Compensatory Responses to Loss of a Sexual Organ in Male Black Widow Spiders

by

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Abstract

Experimental alterations of morphological traits during development can reveal life history tactics and resource allocation patterns. I examined effects of amputation of a structure directly involved with mating compared to one that is less associated with fitness. I amputated one of the paired external copulatory organs (palps) of juvenile male black widow spiders (Latrodectus hesperus), and compared changes in life history traits and fitness to males with amputated legs and controls. I show that palps are more likely to be regenerated than legs, smaller juveniles are more likely to regenerate, and mating success is adversely affected in all adults that suffered early amputation. The pre-existing relationship between juvenile size and development was a critical determinant of regeneration, however no life history costs of regeneration were evident. I conclude that plasticity in life history facilitates regeneration, and that this may mask trade-offs involved in compensatory effects at the population level.
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1 Introduction

Plasticity in life history traits can often reveal the variation in phenotypes and strategies individuals use when faced with changing environmental conditions. Traits such as reproductive rate, body size, age at maturity, and longevity play an integral part in determining an organism’s fitness (Ricklefs & Wikelski 2002, Stearns 1992, Roff 1992), and changes in one or more of these life history traits can alter the developmental trajectory of an organism and the range of behavioural and morphological phenotypes seen in a population. Environmental factors such as predation (e.g. Dahl & Peckarsky 2003, Johnson & Belk 2001), temperature (e.g. David et al. 2006), photoperiod (e.g. Blanckenhorn 1998, Johansson et al. 2001), resource availability (e.g. Kause & Morin 2001) or demography (e.g. Rodd et al. 1997, Kasumovic & Andrade 2006) are all known to affect life history traits, as is the physiology and underlying genetic architecture of the organism itself (e.g. energy use patterns, condition-dependent expression) (Kotiaho et al. 1998, Sibly & Calow 1986). Nevertheless, a tremendous amount of variation in these traits exists in populations and the source of this variation has been the subject of much research.

Life history can be viewed as the net outcome of strategic decisions that incorporate multiple traits important to fitness (Engqvist 2007, Stearns 1992). Because of this, traits that draw from the same pool of resources or influence fitness in complementary ways are often held back from their optimum by an organism’s strategic allocation to competing functions (Stearns 1989, van Noordwijk & de Jong 1986, Engqvist 2007). The result is a set of complex relationships among traits that give rise to tradeoffs shaped by the organism’s adaptive responses to environmental variation. The shape and strength of the relationship between two given life
history traits can vary for many reasons (Stearns & Koella 1986, Day & Rowe 2002, Roff et al. 2002), and this makes it difficult to assess trade-offs. One reason for this is that growth conditions often differ between individuals and this can produce vastly different reaction norms for the same traits (Stearns 1989, Engqvist 2007). Another way that trade-offs may be complicated is when individual variation in resource accumulation produces positive correlations at the population level between traits that normally compete for these resources at the individual level (van Noordwijk & de Jong 1986, Stearns 1989). For example, sexual signaling in a wolf spider is positively correlated with immunocompetence at the population level, providing support that drumming with the abdomen is a good indicator of male quality in the context of female choice (Ahtiainen et al. 2004). However, at the individual level males that increase drumming rate suffer reduced immune function, suggesting a physiological tradeoff between these two functions (Ahtiainen et al. 2005).

Variation in resource allocation and trade-offs between competing functions may also explain many morphological patterns in organisms (Zera & Harshman 2001). Different selection pressures acting on different body parts can produce variation among individuals as they optimize the combination of values that will yield the highest fitness (Stearns 1989). A common approach to investigate dynamic linkages between correlated traits is to damage or remove a body part and measure the ensuing compensatory responses. For example, removing hindwing imaginal discs in caterpillars results in larger forewings and legs in the emerging butterfly (Klingenberg & Nijhout 1998, Nijhout & Emlen 1998), while losing a leg changes the wing size and area of stick insects (Maginnis 2006a). The vast majority of studies on resource allocation have focused either on tradeoffs between morphological features (e.g. Klingenberg & Nijhout 1998, Nijhout & Emlen 1998, Emlen 2001, Radwan et al. 2002, Moczek & Nijhout 2004) or
between classic life history traits (fecundity and growth, maturation time and body size, e.g. Roff et al. 2002, Engqvist 2007). Few, however, have looked at how morphology can interact with life history to modulate the variation in both types of traits (see Stevens et al. 1999, Stevens et al. 2000), or how tradeoffs inherent to these interactions can influence fitness (Boggs 2009).

Morphology and life history traits are particularly likely to interact when compensatory responses to environmental challenges include regeneration after appendage loss. Partial or full regeneration of a lost body part is one strategy to mitigate the costs associated with missing a particular structure, and has been documented in many taxa (Maginnis 2006b). In addition, the potential to replace a body part and the required additional investment can alter pre-existing relationships between morphological features, as well as induce costs that impact the organism’s growth trajectory and development. These can be allocation costs that arise due to reduced resource availability for growth and reproduction, or performance costs if the regenerated appendage has reduced function (Maginnis 2006b). Thus quantification of effects of limb loss should include costs and payoffs of regenerating assessed in terms of life history as well as functional consequences. A system in which regeneration is possible may allow unique insight into trait development and resource allocation because this effectively increases the range of available compensatory tactics. The possibility of regeneration can also allow inferences about the relative importance of amputated structures to fitness. Moreover, since the occurrence and effectiveness of regeneration often varies among individuals (Maginnis 2006b), studies of limb loss in regenerative species may provide the opportunity to investigate regeneration as a strategy for coping with environmental stressors that have both morphological and life history consequences.
In this study, I ask whether losing a strongly sexually selected appendage (male copulatory organ) produces compensatory effects in life history and regeneration, and compare this to the response when the appendage lost is less directly tied to fitness (legs). As appendage loss is common in nature (Roth & Roth 1984), selection for tactics to alleviate such insults should be strong. Environmental and experimental insults on sexually selected traits can trigger drastic compensatory effects, presumably because the impact on individual fitness is immediate and severe. Altering even one aspect of a sexually important trait or signal can produce changes in morphology (Moczek & Nijhout 2004, Nijhout & Emlen 1998), physiology (Fry 2006, Ahtiainen et al. 2005), or behaviour (Bravery & Goldizen 2007) that mitigate the negative fitness effects of these alterations. For example, removing decorations from the bowers of male bowerbirds results in males compensating for the diminished courtship signal by enhancing bower structure (Bravery & Goldizen 2007), while onthophagine beetles with experimentally ablated horn tissue grow larger by maturity and invest in larger testes (Simmons & Emlen 2006). These changes are thought to result from redirected resource allocation to fitness-boosting traits in order to compensate for the fitness disadvantages borne by another trait. Clearly, drastic compensatory effects should also be seen when a genital structure is lost as these directly influence fertilization success (House & Simmons 2007). Unlike weaponry and ornaments, genitalia are not condition-dependent (Eberhard et al. 1998, but see Arnqvist & Thornhill 1998) and tend to have more canalized growth than other traits, resulting in less individual variation and more stable phenotypic averages within populations (Eberhard et al. 1998, Eberhard 2009). One of several hypotheses for the lack of condition-dependence of genitalia (Eberhard 2009, House & Simmons 2007, Ramos et al. 2005) posits that genital growth is most canalized when the risk of redirecting resources to another plastic trait, particularly one subject to strong sexual
selection, is the highest (House & Simmons 2007, Simmons & Emlen 2006). In general, male genitals are expected to be under stabilizing selection to fit the average female in the population (Eberhard 2009, Eberhard et al. 2009, Ramos et al. 2005, but see Bonduriansky 2007), and as such optimal development of genitalia should be of high priority in any system.

