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The Effects of Condition and Sex on Leg Regeneration in the Wolf Spider *Rabidosa punctulata*

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ABSTRACT
The act of autotomy and subsequent limb regeneration occurs fairly frequently in wolf spiders. Limb regeneration however can have its tradeoffs, causing a decrease in mass and prolonged development; this process can sometimes result in a limb that is shorter, misshaped, and discolored. Differential uses across the legs may influence how spiders invest in their regeneration. In both sexes, the forelimbs are often used to capture prey, while in males the forelimbs also are used in courtship to attract a mate. We autotomized spiders and exposed them to different diets, to examine the effects on the limb's length, the spiders mass and the investment into leg regeneration made between genders. We found that males invest more into regrowing their forelegs despite their conditions, while females invested equally in regrowing both their legs and that across both gender and diet significantly affected the length of the regenerated limb.

Keywords: *spiders, autotomy, regeneration*

INTRODUCTION

Autotomy is described as the act of willingly shedding a limb and or appendage (self-amputation). Self-amputation and regeneration are commonly known to be done by many different species of both vertebrates and invertebrates. While organisms like spiders can amputate a limb willingly and regenerate it back, some animals only possess the ability to either amputate or regenerate; meaning that if they lose the limb, it's gone for good while for some they do not have the ability to choose whether to amputate the limb but it will eventually be regenerated. There are over two hundred species capable of autotomy including: lizards with their tails (Cooper, 2003), blue crabs (Smith, 1990) and field crickets and their legs (Bateman and Fleming, 2007), sea stars who can autotomize their arms (Diaz-Guisado, Gaymer, et al., 2006), spiders (Wrinn, 2005), and geckos also (Webb, 2006). Worms when severed into multiple pieces, regenerate to form whole new worms and salamanders have the ability to regenerate their limbs and even their jaws along with tadpoles who could regenerate their tails (Maginnis, 2006).

There are many advantages of autotomy including severing or shedding a limb to escape a predator or threat. Some of the animals when they autotomize the severed limb have the ability to keep moving or to secrete a toxic substance, this serves as a distraction to assist the animal in its get away. According to Maginnis (2006) in her study of autotomy and regeneration in animals, she states that besides avoiding predation an advantage of autotomy especially in the case of arthropods (like spiders) is that they escape a fouled molt. Normally they crawl themselves out of the old exoskeleton and after they're out the new one hardens, but in the process of climbing out of the old exoskeleton, they lose limbs when they get stuck in the old exoskeleton and have to shed them, so not having the leg can actually benefit the animal. Autotomy is used by several animals to prevent poison from spreading through its body after being stung from predator or other organism whether it was a wasp, a bee or a scorpion etc. (Eisner and Camazine, 1983)

The costs of autotomy vary among just about all of the species; the most obvious would be reduced locomotion. Crickets escape speed were hindered because of autotomy, also their ability to move and jump; this also increased the chances of the organisms being caught by another predator (Fleming and Bateman, 2007). Reduced locomotion also affects organisms in terms of walking, running, swimming, diving, gliding, balance and other movements. Other costs to the organisms are impaired foraging abilities, competitive abilities which affects the animal's fitness and it causes the animal to have to allocate much more energy to regrow the loss body part which can grow to be a problem if the animal doesn't have a sufficient amount of nutrients to help regenerate a fully working limb/appendage (Eisner and Camazine, 1983). With their foraging abilities decreased the animal is forced to make the decision to either forage in a safe place but have less prey to capture or forage in a more dangerous place and risk another confrontation with a predator. Other disadvantages of autotomy are loss of predator defense and loss of stored nutrients and changes in social and reproductive behavior. Loss of a limb can also play a role in the organisms' fitness; as could be seen in a study done with the wolf spider *Schizocosa ocreata* whose male species' forelegs are decorated with bristles to attract a mate and also as a form of species identification. In bilaterally symmetrical species such as spiders, appearing asymmetrical indicates that the individual had experienced developmental trouble or is under stress, or in the female perspective, the individual has “bad genes”. In previous studies, when placed under stress the males regenerated their legs back but they were small misshapen and lacked the bristles that were their prior to the autotomy. They found that asymmetric males had significantly lower mating success than symmetric males suggesting that female choice is based off of this physical trait of having the bristles. The males not finding a mate means that they don’t get to reproduce and pass on their genes which can prove to be quite costly for the individual (Uetz, McClintock, Miller, et al., 1995).

