. valens* during mid-June and were abundant enough that we eventually ceased collecting them during the initial capture phase. Indeed, nearly half of

the unmarked spiders collected (140 individuals, ~45% of the total) were females with egg sacs. Second, work on home range size in this species suggests that a large majority of individuals tend to stay within a 10-15 m linear stretch along the stream over a period of 1-2 wks (B. Baggett and C. Brown, unpublished data), which suggests that large-scale migration into the transect is unlikely. Therefore, the unmarked spiders most likely represent a portion of the resident population of the transect, and the large numbers reflect the more exhaustive searching done during the recapture phase of our study.

Thus, for *P. valens* leg autotomy appears to be a costly behavior, with a substantially decreased survivorship following loss of a leg. This implies that the benefits of autotomy must also be high for this behavior to have evolved, which is almost certainly true; surviving a predatory encounter while losing a leg should always be preferable to keeping the leg but dying. Perhaps of more interest, our results also suggest that these benefits may need to be used rather quickly following autotomy. Adult *P. valens* appear to survive just a single breeding season, so for males even a few extra days survival should greatly increase their chances of successfully mating (or remating). However, for females the decrease in survival may impose a more severe cost than for males. The length of time between copulation and offspring emergence from the egg sac is not known for *P. valens*, but if similar to other like-sized lycosids is probably within the range of 14-21 days (Eason 1964). Thus, relatively high short-term survival costs would prevent many females from completing their reproductive cycle. Females had a greater difference in survivorship between intact and autotomized individuals than did males in our study, and this may provide a partial explanation for why females have

lower natural leg-loss frequencies than do males; females may be more reluctant to sacrifice a leg and then incur this cost. Future studies examining the exact causes of leg autotomy, and any differences among sexes in pre- or post-autotomy behavior, should help elucidate the potential survival costs and benefits of this common antipredator behavior.

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Table 1. Frequency of *Pardosa valens* from each of two populations that had all legs intact (8 Legs), were missing a single leg (7 Legs), or were missing two or more legs (<7 Legs). Counts are provided separately for males, females without an egg sac (Females), and females carrying an egg sac.

Population (Year)	Males			Females			Females w/ Egg Sacs		
	8 Legs	7 Legs	<7 Legs	8 Legs	7 Legs	<7 Legs	8 Legs	7 Legs	<7 Legs
Cave Creek									
2003	130	68	17	94	34	9	96	20	9
2004	71	28	10	93	30	14	87	17	12
2005	45	6	9	44	8	6	100	31	9
2006	11	3	0	63	20	6	25	12	7
Total	257	105	36	294	92	35	308	80	37
Turkey Creek									
2003	141	64	26	122	32	6	67	13	8
2004	59	18	14	65	10	13	25	10	4
2005	42	21	8	36	11	3	105	41	10
2006	0	1	0	19	8	3	9	0	0
Total	242	104	48	242	61	25	206	64	22

Figure 1. Percent recapture (+SE) of intact (black) and autotomized (white) *Pardosa valens* from the 2005 field experiment at (A) Cave Creek and (B) Turkey Creek. Data from each location are provided separately for males, females without an egg sac, and females with an egg sac.

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