In this study I conduct experimental removal of appendages in male western black widow spider (Latrodectus hesperus), and track development, adult size, and mating performance. In many spiders, growth and development are known to be highly plastic traits (Schneider 1997, Uhl et al. 2004, Vertainen et al. 2000, Fernández-Montraveta & Moya-Laraño 2007, Foellmer & Fairbairn 2005, Kasumovic & Andrade 2006, Kasumovic et al. 2009a, Kasumovic et al. 2009b, Blanckenhorn et al. 2007, Kaston 1970), allowing for a broad range of compensatory effects. Manipulation of the copulatory organs of male spiders (pedipalps) is relatively easy since these are external and separated from the testes (Foelix 1996), allowing one to impede reproductive function without performing invasive procedures. Moreover, male spiders have two palps and the reproductive cost of losing one is clear and measurable. In Latrodectus spiders, each palp accounts for about 50% of a male’s potential paternity (Snow & Andrade 2005). While one palp is sufficient for mating and transfers enough sperm to fertilize a female’s eggs over her lifetime, two are needed to optimize performance under sperm competition (Snow & Andrade 2005). Females have paired sperm storage organs (spermathecae) (Foelix 1996), and males must insert each palp in a spermatheca and deposit a sperm plug in order to prevent rival insemination (Snow et al. 2006). Partly for this reason, sexual selection on palp function is expected to be relatively strong. Another reason selection for palp structure may also be strong is that palp shape and size are likely to be constrained by the extreme sexual size dimorphism (Ramos et al. 2005, Kuntner et al. 2009) of widow spiders (Kaston 1970). In
addition to their obvious primary function, palps can shape male fitness in more indirect ways as well (e.g. as part of courtship drumming displays in wolf spiders, Fernández-Montraveta & Simó 2002; jumping spiders, Elias et al. 2005; and redback spiders, Lee et al. in prep).

While pedipalp loss in the field has only been documented in one spider species (5% in male wolf spiders, Lynam et al. 2006), it has been observed in other species, including western black widows (M.C.B. Andrade, pers. comm.). In comparison, the frequency of leg loss in field-caught spiders ranges from 7.5% to nearly 40% (Brueseke et al. 2001, Lutzy & Morse 2008, Brautigam & Persons 2003, Apontes & Brown 2005, Wrinn & Uetz 2007, Uetz et al. 1996, Johnson & Jakob 1999, Dodson & Beck 1993). Leg loss usually happens due to autotomy, where the leg is voluntarily separated from the cephalothorax at the coxa-trochanter joint (Wood 1926, Foelix 1996, Parry 1957, Randall 1982) to avoid harm to the rest of the organism. Autotomy has been shown to be an adaptive strategy to limit the spread of toxins during predator attacks (Eisner & Camazine 1983, Eisner et al. 2006, Klawinski & Formanowicz 1994, Formanowicz 1990, Punzo 1997), minimize injury during conspecific fighting (Dodson & Beck 1993), or reduce mortality due to molting accidents (Rovner 1967, Maginnis 2006b, Modanu pers. obs.). Although autotomy increases the probability of immediate survival (Apontes & Brown 2005, Fromhage & Schneider 2006), the long-term effects of autotomizing legs are less clear (see Table 1). While legs can be subject to sexual selection when they are used for courtship signaling (e.g. Foellmer & Fairbairn 2005, Taylor et al. 2006), or via scaling relationships with body size when size is subject to intersexual selection (Foellmer & Fairbairn 2005, Tomkins et al. 2005), sexual selection on legs is likely to be weaker than on palps.

In this study, I induced the loss of one pedipalp or one leg in juvenile male black widow spiders, followed their development and examined their regenerative ability and mating success
as adults. Imposing this cost experimentally forces every individual to adjust and may reveal a greater variety of compensating strategies, whereas with voluntary autotomy only the subset of individuals that successfully executes the autotomy may remain. This naturalistic paradigm allowed me to examine variation in regeneration patterns and life history responses to provide insight not only into the decision rules that individuals use to determine optimal resource allocation (Boggs 2009), but also to assess whether different rules apply for strongly versus weakly sexually selected structures (genitals vs. legs). Because the palp is so tightly coupled with an individual’s fitness, I predict that male black widows who lose a palp early in development will regenerate it more often than males that lose a leg. However, all males that regenerate should have smaller adult body size due to allocation of resources to regenerating the lost appendage (Maginnis 2006b). Since adult body size is known to correlate positively with development time in widow spiders (Kasumovic & Andrade 2006, Kasumovic et al. 2009a, Stoltz et al. in prep), I expect that slow-growing control males should be larger at adulthood than rapidly-growing males. However, males that show evidence of regeneration should delay maturity relative to those that do not regenerate and to controls, as additional time may be required to regrow the lost appendage (Maginnis 2006b). Mating success should be lowest for palp-amputated males that do not regenerate and highest for leg-amputated and control males, with regenerators approaching control success.

The strength of this study is its global life history approach which combines both morphological and fitness measures to investigate appendage loss and regeneration, using both sexual and non-sexual appendages. In contrast, most previous work has focused on non-sexual appendages only (Maginnis 2006b) and has documented relatively few measures of the effects of this loss on individual development and fitness.
2 Methods

2.1 Procedure

Spider eggs were obtained from laboratory-reared females descended from adult spiders caught near San Diego, California in May 2007. Eggs from 14 different females were individually raised in 4 x 4 x 6cm clear plastic cages (Amac Plastic) to prevent juvenile cannibalism. Feeding commenced two weeks after hatching, when yolk sac reserves are exhausted and juveniles can build a web to catch prey (Foelix 1996). Spiderlings were each fed one fly twice a week for two weeks, and this was increased to two flies twice a week until the spiderling reached the fourth instar. Preliminary trials indicated that the fourth instar was optimal for amputations because spiderlings can be reliably sexed (Mahmoudi et al. 2008) and attrition from the procedure is minimal. At the fourth instar, spiderlings were sexed (Mahmoudi et al. 2008) and males were randomly assigned to one of four treatments: control (n = 26), palp-amputated (P-Amp, n = 48), first-leg amputated (L1-Amp, n = 17), and third-leg amputated (L3-Amp, n = 19). The first and third legs were chosen because they are the longest and shortest legs, respectively, of male widow legs and, while first legs can play a role during courtship (Foellmer & Fairbairn 2005, Taylor et al. 2006), both first and third legs have relatively little apparent function other than fine-tuning locomotion. Males in the P-Amp group were anesthetized with CO₂ and one randomly chosen pedipalp was amputated proximal to the patella using a pair of iridectomy microdissection scissors. The same protocol was followed for the L1-Amp and L3-Amp groups, which had one leg amputated distal to the coxa. Autotomy of the leg usually happens at the coxa-trochanter joint (Wood 1926, Foelix 1996, Parry 1957, Randall 1982) and leaving the coxa intact is critical for operation of the mechanism that reduces hemolymph loss.
from the wound (Randall 1982) and thus reduces mortality (see Randall 1981). Control males were also anesthetized and their palps and legs were similarly handled, but no amputation was performed. All amputations were performed within 2-3 days of the moult into the fourth instar (control spiderlings spend an average of 9.5 ± 3.8 (mean ± sd) days in the fourth instar, n = 31). Treatment group assignment ensured that early- and late-developing spiderlings were distributed across treatments (hatchlings reach the fourth instar 44 ± 10 days (mean ± sd) after hatching, n = 133). Hemolymph loss after amputation was minimal and brief, and spiders recovered from the anesthetic within minutes.