Losing a limb can be quite costly and can hinder an individual’s ability to retrieve food and capture prey. The conditions they are in are particularly important to help them regenerate their lost limbs more quickly and fully. Individuals that don’t feed much are in poor condition having low energy levels and aren’t expected to regenerate legs as quickly causing them to go into a downward spiral making the condition they’re in worse and worse, this paired with reduced foraging abilities can prove to be detrimental to the organisms.

Arthropods were used as the model organisms for the experiment for more than just their ability to autotomize and regenerate their limbs. Arthropods go through the process of molting to grow, they shed their exoskeleton frequently as they grow throughout their lifetimes; in addition to shedding their exoskeleton they form a whole new and flexible exoskeleton. Although arthropods are special for their molting and regenerating, it takes a few molting periods to regenerate an autotomized limb which would cause the underdeveloped leg to be smaller than the other legs. Similar to the organisms who possess this ability, autotomy is especially important to arachnids like spiders because they depend on their legs for most actions including prey capture and restraint and sensory detection (Wrinn and Uetz, 2008). According to Eisner and Camazine, when a limb is injured or pulled, autotomy “occurs at the level of the coxa-trochanter joint near the base of the leg.” They state that spiders possess a special trait that can assist the organism in reducing the amount of bleeding at the specific location where the limb was loss (1983). In terms of regeneration, Wrinn and Uetz found that the spiders have some sort of tradeoff between their development and their mass that happens after the autotomy. They saw that the spiders wasted no time in regenerating the limb, decreasing the costs related to missing a limb and regenerating it back to only one molt. Seeing that the regeneration of the missing limb caused the spider to either take a longer time to molt or have a smaller mass showed them that there indeed is a tradeoff happening (Wrinn and Uetz, 2007).

The study organism for this research is the Dotted wolf spider, *Rabidosa punctulata.* These spiders are ground dwelling spiders who usually can be found living in tangles of grass and vegetation. The dotted wolf spider can also be identified by the pattern of black spots underneath its abdomen. The average maturation size (body length) of this species is seen to be 12.8 millimeters for males and 15.2 millimeters for females. The female dotted wolf spiders are seen to mature in October while the males appear to mature slightly earlier around the month of September. Male wolf spiders are known to use their front legs in courtship and both sexes use these limbs in capturing prey while the hind legs are mostly used for mobility purposes. In reference to this experiment, we understand that spiders use their legs for different reasons and that there are costs to regenerating a limb, so we will explore whether condition would have an effect on the regeneration of the limb and whether the different sexes will invest differently in regenerating their limbs. In males, loss of their front legs would impair their abilities to perform courtship to females and loss of the hind legs in either male or female would have some effect on the spiders’ mobility.

The objectives of the experiment are to investigate whether there is a relationship between spider body conditions, sex and investment into the regeneration of different legs We are going to see how the spider reacts to missing a front and hind limb and which limb do they compensate energy to regenerate (whether they find one to be more important or both).

MATERIALS AND METHODS

In preparation to begin the different aspects of the experiment, one hundred seventeen Rabidosa punctulata wolf spiders were collected from around the McPherson, Kansas area; all of which were kept in individual visually isolated cages (AMAC 83mm x 83mm x 109mm). They were also kept on a 14:10 L:D cycle at 27˚ C.

