

Limb autotomy and regeneration affect vibratory/seismic courtship signaling and female receptivity in wolf spiders

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Abstract. In many spiders, limb autotomy (self-amputation) is a common anti-predator behavior. While many species are able to regenerate lost limbs without apparent fitness costs, there are demonstrable costs in others. Previous studies have shown males of the brush-legged wolf spider *Schizocosa ocreata* (Hentz, 1844) incur reduced mating success after autotomy and/or regeneration of their decorated forelimbs, which affects visual courtship displays. However, because courtship of male *S. ocreata* is multimodal and contains vibratory/seismic signals, communication in this channel might also be affected. We recorded female receptivity to isolated male vibratory/seismic courtship signals from: males with intact forelimbs (control), males with a regenerated forelimb, and males missing a forelimb. Females were more receptive to isolated vibratory/seismic signals of intact males over males missing a foreleg, but not males that regenerated a foreleg. Although initial size and body condition did not differ among treatments, regeneration of a limb had a significant negative impact on growth increment from penultimate instar to adult, suggesting a possible physiological cost of regeneration. To investigate the impact of autotomy and regeneration on vibratory/seismic signals, we used laser Doppler vibrometry (LDV). Analysis of overall signal amplitude across treatments revealed significantly reduced amplitude for males with regenerated limbs, despite these males having receptivity responses statistically similar to control males. Analyses of component elements of vibratory/seismic signals showed three (of four) had significantly reduced amplitude in males regenerating limbs. These results demonstrate a potential fitness impact of autotomy and regeneration on the vibratory/seismic component of male courtship signals.

Keywords: Vibration, vibratory/seismic communication, mate choice, Lycosidae, appendotomy

Autotomy, the voluntary detachment of a body part, is used to avoid predation or escape entrapment (Eisner & Camazine 1983; Fleming et al. 2007). Autotomy is seen in both vertebrates (Arnold 1984; Shargal et al. 1999; Bernardo & Agosta 2005; Clause & Capaldi 2006) and invertebrates (Amaya et al. 2001; Brautigam & Persons 2003; Diaz-Guisado et al. 2006; Fleming et al. 2007) and is thought to have evolved independently several times (Bely & Nyberg 2010). Despite the obvious anti-predation benefit of limb autotomy for spiders, consequences of this response in terms of other potential fitness costs are not always clear (Guffey 1999; Johnson & Jakob 1999; Amaya et al. 2001; Brueske et al. 2001; Lutzy & Morse 2008; Pasquet et al. 2011; Brown & Formanowicz 2012; Steffenson et al. 2014; Gerald et al. 2017; Ramirez et al. 2017; Brown et al. 2018).

Spiders are excellent models with which to study autotomy as they not only use their limbs for locomotion, but for sensory perception as well as communication (Seyfarth & Barth 1972). Communication is essential in mating, and in the case of spiders, efficacy in conveying messages can often mean the difference between successful mating and cannibalism. For spiders that use their limbs for communication (e.g., between conspecific males and females during mating), limb autotomy may affect behaviors subject to sexual selection (Brautigam & Persons 2003; Taylor et al. 2006, 2008).

The brush-legged wolf spider *Schizocosa ocreata* (Hentz, 1844) (World Spider Catalogue 2018) is a well-studied model used to investigate questions about communication and female mate choice (Uetz 2000; Uetz & Roberts 2002; Uetz et al. 2016). Male *S. ocreata* court using multimodal signaling (i.e., visual and vibratory/seismic vibration signals - Uetz et al.

2009). Male forelimbs are used for visual courtship displays, and are decorated with conspicuous tufts of bristles that increase background contrast and detectability (Uetz et al. 2011). In addition, a substratum-coupled vibration component of courtship (vibratory/seismic signaling) is created by percussion (striking the legs and chelicerae on the substratum) and stridulation (produced by organs in the tarsal joint of the male pedipalps) (Rovner 1975; Gibson & Uetz 2008).