After treatment, spiderlings were fed twice a week until adulthood under the following feeding regime: # flies = current instar # - 1. This regime accounted for individual differences in moult dates and ensured that feed amounts were gradually increased with age and scaled to each individual’s development time. Spiders were not fed after the adult moult for two reasons: a) to mimic natural conditions in the field where males begin searching for females and cease building webs (Foelix 1996); and b) to maximize longevity after maturity which has been shown to decrease with adult diet in male widow spiders (Segoli et al. 2007).

All individuals were checked for moult skins at least every second day, and moult dates were recorded to measure time spent in each instar. Three cohorts of spiderlings were raised in this study, one hatching in the winter of 2008 (n = 31) and fed primarily *Drosophila melanogaster* flies, and the other two hatching in the late summer of 2008 (n = 68) and winter of 2009 (n = 11), and fed primarily *Drosophila hydeii* flies. All spiders were maintained on a 12:12 light:dark cycle at 25°C.
2.2 Regeneration and Development

To determine the effects of amputation, I documented development time, size, mating success and regeneration success in all groups. Upon reaching maturity, each male had his development time to adulthood recorded and was assigned to one of two regeneration categories: a) no regeneration attempt if there was no structure at the site of appendage amputation; or b) regeneration attempt if there was any evidence of a substantial structure grown at the amputated location (see Fig 1 for least and most obvious regeneration attempts). This category included both complete and partial growth because assessing regenerative effort can be problematic and the focus was on any reallocation of resources that might have affected development.

2.3 Morphometrics

All males were photographed at the fourth instar before being assigned to groups. Spiders were briefly anesthetized with CO₂, and placed under the camera to ensure a top-down view of the body parts photographed. Digital images of the cephalothorax, all legs, and both palps were taken with Act 1 image acquisition software (Nikon Corp., 2000) using a Nikon DXM 1200 camera mounted on a dissecting microscope (Zeiss Stemi 2000-C). Measurements were obtained from the images using ImageTool (UTHSCSA ImageTool Version 3.0, University of Texas Health Science Centre in San Antonio), and the average of 3-5 different images was taken to minimize measurement error. Prosoma width was measured as the widest point of the cephalothorax, the total length of the patella and tibia of each leg was used for leg length, and palp width was measured at the widest point near the patella of the palp (Brandt & Andrade
The same body parts were photographed and measured at adulthood, except for palp width which was measured as the widest part of the bulb where the sclerotized embolus coil begins. The researcher was blind to the experimental group of the spider during all measurements.

2.4 Mating trials

To quantify the fitness consequences of these treatments, adult males from each treatment were paired with virgin females and mating success and reproductive output were assessed. Male widows must fill their palps with sperm after they reach adulthood, and because this sperm induction can occur days after their adult moult (Foelix 1996), I conducted mating trials at least seven days after a male reached maturity. Since black widows are nocturnal, mating trials were conducted under red light and viewed with low-light CCTV cameras (Panasonic WVBP330) fitted with a time code generator (Horita VG-50). Unrelated adult virgin females were placed on metal frames and given 24-48 hours to construct a web. Males were then introduced to the web and allowed to court the female for up to 6 hours, at which point the trial was terminated. Black widow mating typically occurs 2-3 hours after courtship begins (MacLeod et al., in prep; Modanu pers. obs.). Males were observed during the trial for courtship and copulation events, and weighed after each trial with an Ohaus Explorer balance (accurate to 0.01 mg). Since it is common for normal (unmanipulated) males to fail to mate on their first attempt (MacLeod et al., in prep), males who did not mate in their first trial were given a second opportunity with a different virgin female at least two days after the first trial. Adult males are relatively short-lived
(about 20 days, Modanu pers. obs.) and some unmated experimental males died before their second mating opportunity (n = 16).

A second group of control males from the laboratory population was also paired with virgin females to determine the baseline mating success expected for males that are missing one palp in the absence of compensatory effects that may arise during development in the experimental groups (particularly P-Amp). These males (Adult-Amp) had one palp amputated at adulthood on the day before their mating trial.

If mating occurred, females were collected and fed one cricket per week (Acheta domesticus) until they produced at least three egg sacs. Eggs in each egg sac were examined and classified as viable (translucent and spherical) or inviable (irregular shape and opaque, see Snow & Andrade 2005), and the fertility of each male’s mate calculated.

2.5 Statistical analyses

All analyses were performed in R (R Development Core Team 2008). If data did not conform to assumptions of normality and homoscedasticity with the Shapiro-Wilk test and Levene’s test, respectively, non-parametric tests were used where indicated. Model II regression (Ranged Major Axis) was used where the error in the x variable was presumed to be equivalent or unknown relative to the error in the y variable (Legendre & Legendre 1998, Legendre 2008). Natural logarithm transformations were performed on variables in order to calculate allometric slopes between two morphometric traits at the same life stage (static allometry) or between the same trait over an individual’s development (ontogenetic allometry) (Shingleton et al. 2007).
All morphometric size measurements were highly correlated, so a principal component analysis (PCA) using a correlation matrix was used to create size scores for each individual spider. Separate PCAs were performed on spiders at the juvenile (fourth instar) stage before treatment commenced, and at adulthood. The thorax and all legs loaded strongly onto the first component of each PCA (Table 2), hence the PCA scores from the first axes are used throughout this study as indicators of juvenile and adult size, unless otherwise indicated.

3 Results

3.1 Regeneration

Nineteen percent (16/84) of spiders regenerated, but the occurrence of regeneration varied among treatment groups. The likelihood of regeneration was variable but not significantly different across the three P-Amp cohorts (G-test with Williams’ correction, G = 4.14, p = 0.13, Table 3), and they are treated as one group in all comparisons of regeneration. P-Amp males were most likely to attempt regeneration and L1-Amp the least (29% and 0% respectively, G-test with Williams’ correction, G = 10.49, p = 0.005, Table 3).

To identify factors that might predict regeneration in amputated spiders, I used all potentially important variables (development time before treatment, juvenile size, treatment group) in a multiple logistic regression with regeneration as the outcome, and chose the simplest model based on the lowest Akaike Information Criterion (AIC) (Crawley 2007). The simplest model only included juvenile size as a predictor, with a significant negative relationship between
juvenile size and the likelihood of regeneration \((Wald z = -3.83, p = 0.00013)\). To determine the value of juvenile size alone as a predictor of regeneration by adulthood, I used a discriminant function analysis (DFA) with a jackknife procedure to estimate the probability of each amputated spider being correctly classified as a regenerator or a non-regenerator based solely on their juvenile size. I performed two separate analyses, one using 4th instar size scores from the PCA, and one using raw measures of average first leg length at the 4th instar, as the latter is often used in spider field and laboratory studies and provides a more easily interpretable result. Both DFAs correctly classified 82% of experimental spiders \((69/84)\) as regenerators or non-regenerators based on their 4th instar size, confirming that smaller juveniles are more likely to regenerate by adulthood (Fig 2).

Smaller males might be more likely to regenerate if their palp is proportionately smaller than the appendage larger males carry. Consistent with this, juvenile palp size had a strong positive allometric relationship with thorax size (allometric slope of 2.25, RMA regression 95% confidence interval 1.96 - 2.6, \(n = 110\), \(r = 0.80\), \(p < 0.001\)), indicating that large males have proportionately larger pedipalps for their body size at the juvenile stage. In comparison, the means of the allometric slopes for all the legs at the same juvenile stage ranged from 1.10 to 1.41 (Table 4).