Condition vs Limb Regeneration

This aspect of the experiment was to test whether the spider’s environmental conditions had an effect on whether the spider would regenerate the missing limb back or not. The spiders collected were split into two different treatment groups: one of good condition and the others were considered poor conditioned. The variables that we will observe are (1) whether the limb regenerated or not, (2) the length of the regenerated limb, (3) the amount of time it took for the limb to be regenerated and (4) the number of molts it took for the leg to be regenerated. Fifty-eight spiders were randomly assigned to each group to avoid bias.

The “good” conditioned spiders were fed two crickets two times a week and the “poor” conditioned spiders received two crickets every two weeks. After capture, the spiders from each treatment underwent a form of artificial autotomy. The “artificial” autotomy was performed by taking each spider and placing them into a small plastic bag with a small hole cut into the corner of the back. The spiders were then coaxed into the corner of the bag where the spider would attempt to escape the bag by sticking its legs through the hole. Once this happened, we pressed gently on the bag to immobilize the spider and to prevent it from escaping; we then held onto the desired leg and softly tugged on said leg to force the spider to autotomize. For the autotomy we randomly assigned which leg was to be removed (left or right) each spider had both a front leg (leg 1) and a hind leg (leg 4) removed, this was decided randomly by rolling a die to determine which limb.

The spiders were then then placed back in their pens, and their treatment was continued for eleven weeks, over this period of time they were checked for molt two times a week, and their molting periods were recorded along with the number of days that passed until the limb was fully regenerated. Once the spiders reached maturity, they were each weighed using a digital scale, sacrificed and placed at -20 C until measuring. Using a digital caliper, we measured both the regenerated legs and the legs from the opposite side that hadn’t been removed two or three times before averaging them to perform the comparison.

Statistical Methods:

After collecting all of the measurements, we then recorded the following variables from both conditions: whether they regenerated the limb or not, the length of the regenerated limb, the length of the limb that wasn’t removed, the number of molting periods for each spider and the number of days since the spider autotomized and the weight of the spider. Comparisons that were performed when analyzing the data included: the regenerated leg lengths between the diet treatments, the regenerated leg lengths between the legs of each individual, the regenerated legs between sexes and the relative leg lengths between the sexes. We calculated the Relative leg differences by dividing the length of the leg that was removed by the absolute leg length difference in order to do a comparison.

We then obtained a relative leg difference for the front leg and hind leg. A Repeated Measures ANOVA was performed to analyze leg investment in both sexes and conditions.

RESULTS

After completing the experiment, we found that of the 59 spiders placed in the high conditioned treatment group, 97% of them regenerated their limb in the given time; and of the 58 spiders in the low conditioned group, we saw 85% regeneration. Analysis of the data on the *Rabidosa punctulata* revealed that there was indeed a significant difference between the sexes in this experiment, but in terms of whether they regenerated the missing limb back, there was not any significance. Simply put, both conditions regenerated their limbs; in fact approximately 88% of the spiders used in this experiment regenerated at least one of their legs back, and approximately 87% regenerated both of the legs.

Table 1: Repeated Measures ANOVA testing for differences both within subjects (Leg) and between subjects (Sex, Diet).

	df	Mean Square	*F*	*p*
Leg	1	0.061	28.091	<0.001
Leg ✻ Sex	1	0.017	7.835	0.006
Leg ✻ Diet	1	0.001	0.657	0.420
Leg ✻ Sex ✻ Diet	1	0.006	2.916	0.091
Sex	1	0.113	17.756	<0.001
Diet	1	0.030	4.662	0.033

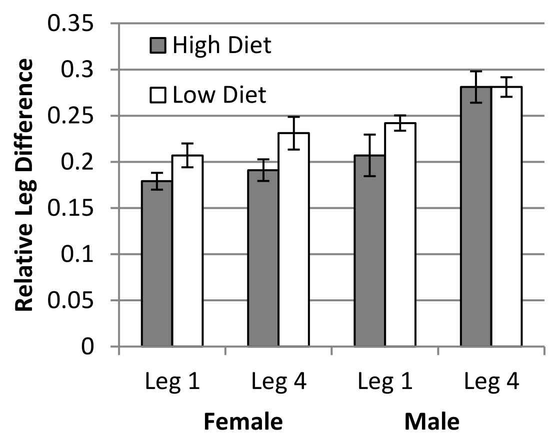

Figure 1: Comparison of the relative leg differences among sexes.