In terms of absolute size, a one-way ANOVA indicated significant differences in adult size \((F_{2,107} = 5.01, p = 0.008)\), with regenerated spiders larger than both non-regenerators (Tukey post hoc \(p = 0.056\)) and controls (Tukey post-hoc \(p = 0.006\)). Adult size correlates strongly with juvenile size in control spiderlings \((r = 0.64, p = 0.0099, n = 26\), RMA line of best fit is \(y = 0.722633x - 0.2109668\)), but this relationship does not hold for the amputated spiders \((P-Amp r = -0.09, p = 0.55; L1-Amp r = -0.41, p = 0.098; L3-Amp r = -0.44, p = 0.056)\). To compare
relative adult growth across treatments, I calculated residual growth of experimental spiders relative to the control spider regression line (Fig 3A and C). This approach quantifies the deviation in growth as a result of amputation and allows comparison of growth differences across treatments. Regenerating spiders had significantly larger and more positive residuals than non-regenerating spiders (Mann-Whitney W = 847, p = 0.0006, Fig 3B), and residuals also differed among treatment groups (F_{2,81} = 5.06, p = 0.0085, Fig 3D). A Tukey post-hoc analysis revealed that P-Amp males had significantly more positive residuals than L1-Amp (Fig 3D), with all other pairwise comparisons non-significant.

### 3.2 Development

Black widow males go through an average of 6-7 moults to reach maturity (Modanu pers. obs., Kaston 1970). Regenerating spiders moulted through more instars than non-regenerating or control spiders (ordinal logistic regression Wald Z = 4.84, p < 0.0001, Figure 4). However, this may arise as a correlated effect of their juvenile size, rather than as an effect of regeneration. Control males whose development was not altered by amputation showed a negative relationship between their size at the fourth instar and the number of instars to maturity (r = -0.78, t = -6.4, p < 0.0001, Figure 5). However, despite the additional instars, smaller males do not achieve the same size by adulthood as males that are relatively large at the fourth instar. This is indicated by the isometric relationship between juvenile and adult size in control males (log-log plot slope = 0.86, RMA 95% confidence interval 0.45-1.62, n = 26, p = 0.0099, r = 0.58). Thus small males maintain their relative position in the size hierarchy despite undergoing more moults.

Development time measured in total days from amputation to maturity also differed among groups (Kruskal-Wallis chi-squared = 22.4, df = 2, p < 0.0001), with regenerating males
taking longer to reach maturity \((71.7 \pm 27 \text{ days, mean \(\pm\) sd})\) than control males \((49 \pm 25 \text{ days})\) or non-regenerating males \((40.4 \pm 25 \text{ days})\). These differences arose as a result of the treatment, as there were no significant differences in growing time from hatching to amputation for males that were later placed in these three groups (Kruskal-Wallis chi-squared = 4.5, df = 2, \(p = 0.1\); regenerators: \(40.8 \pm 10.5 \text{ days pre-amputation}\), non-regenerators: \(44.5 \pm 7.2 \text{ days pre-amputation}\), controls: \(43.7 \pm 7.6 \text{ days}\)).

There were also differences in development time between amputation treatments. P-Amp males took longer to mature \((55 \pm 32.8 \text{ days})\) than all other groups (L3-Amp: \(36.7 \pm 16.6\); L1-Amp: \(32.6 \pm 12.6\), controls: \(49 \pm 25.2 \text{ days}\); Kruskal-Wallis chi-squared = 10.95, df = 3, \(p = 0.012\)). A general linear model with post-amputation development time as a Poisson-distributed response variable found significant effects of both treatment group (P-Amp, L1-Amp, L3-Amp, or Control; \(z = 8.9, p < 0.001\)) and regeneration status (\(z = 10.2, p < 0.001\)), as well as an interaction between the two (\(z = 3.9, p < 0.001\)). However, this interaction is likely driven by the fact that almost all regeneration attempts \((14/16)\) occurred in the P-Amp group.

### 3.3 Mating

Mating success in the first pairing for experimental groups was about half that of controls (Table 5). Among experimental groups P-Amp males tended to have lower mating rates, but this was not significantly different from other experimental groups (G-test with Williams’ correction, \(G = 4.1, df = 4, p = 0.39\)). Mating success was also not significantly affected by the regeneration status of amputated males although regenerators tended to have higher success (G-test with Williams’ correction, \(G = 4.6, df = 2, p = 0.098\), Table 5).
Since males are likely have more than one opportunity to mate in the field (MacLeod et al. in prep), I also assessed summed mating success as the proportion of males who mated once across the two attempts. This sample does not include males who did not mate on the first attempt, but died before getting a second opportunity to mate (n = 16). Summed mating success across both mating opportunities was still lower in experimental groups than controls (G-test with Williams’ correction, G = 9.18, df = 3, p = 0.027, Table 5), with P-Amp males showing the lowest success at 19%. Among the amputated males, regenerating males had higher mating success than non-regenerating males when both were compared to controls (G-test with Williams’ correction, G = 8.75, df = 2, p = 0.013). Interestingly, none of the regenerated males used their regenerated palp in these matings, but instead only copulated once with the palp that was not regenerated.

Using a logistic regression, I then examined whether adult size, regeneration status, treatment group, or male condition (see Kasumovic & Andrade 2006 suppl. for calculation of condition scores), and all possible interactions predicted variation in summed mating success. Using AIC to simplify the model resulted in a strong trend for regenerated males to be more likely to mate successfully (z = 1.91, p = 0.056, Table 6).

Although cannibalism is rare in *L. hesperus* (MacLeod et al., in prep), eight instances of precopulatory cannibalism occurred during mating trials. In seven of these cases, the male was wrapped in silk by the female and bitten within 30 minutes of being placed on the web and initiating courtship. In the eighth case, the male was wrapped but fell off the female’s web. Six of the cannibalized males were from the P-Amp group, but this proportion was not significantly different from the cannibalism rate in the other groups (G-test with Williams’ correction, G = 6.04, df = 3, p = 0.11, Fig 6).
Due to the low mating success of experimental males (Table 5), few females were inseminated and no statistical tests were performed on potential differences in fertilization across treatment groups. However, fertilization success was high in all mated females (over 90% in the first egg sac) in all groups, confirming that copulation did result in successful insemination whenever a male achieved a mating.

4 Discussion

Male black widow spiders show different compensatory responses to the loss of copulatory and locomotor structures. Consistent with predictions based on the relative importance of strongly sexually selected structures, males that lose a pedipalp early in development show a significantly higher frequency of regeneration relative to those that lose a leg (Table 3). Regeneration was more likely for males that were smaller at the time of the amputation, and these males took longer to mature and reached adulthood after more instars than males that did not regenerate (Fig 4). This difference in development time appears to be a general feature of the life history of small males, since controls also showed a negative relationship between juvenile size and number of instars to adulthood (Fig 5). Nevertheless small control males were still relatively small as adults (Fig 3A,C) and non-regenerating males showed a similar pattern, but regenerated males (particularly those with amputated palps) were significantly larger than expected as adults (Fig 3B,D). This is contrary to predictions that body size would suffer from a shift in allocation to regeneration. However, males clearly paid a fitness cost of amputation, as all amputated males had reduced mating success relative to controls (Table 5). Regenerated males, particularly those that regenerated a palp, tended to be more
successful than non-regenerators, and regenerative ability showed a strong trend towards predicting copulation among amputated males (Table 6). This was true despite the fact that regenerated palps were never used for copulating.

Very few male spiders who lost a leg at the fourth instar regenerated (Table 3). These results are consistent with previous investigations of appendage loss in this genus, which have reported poor regenerative ability in *Latrodectus* (*L. mactans*, Vollrath 1990; *L. variolus*, Randall 1981) but have focused only on leg loss. In contrast, here I demonstrate that more than one quarter of males that lose a pedipalp show evidence of regeneration by adulthood (Table 3). This may underestimate regenerative ability as some males attempting regeneration may have died before adulthood, or failed to regenerate a detectable appendage by their final moult. In many taxa, regeneration of a lost body part by maturity is strongly dependent on the amount of time an organism has to regrow the lost appendage (Maginnis 2006b, Wrinn & Uetz 2007). The data here are consistent with this conclusion since regenerators were almost exclusively those males that were small as juveniles and likely to have had a higher number of instars regardless of amputation (see controls Fig 5). Thus, the prevalence of regeneration attempts is likely affected by the instar at which the appendage was lost. Nevertheless, these results show clearly that regeneration of lost appendages is possible in *Latrodectus*, but the likelihood of this occurring depends on the function of the structure. Sexual organs are directly implicated in an organism’s fitness and selection for regrowth of these structures should be stronger than for locomotory appendages. There are currently no data on the incidence of palp loss in the field in any spider species but one (*Pardosa milvina*, Lynam et al. 2006), and no indication that regeneration of this organ has ever been documented (but it is possible in a pholcid spider, A. Peretti pers. comm.;
see Rovner 1967 for mention of palpal stumps in a wolf spider). If regeneration of palps is common though, it may be challenging to assess the prevalence of palp loss in the field.

Males who attempted to regenerate also grew larger by maturity, both in relative and absolute terms, compared to non-regenerating and control males (Fig 3B). This finding contradicts the prediction that stressors during development should result in smaller body size at adulthood (e.g. De Block & Stoks 2003, De Block et al. 2008, Stevens et al. 1999, Stevens et al. 2000, Reim et al. 2006, Teuschl et al. 2007, Mordue 1977, Wrinn & Uetz 2007). Theoretical models of optimal resource allocation suggest that increased allocation to sexual ornaments should occur at the expense of body size if the ornaments contribute equally or more to individual fitness (e.g. Lindstrom et al. 2005, Kodric-Brown et al. 2006). Although pedipalps are not ornaments, they do significantly affect fitness regardless of body size, and diverting resources to regenerate a palp ought to have reduced the energy available for somatic growth. It is noteworthy that relatively small juvenile males comprised the majority of regenerators, yet regenerating males grew to be largest among adults. Two hypotheses may explain why this pattern was observed. One is that mortality may have eliminated the males that allocated resources to regeneration at the expense of somatic growth, such that large males that would have regenerated died as a result of impaired somatic function. If this is the case, then males that survived and regenerated may be a self-selected group of higher quality individuals that were more adept at resource allocation and less affected by this tradeoff between regenerative and somatic growth than the population average. Such a group might be expected to do well on other metrics of fitness, which could explain why regenerating males had higher mating success than non-regenerators (Table 5). Unfortunately, morphometric data on spiderlings that died after
being assigned to a treatment (n = 21) is unavoidably limited, so it is difficult to assess support for this explanation.

Another hypothesis for the observed relationship between size and regeneration is that preferential resource allocation toward palp regeneration only occurs near maturity and may not compromise somatic growth throughout development. Most of the sexually dimorphic growth and enlargement of the pedipalps of male \textit{Latrodectus} spiders occurs during the last two instars (Mahmoudi et al. 2008, Kaston 1970). If resources are typically committed to palp growth as sexual maturity approaches, then somatic growth may not be reduced significantly even when one palp is compromised at an early instar. If this is the case, males who regenerated may be those that had sufficient time and resources to devote to all growth, including palp growth, at the final instar. Support for this hypothesis comes from two related facts: (1) the longer development time and increased instar numbers of control males that were relatively small as juveniles (Fig 5) and (2) the increased probability of regeneration among smaller juveniles in the palp amputated treatment (Fig 2). The relationship between maturation time and juvenile size suggests that small males in general may be following a decision rule to delay maturity and accumulate resources, and regeneration by these males may simply be a beneficial side-effect of following this decision rule. Males that are already following this developmental trajectory may then be more likely to regenerate if they accumulate sufficient resources to do so without compromising other traits, and may augment their growth beyond their expected adult size if additional resources are available. Thus regeneration could be a marker of a subset of small juvenile males that are able to bear the cost of palp loss due to their pre-existing growth strategy, and due to the strong positive allometric relationship between juvenile male palps and juvenile body size. The latter implies that the cost of developing the palp is proportionately lower for smaller juvenile males,
but whether this relationship is maintained throughout development is unknown. The potential to regenerate may thus be part of a more global life history strategy, which includes delayed maturation and longer resource accumulation, but may also depend on initial condition or size.

Size-dependent costs of palp regeneration may be an important feature of this life history pattern. In wolf spiders that drum with their abdomen during female courtship, small males have lower CO₂ use per unit of mass and therefore pay a lower cost for drumming than large males (Kotiaho et al. 1998). If developmental costs are similarly size-dependent in black widow males, then varying resource levels should temper both growth and regeneration effects. The present study did not manipulate diet levels and a relatively rich diet was chosen to maximize the likelihood of revealing variation in resource allocation (van Noordwijk & de Jong 1986) as well as to minimize mortality (Uhl et al. 2004). Despite the consistency of diet across individuals, there are two reasons why diet differences between males could have produced some variance in resource acquisition across treatments. One is that spiders are fed live prey and it is the male’s ability to capture and immobilize his prey that determines the amount of food obtained. The other reason is that diet was tailored to each individual’s developmental stage, but not to their size, allowing a small male to get proportionally more food given his mass at a specific instar compared to a large male. In addition, the increased number of instars in small males (Fig 5) and regenerating spiders (Fig 4) meant that these males were fed over a longer period and could accumulate more resources before maturity. One way to examine the effect of variation in the total duration and amount of feeding would be to include total development time of each male as a covariate in analyses of body size as a function of regeneration status. This analysis is not possible here because of limited sample sizes.
Another way to assess the effect of variation in resource intake would be to replicate this experiment under a more limited diet. Although this could increase mortality, it would provide a good test of whether the likelihood of regenerating is resource-dependent and whether somatic growth can be sacrificed for palp growth across all size classes of males. I completed a pilot study of this type, and results are described in the Appendix. Pilot data show that diet differences between males are not a major factor driving regeneration attempts (Appendix table A1), suggesting variation in food intake is unlikely to explain the results found here. Mortality in this additional study was high, however, and it was unclear whether regenerating males tended to be those that were smaller as juveniles (e.g. Fig. 2). However, the difference in developmental trajectory (instar number) as a function of juvenile size was replicated for control males in the pilot study (Fig. A1). Unlike this study though, in the pilot data development time was unrelated to regeneration and instead showed a strong association with diet (Fig A3). Low diet males did not alter the number of instars prior to maturity (Fig. A1), but their post-amputation development time was prolonged relative to high diet males (Appendix Fig. A2). These data suggest that resource availability in itself is not what allows males to regenerate a palp, and neither are changes in development time linked to growth decisions that are driven by diet. Instead, these data support the conclusion that, for a given diet level, a subset of males is able to apportion resources throughout development in a way that allows both regeneration and sufficient energy for somatic growth.