As can be seen in the table above, there was a significant difference between leg length and sex (P= <0.001), we saw that the males (both high and low treatment groups had a larger mean leg difference when compared to the females. As seen in the figure 2 the length of the regenerated legs differed across the diet treatments also with the spiders in the high diet groups being able to produce a longer limb, but remarkably the low diet group was able to reproduce their leg 4 the same as the high treatment group. The main finding of this research was that the males were seen to invest more into leg 1 rather than leg 4, while the females invested equally as seen in Figure 1.

Although the number of days to mature varied greatly amongst genders and leg lengths, we found

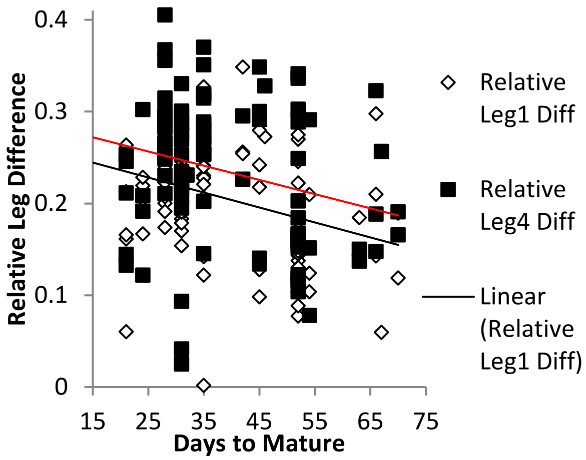

Figure 2: Comparison of the number of days it took the spiders to mature versus the relative leg length differences of spiders from both sexes. As can be seen, the numbers varied greatly for both legs.

that there was a significant difference in the amount of days it took the high conditioned spiders to regenerate compared to those of the low conditioned treatment group. The difference showed that those in the high conditioned groups matured significantly faster than those of the other treatment group producing a P value of <0.01

DISCUSSION

Sex and exposure to different condition appears to have an effect on the *Rabidosa punctulata*'s ability to regenerate autotomized limbs. Many studies have talked about and demonstrated the costs and consequences of autotomy on an individual prior to this experiment. As stated in the results we saw a significant difference amongst the sexes in whether they regenerated the limb or not, when we looked at it across the diets, there wasn't a significant difference meaning that both the spiders in the high diet and low diet regenerated their legs, but there was a difference in the leg lengths amongst the groups.

When exposed to different stresses and feeding schedules, energy is limited and has to be invested specifically to help aid the individual in surviving, we saw the male spiders invest into their front legs, which supports our hypothesis that they would do so seeing that their foreleg aids them in finding a mate, capturing prey etc. The spider's body condition also plays a role in the length of the regenerated leg, the sexes displayed significance in the lengths of the regenerated legs compared to their autotomized limb although there wasn't any significance amongst the two diet groups.

When comparing all of the measurements it was clear that the males indeed compensated for their front legs while the females didn't have a specific preference, they focused more on growing back both legs rather than choosing a favorite. In terms of evolution, it is typical for the female to not prefer one specific leg over another because; males participate in sexual selection by females. Their forelegs serve as ornaments, to attract a mate, the female doesn't have ornaments so they don't have a preference, and they just invest equally to have all of their legs. As Wrinn (2005) stated in her paper on the Impacts of leg loss and regeneration on body condition, growth, and development time in the wolf spider, leg loss in addition to a specific diet treatment could have noticeable effects on the growth and regeneration of the autotomized limb, and that was supported here where we provided the spiders with a specific source of food to limit the amount of energy they could gain to regenerate the specific limb, and also to put stress on the spiders. The more energy the spider had they could compensate or invest toward regenerating a fully functional leg, with the high condition spiders taking a shorter time to mature while the low conditioned spiders were accumulating energy from the little amount of food they were receiving having less molting period and taking more time to mature. Overall no matter the condition, the tradeoff for spiders when it comes to regenerating a missing limb is between mass and development time.