The difference in regeneration rates between palps and legs, however, cannot be explained by resource use patterns governed solely by diet or growth strategy. The lower regeneration rates for legs could mean that legs are more expensive to regrow, leg loss impairs resource acquisition, or legs have a lower allocation priority. Only two spiders regenerated a leg
and in both cases it was the third and smallest leg (Table 3), suggesting that the energetic cost of regeneration could be preventing leg-amputated males from attempting to regrow the limb. However, the complexity of the enlarged pedipalp relative to the leg suggests this may not be the best explanation for the difference in regeneration we found in our study. Another possibility is that missing a leg impairs prey capture (Vollrath 1987). Wrinn & Uetz (2007) found that field-caught wolf spiders with a missing or a regenerated leg had lower body condition, mass, and size, which they hypothesized to be due to males’ reduced foraging ability and therefore fewer resources during development. If this were the case for black widows, leg loss could have prevented males from acquiring equivalent amounts of energy as palp-amputated males. However, black widows primarily use their hind (fourth) pair of legs to wrap and subdue prey (Modanu pers. obs.), making it unlikely that feeding was adversely affected. A more likely explanation for our results is that investing in leg growth may not be a cost-effective strategy if an additional leg does not significantly increase fitness. Because very few spiders regrew their leg, it is unclear whether possessing all eight legs would benefit male lifetime reproductive success, and the literature on the effects of leg loss is equivocal (see Table 1). One reason for this is that broad comparisons of leg loss may not be appropriate across different spider systems because the function of the appendages vary. Loss of a leg bearing sexual ornaments (e.g. Hebets & Uetz 2000), used for courtship (e.g. Taylor et al. 2006), threat displays (e.g. Wrinn & Uetz 2007), or for subduing prey (e.g. Wrinn & Uetz 2008) may not result in the same fitness costs as loss of a different leg used for web-building (e.g. Vollrath 1987) or mobility (e.g. Lutzy & Morse 2008). In addition, the costs of losing a prey-capturing foreleg for a hunting spider (e.g. wolf spiders, Wrinn & Uetz 2008) may be substantial compared to the same leg being lost in a sit-and-wait predator (e.g. female widow spiders).While it is not possible to generalize to other spider
species, male black widows are clearly able to move, capture prey and court even while missing one or more legs (this study, Andrade pers. comm.). Thus the rarity of leg regeneration in *Latrodectus* reported here and in the literature (Vollrath 1990, Randall 1981) seems more likely to represent an allocation decision rather than a developmental constraint, particularly when contrasted with the successful regeneration of the pedipalp.

Although palp regeneration may lead to fitness benefits, increased development time may have significant costs in terms of both natural and sexual selection pressures, and regenerating appears to be associated with both. Delaying development can be costly because it increases predation risk and decreases the likelihood of survival to sexual maturity (Uhl et al. 2004, Engqvist 2007, Abrams et al. 1996, Stearns & Koella 1986, Stearns 1989). Developmental delays can also affect mate availability and increase the risk of sperm competition. Males that mature later in the season are less likely to encounter virgins (e.g. Anava & Lubin 1993) and these males may have decreased success in the scramble competition to reach available females (Kasumovic & Andrade 2006) which is typical for these size dimorphic spiders (Vollrath and Parker 1992). Kasumovic & Andrade (2008) found that even a one day advantage in development time can result in profound differences in mating success for male *L. hasselti* seeking virgin females, particularly at high population densities. This is a considerably shorter interval than the 20-30 day developmental delay that regenerating males experienced in the current study. However, this significant delay may also mean that regenerating males compete in a different context than rapidly-maturing males. While rapid non-regenerators compete for virgins, regenerators may mature later in the season once mated females are sperm depleted. These larger males may then be able to gain significant success by pairing with previously mated females. For example, female *L. hasselti* that cease sex pheromone production after their first mating readvertise
receptivity months later (Perampaladas et al. 2008), and the paternity of males that copulate at this point may approach 75% (Stoltz & Andrade, in prep). In addition, longer maturation time may confer an added benefit if increased size at maturity provides males with a competitive advantage over rivals (Stoltz et al. 2008, 2009, Kasumovic & Andrade 2008) or improves male longevity and condition (Kasumovic et al. 2009a) to increase endurance during risky mate search for females (Kasumovic et al. 2007, Andrade 2003).

One interesting alternative to these adaptive scenarios is that regeneration may be a maladaptive developmental trap that is a feature of the life history trajectory of particular males. This may be the case if development of the mature palp occurs during some window in the last instar, and males that reach this window with sufficient resources inevitably undergo the developmental processes that lead to palp production. Thus, palps may be regenerated even though they are not used in mating, and despite the fact that regeneration incurs a possibly costly increase in development time.

The evolutionary advantage of regenerating palps is unclear given that none of the experimental males used their regenerated palp to mate. Based on sperm use patterns in females, this means that regenerated males would achieve a maximum of 50% paternity under sperm competition (Snow & Andrade 2005, Stoltz & Andrade in prep). Although this is clearly better than not mating at all, double insertions are the norm in black widows (MacLeod et al. in prep) and males would benefit greatly from using the regenerated palp. Whether this mating pattern occurred because the palp was not functional or because the female did not allow a second copulation is unclear. However, behavioural data from the mating trials hints at some of the selective pressures that might affect regeneration. Males that regenerated had a slightly, though not significantly, higher mating success than those that did not regenerate, and successful
copulation was best predicted by the regeneration status of males (Table 6). Thus, females may discriminate against impaired males, or males may perform courtship differently with two palps. Consistent with the female choice hypothesis, all eight cases of pre-copulatory cannibalism (which is otherwise rare in this species) occurred in amputated males (Fig 6). Unfortunately, the rarity of this event precludes us from determining whether regeneration played a role in preventing cannibalism. However, this study suggests that regenerating may allow males to feign functionality. This mirrors Maginnis’s (2006b) notion that the strategy of regenerating vs. not regenerating is analogous to the alternative mating tactics that males employ when expressing an unfavorable phenotype – i.e. sneaky mating to make the best of a bad job. Alternative mating tactics, especially for males in poor condition, often work to reduce female assessment (Shuster & Wade 2003). If regenerating allows some female-mediated improvement of mating success relative to non-regenerating (Table 5), then it may pay amputated males to invest in regeneration even if the new palp is not used to transfer sperm.

The second hypothesis that males who develop after an injury perform differently during courtship has some support from the mating trials with population controls (Table 5). Mating success of males who lost a palp as adults was equivalent to that of controls (~30%) and twice as high as all amputated males. This suggests that females may not be selecting against missing appendages per se, but against the courtship ability of males that have been subjected to the developmental effects of amputation. Although courtship was not quantified in the present study, experimental males were observed to court females during most trials and fertilization success was high in all males that achieved mating, indicating that amputated males were at least functionally capable of achieving copulation. However, courtship is an energetically costly activity in Latrodectus (Stoltz unpub.) and it is possible that the body condition of experimental
males was reduced in a manner that affected the quality of courtship provided to females (i.e. a performance cost, Maginnis 2006b). If this is the case, then the fitness implications of injury, whether structurally remedied over time or not, are profound. This implies that sexual selection for regenerative ability may not be sufficient to counter any costs of altered resource allocation. It also suggests that, while amputation and regeneration may not lead to observable costs in adult body size (this study), examinations of body condition, energetic reserves, or other measures of adult performance may provide essential information on the underlying life history trade-offs involved in this developmental tactic.

In conclusion, individuals that lost a palp were more likely to regenerate it than those who lost a leg, but this did not result in significant fitness gains nor did it lead to reduced somatic growth as predicted. On the contrary, the expectation that selection should lead to this sexual organ being prioritized during development was not met because regenerated palps were not used in mating and palp-less males from the general population achieved equal mating success as unmanipulated controls. Instead, it seems likely that regeneration may have occurred due to the slower growth trajectory of smaller individuals and not as a strategy to mitigate fitness consequences of appendage loss. Male regeneration attempts along with increased size and delayed maturation effects demonstrate that multiple life history strategies can exist to achieve a functional phenotype and to optimize resource use in unfavorable circumstances (Kotiaho et al. 1998, Stearns 1992). This highlights the importance of studying alternative strategies in the context of life history because patterns may be complicated or obscured by pre-existing variation in life history traits (Blanckenhorn 1998). The relationship between size at maturity and development time has received much attention in the literature and the current study reinforces the notion that integration of multiple traits in an organism’s developmental trajectory can lead to
Literature Cited


Behavioral Ecology 14(4):531-538


MacLeod, E. C., Modanu, M. & M. C. B. Andrade. Multiple mating in male Latrodectus hesperus despite genital damage. In prep.


Table 1. Summary of studies examining functional consequences of leg autotomy in spiders.

Asterisk (*) refers to positions of legs lost numbered from anterior to posterior pair, where ‘any’
means the study examined removal or loss of any one of eight spider legs.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Species</th>
<th>Leg lost*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower running speed</td>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>) and wolf spider (<em>Varacosa terricola</em>)</td>
<td>Any</td>
<td>Amaya et al. 2001</td>
</tr>
<tr>
<td></td>
<td>Wolf spider (<em>Pirata sedentarius</em>)</td>
<td>Any</td>
<td>Apontes &amp; Brown 2005</td>
</tr>
<tr>
<td>Lower prey capture</td>
<td>Grass spider (<em>Agelenopsis aperta</em>)</td>
<td>Any</td>
<td>Hammerstein &amp; Riechert 1988</td>
</tr>
<tr>
<td></td>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>)</td>
<td>First</td>
<td>Wrinn &amp; Uetz 2008</td>
</tr>
<tr>
<td></td>
<td>Grass spider (<em>Agelenopsis aperta</em>)</td>
<td>Any</td>
<td>Riechert 1988</td>
</tr>
<tr>
<td>Lower competitive ability</td>
<td>New Zealand jumping spider (<em>Trite planiceps</em>)</td>
<td>First</td>
<td>Taylor &amp; Jackson 2003</td>
</tr>
<tr>
<td></td>
<td>Grass spider (<em>Agelenopsis aperta</em>)</td>
<td>Any</td>
<td>Riechert 1988</td>
</tr>
<tr>
<td></td>
<td>Crab spider (<em>Misumenoides formosipes</em>)</td>
<td>First</td>
<td>Dodson &amp; Beck 1993</td>
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<tr>
<td>Lower body condition</td>
<td>Crab spider (<em>Misumena vatia</em>)</td>
<td>First</td>
<td>Lutzy &amp; Morse 2008</td>
</tr>
<tr>
<td></td>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>)</td>
<td>First</td>
<td>Wrinn &amp; Uetz 2007</td>
</tr>
<tr>
<td>Lower mating success</td>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>)</td>
<td>First</td>
<td>Uetz et al. 1996</td>
</tr>
<tr>
<td>Changes in courtship</td>
<td>Species</td>
<td>Leg lost*</td>
<td>Reference</td>
</tr>
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<td>---------</td>
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<td>-----------</td>
</tr>
<tr>
<td>First or second</td>
<td>Wolf spider (<em>Pardosa milvina</em>)</td>
<td>Brautigam &amp; Persons 2003</td>
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<th>Higher female cannibalism</th>
<th>Species</th>
<th>Leg lost*</th>
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<td>Wolf spider (<em>Pardosa milvina</em>)</td>
<td>Brautigam &amp; Persons 2003</td>
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<table>
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<th>Reference</th>
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<tr>
<td>Running ability</td>
<td>Crab spider (<em>Misumena vatia</em>)</td>
<td>First</td>
<td>Lutzy &amp; Morse 2008</td>
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<td>Wolf spider (<em>Pardosa milvina</em>)</td>
<td>First</td>
<td>Brueseke et al. 2001</td>
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<td>Foraging ability</td>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>) and wolf spider (<em>Varacosa terricola</em>)</td>
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<td>Amaya et al. 2001</td>
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<td>Wolf spider (<em>Pardosa milvina</em>)</td>
<td>First</td>
<td>Brueseke et al. 2001</td>
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<tr>
<td>Brush-legged wolf spider (<em>Schizocosa ocreata</em>)</td>
<td>First</td>
<td>Wrinn &amp; Uetz 2008</td>
<td></td>
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<tr>
<td>Jumping spider (<em>Phidippus princeps</em>)</td>
<td>Second, third or fourth</td>
<td>Stankowich 2009</td>
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<tr>
<td>Competitive ability</td>
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<td>First</td>
<td>Dodson &amp; Schwaab 2001</td>
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<tr>
<td>Pholcid spider (<em>Holocnemus pluchei</em>)</td>
<td>First</td>
<td>Johnson &amp; Jakob 1999</td>
<td></td>
</tr>
<tr>
<td>Harvestmen (<em>Leiobunum nigripes &amp; Leiobunum vittatum</em>)</td>
<td>Any</td>
<td>Guffey 1998</td>
<td></td>
</tr>
<tr>
<td>Juvenile growth rate</td>
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<td>First</td>
<td>Johnson &amp; Jakob 1999</td>
</tr>
<tr>
<td>Mating success</td>
<td>Golden orb spider (<em>Nephila fenestrata</em>)</td>
<td>First</td>
<td>Fromhage &amp; Schneider 2006</td>
</tr>
</tbody>
</table>
Brush-legged wolf spider 

(*Schizocosa ocreata*)

First Taylor et al. 2008
Table 2. Results of separate Principal Component Analyses on juvenile and adult size measurements. The first axis of each PCA captured most of the variation in both instances and is used as a composite measure of juvenile or adult size in subsequent analyses.

<table>
<thead>
<tr>
<th></th>
<th>Adult Loadings</th>
<th>Juvenile Loadings</th>
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<tbody>
<tr>
<td>First leg</td>
<td>0.499</td>
<td>0.503</td>
</tr>
<tr>
<td>Second leg</td>
<td>0.504</td>
<td>0.505</td>
</tr>
<tr>
<td>Fourth leg</td>
<td>0.504</td>
<td>0.504</td>
</tr>
<tr>
<td>Thorax</td>
<td>0.493</td>
<td>0.488</td>
</tr>
<tr>
<td>PC1 % variance</td>
<td>97.6%</td>
<td>96.6%</td>
</tr>
</tbody>
</table>
Table 3. Number of male spiders showing partial or complete regeneration of Leg 1, Leg 3, or a pedipalp (P-Amp) after amputation at the 4th instar, where palp-amputated males were raised in three cohorts.

<table>
<thead>
<tr>
<th>Group</th>
<th># regenerating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg1-Amp</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Leg3-Amp</td>
<td>2 (10.5%)</td>
</tr>
<tr>
<td>Total P-Amp</td>
<td>14 (29.2%)</td>
</tr>
</tbody>
</table>

P-Amp by cohort:

<table>
<thead>
<tr>
<th>Cohort</th>
<th># regenerating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td>7 (42.8%)</td>
</tr>
<tr>
<td>Cohort 2</td>
<td>3 (14.3%)</td>
</tr>
<tr>
<td>Cohort 3</td>
<td>4 (36.4%)</td>
</tr>
</tbody>
</table>
Table 4. Static allometry of legs at the juvenile and adult stage, showing 95% confidence intervals for Reduced Major Axis (RMA) slopes of log (leg length) relative to log (thorax width) as a function of regeneration status (control C; regenerator R; non-regenerator NR).