In conclusion, from this experiment we can say that there is indeed a correlation between regenerated leg lengths of spiders of different genders and of those who are on different diets. There was also the proof of differential investment amongst the genders, where we found that males invested more in their forelegs with the results from the low conditioned males displaying that the relative leg difference between the two conditions was the same; while females invested equally in both legs despite the conditions they were place in.

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LITERATURE CITED

Cooper, W.E. 2003. Shifter balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (Holbrookia propinqua). *Behavioral Ecology and Sociobiology.* 54 (2), 179-187

Diaz- Guisado, D., C.F. Gaymer, K.B. Brokordt, J.M. Lawrence. 2006. Autotomy reduces feeding energy storage and growth of the sea star Stichaster striatus. *Journal of Experimental Marine Biology* *and Ecology.* 338(1) 73-80.

Eisner, T., S. Camazine. 1983. Spider leg autotomy induced by prey venom injection: An adaptive response to “pain” *Proceedings of the National Academy of Sciences* (80) 3382-3385

Fleming, P.A, P.W. Bateman. 2007. Just drop it and run: the effect of limb autotomy on running distance and locomotion energetics of field crickets (Gryllus bimaculatus). *The Journal of Experimental Biology* (210) 1446-1454

Gong, J., K. Yu, L. Shu, H. Ye, S. Li, and C. Zeng. "Evaluating the Effects of Temperature, Salinity, Starvation and Autotomy on Molting Success, Molting Interval and Expression of Ecdysone Receptor in Early Juvenile Mud Crabs, Scylla Paramamosain." Journal of Experimental Marine Biology and Ecology 464 (2015): 11-17. Web. 23 Feb. 2016.

Maginnis, T.L. 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology.* 17(5) 857-872

Miyashita, K. 1968. Growth and Development of Lycosa T-insignita Boes. Er Str. (Aranae: Lycosidae) under Different Feeding Conditions. *Applied Entomology and Zoology.* (2) 81-88

Slos, S., M. De Block, R. Stoks 2009. Autotomy reduces immune function and antioxidant defense. *Biology Letters.* 5, 90-92

Steffenson, M. M., D. R. Formanowicz, and Christopher A. Brown. "Autotomy and Its Effects on Wolf Spider Foraging Success." *Ethology* 120(11) (2014): 1128-136. Web. 23 Feb. 2016.

Tan, L., Y. Zhao, and C.Lei. "Development and Integrality of the Regeneration Leg in Eupolyphaga Sinensis." *Bulletin of Insectology* 66 (2) 2013: 173-80. Web. 23 Feb. 2016.

Webb, J.K. 2006. Effects of tail autotomy on survival, growth and territory occupation in free-ranging juvenile geckos (Oedura lesueurii). *Austral Ecology.* 31 (4) 432-440.

Wrinn, K.M. 2005. The effects of leg loss and regeneration on prey capture, growth, and development time in wolf spiders.

Wrinn, K., G.W. Uetz, G. 2007. Impacts of leg loss and regeneration on body condition, growth, and development time in the wolf spider *Schizocosa ocreata.* Canadian Journal of Zoology, 85 (7), 823-831

Wrinn, K.M., G.W. Uetz. 2008. Effects of autotomy and regeneration on detection and capture of prey in a generalist predator. *Behavioral Ecology* 19 (6) 1282-1288

Uetz, G. W., W. J. Mcclintock, D. Miller, E. I. Smith, and K. K. Cook. "Limb regeneration and subsequent asymmetry in a male secondary sexual character influences sexual selection in wolf spiders." Behavioral Ecology and Sociobiology 38.4 (1996): 253-57. Web. 20 Feb. 2017.