<table>
<thead>
<tr>
<th>Leg</th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
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<tr>
<td></td>
<td>0.92-1.31 C</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>1.28-1.50</td>
<td>0.85-1.25 R</td>
</tr>
<tr>
<td></td>
<td>0.92-1.13 NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.98-1.25 C</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>1.24-1.44</td>
<td>0.94-1.29 R</td>
</tr>
<tr>
<td></td>
<td>1.09-1.26 NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.80-1.56 C</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>0.94-1.27</td>
<td>0.96-1.45 R</td>
</tr>
<tr>
<td></td>
<td>1.09-1.27 NR</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Mating success of males in their first mating opportunity and summed mating success across both opportunities as a function of (A) treatment group and, for experimental males only, (B) regeneration status.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number mated (%)</th>
<th>Number mated (%)</th>
<th>Number mated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First mating</td>
<td>Summed mating</td>
<td></td>
</tr>
<tr>
<td></td>
<td>success</td>
<td>success</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg1-Amp</td>
<td>2 (16.7%)</td>
<td>4 (36.4%)</td>
<td></td>
</tr>
<tr>
<td>Leg3-Amp</td>
<td>3 (15.8%)</td>
<td>4 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>P-Amp</td>
<td>4 (11.4%)</td>
<td>5 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5 (31.3%)</td>
<td>10 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Population controls</td>
<td>6 (30%)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regenerating</td>
<td>3 (25%)</td>
<td>4 (36.4%)</td>
<td></td>
</tr>
<tr>
<td>Non regenerating</td>
<td>5 (9.3%)</td>
<td>9 (22.5%)</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Logistic regression analyzing predictors of summed mating success for experimental males.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.469</td>
<td>0.6687</td>
<td>-0.701</td>
<td>0.4831</td>
</tr>
<tr>
<td>Adult size</td>
<td>0.3129</td>
<td>0.2224</td>
<td>1.407</td>
<td>0.1595</td>
</tr>
<tr>
<td>Condition</td>
<td>-6.7198</td>
<td>7.2266</td>
<td>-0.930</td>
<td>0.3524</td>
</tr>
<tr>
<td>Group</td>
<td>-0.2974</td>
<td>0.1698</td>
<td>-1.752</td>
<td>0.0798</td>
</tr>
<tr>
<td>Regeneration</td>
<td>1.9618</td>
<td>1.0247</td>
<td>1.914</td>
<td>0.0556</td>
</tr>
</tbody>
</table>
Figures

Figure 1. Image of anterior portion of cephalothorax and pedipalps of adult males that attempted regeneration of a palp amputated at the 4th instar. Regenerated palps ranged from apparently functional copulatory organs (A) to non-functional, reduced appendages (B).
Figure 2. Number and size of juvenile males reaching adulthood and regenerating (grey) or not regenerating (white) a pedipalp or leg following amputation during the 4th instar (n = 84).
Figure 3. Relationship between juvenile and adult size for all experimental males (controls not shown) as a function of the appendage amputated and whether or not regeneration occurred. Lines in A and C indicate line of best fit for control males (RMA regression $y = 0.722633x - 0.2109668$, data not shown for clarity) and are used as a reference for calculation of residuals shown in B and D. (A) Data separated by regeneration outcomes. Open circles = non-regenerated, filled circles = regenerated; (B) Mean residual size ± SE for regenerated and non-regenerated males, $W = 847$, $p = 0.0006$; (C) Data separated by amputation treatment. Open circles = Leg1-Amp, crossed squares = Leg3-Amp, filled triangles = P-Amp; (D) Mean residual size ± SE for amputated males in each treatment, $F_{2,81}=5.06$, $p = 0.0085$. A Tukey post hoc test reveals a significant difference in residuals between one pair (L1-Amp and P-amp $p = 0.015$; L1-Amp and L3-Amp $p = 0.77$; L3-Amp and P-Amp $p = 0.09$).
Figure 4. Frequency histogram of the number of instars prior to maturity for control males and experimental males as a function of whether or not they regenerated an appendage.
Figure 5. Negative relationship between juvenile size and number of instars prior to adulthood for control males ($r = -0.78$, $t = -6.4$, $p < 0.0001$).
Figure 6. Incidence of pre-copulatory cannibalism during mating trials in each group.
5 Appendix

5.1 Summary

In a separate experiment, I repeated the palp amputation manipulation on male and female spiderlings that were fed on a high or low diet (see below). The goals of this study were to examine whether (1) variation in food intake (resource acquisition) might explain the variation in regeneration or growth trajectories (number of instars) seen in the main study and (2) whether palp regeneration varies with sex. For females, palps are feeding appendages (naturally selected) with no sexual function. Results show that (1) regeneration attempts cannot be explained by variation in diet (in either sex, table A1), and growth trajectories are best explained by juvenile size rather than diet; and (2) females are more likely than males to regenerate a palp.

5.2 Methods

Adult female spiders were collected from Hastings Natural Reserve System (N 36° 26' 35.67", W 121° 39' 55.86, University of California Natural Reserve System) in May 2008 and eggs from seven females were individually reared in a 4 x 4 x 6cm clear plastic cage (Amac Plastic) to avoid juvenile cannibalism. Two weeks after hatching, spiderlings were fed *Drosophila hydeii* flies under one of two feeding regimes: a) one fly per week until the fourth instar, then (current instar # - 2) once a week until adulthood (low diet); or b) two flies per week until the fourth instar, then (current instar # - 1) twice a week until adulthood (high diet). The high diet treatment was equivalent to the feeding regime of spiders in the main study (see Methods p.9). At the fourth instar, spiderlings were sexed (Mahmoudi et al 2008) and both males
and females were randomly assigned to a palp-amputation group (P-Amp) or control. Each spiderling was briefly anesthetized with CO₂ and spiderlings in the P-Amp group had one palp amputated proximal to the patella using a pair of iridectomy microdissection scissors. Control juveniles were also anesthetized and similarly handled but no amputation was performed. Male spiders were maintained on a high or low diet until adulthood. Females were maintained on these same diets until they reached the 8th instar, a comparable stage to adult males in terms of size and development time (most *L. hesperus* males reach adulthood before or at the 8th instar Modanu pers. obs., while females are still juveniles). After reaching the 8th instar, females were photographed and removed from the study. Moult dates and palp regeneration attempts were monitored in all spiderlings, and morphometric measurements were performed as in the main study (see Methods p.10).

### 5.3 Results

Here I summarize results from this pilot study that are relevant to interpretation of results of the main study. Mortality during the course of the experiment reduced sample sizes in some groups and made statistical tests of detailed predictions impractical due to low power. Where statistical analyses are not practical, written descriptions of apparent patterns are provided although these are insufficient to draw conclusions.

1. Treatment affected regeneration (Overall G test with Williams correction: G = 11.87, chi-squared df = 3, p = 0.0078, Table A1). However, while regeneration was more likely for females than males within each diet treatment (high diet: Fisher’s exact test: p = 0.054; low diet: 0.0162), diet itself had no significant effect on regeneration within each sex (males: Fisher’s exact test: p = 0.533; females: Fisher’s exact test: p = 0.675).
2. To examine effects of diet on instar number and duration, I examined only males (which were allowed to reach sexual maturity). As in the main study, smaller control males went through more instars prior to maturity than larger controls, in both the high and low diet groups (Fig. A1). This confirms that growth trajectory (number of instars) is juvenile size-dependent, and also shows instar number is not diet-dependent.

3. Development time depended mainly on diet (Fig. A2) with little apparent effect of regeneration (Fig. A2).
Table A1. Regeneration attempts in males and females for low and high diet treatments.

<table>
<thead>
<tr>
<th></th>
<th>Regeneration attempts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High diet</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>8/21 (38.1%)</td>
</tr>
<tr>
<td>Females</td>
<td>8/10 (80%)</td>
</tr>
<tr>
<td><strong>Low diet</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>7/26 (26.9%)</td>
</tr>
<tr>
<td>Females</td>
<td>13/20 (65%)</td>
</tr>
</tbody>
</table>
Figure A1. Relationship between number of instars prior to maturity and a measure of juvenile size for males on high (filled circles) and low diets (open circles).
Figure A2. Development time of males as a function of diet and regeneration status. Diet, but not regeneration attempts, had a strong effect on development time.