In *S. ocreata* wolf spiders, autotomy of legs is used as an anti-predation behavior that allows for escape, and on average 11%–19% of these spiders collected from the field are missing limbs (Uetz et al. 1996; Wrinn & Uetz 2007). Because of the importance of the decorated forelimbs in visual courtship signaling, injury from autotomy and subsequent regeneration of limbs (with reduced foreleg tufts) has been studied and shown to have a negative impact on mating success (Uetz et al. 1996; Uetz & Smith 1999; Taylor et al. 2006). However, because the courtship of male *S. ocreata* is multimodal, and also contains vibratory/seismic signals, questions arise whether communication in this channel might also be affected. Specifically, can females recognize males that are missing or regenerating their forelimbs based on vibratory/seismic signals alone, and does this affect receptivity? If so, how might vibratory/seismic signals be affected by loss and/or regeneration of forelimbs, which appear to play only a minor role in production of vibratory/seismic signals (Gibson & Uetz 2008)? In this study, we experimentally autotomized forelegs of male *S. ocreata* to examine the potential impact of limb loss and regeneration on isolated male vibratory/seismic signals and female responses to these signals.

METHODS

General methods: Spider collection and maintenance.—

Spiders used in the several experimental studies here were collected from the Cincinnati Nature Center Rowe Woods property in Milford, Ohio in the fall of 2012 and spring of 2013. Therefore, they were from the same generational cohort. All individuals were maintained under the same controlled laboratory conditions with a springtime light cycle (13/11 hrs L: D), controlled humidity (>55% R.H.), and controlled temperature (20–22° C). Spiders were housed in individual deli dish containers (11.8 cm diam., 7.4 cm ht.) with a moist dental wick to provide hydration. Spiders were fed 2–3 crickets (10-day old *Acheta domesticus* appropriately sized to spider size) twice per week and molts were checked and recorded daily until maturity. At the end of this study, spiders were humanely euthanized with CO₂ and freezing.

Experimental protocol: limb autotomy.—In each experiment, males were assigned randomly to one of the three treatment groups: (1) control, (2) missing forelimb, or (3) regenerated forelimb. Male spiders of the regenerated treatment group were encouraged to autotomize a forelimb five to six days after the penultimate molt (as in Taylor et al. 2006). Males were anesthetized with carbon dioxide and a pair of forceps was used to apply a mechanical stimulus to the femur of either the right or left forelimb (chosen randomly). The carbon dioxide was used only to allow for appropriate placement of forceps or sham stimulus (control); males were conscious when the autotomy occurred (i.e., limbs were autotomized and not amputated). Males from the treatment group that were missing a limb entirely were subjected to the same leg autotomy process 5–7 days after they reached maturity. Males were allowed to recover from the autotomy process for 2 days with food and water *ad libitum*.

Experiment 1: Effect of autotomy and regeneration on male condition and female receptivity.—At 10–13 days post maturity date, 66 female spiders were paired randomly with males of similar age from the three treatment groups above. A total of 26 control males, 20 regenerated males and 21 males missing one forelimb were paired with females and used in behavior trials (original sample sizes were 25–26 each, but some were lost due to mortality). Spiders were fed 2–3 crickets two days prior to trial and females were left overnight on parchment paper in order to deposit chemical cues to stimulate male courtship.

Male and female pairs were recorded in trial arenas (Fig 1.) consisting of two adjacent plastic boxes (13 cm L x 7 cm W x 6.5 cm H) in which spiders were isolated. Boxes were placed on a foam backed-poster board substratum, with the entire apparatus placed atop a large granite block with a Sorbothane® pad beneath (to reduce vibration from outside the arena). Parchment paper with female silk cues was used as the substratum atop the poster board but underneath the side-by-side bottomless plastic boxes with a cardboard visual barrier between them (as in Scheffer et al. 1996; Gibson & Uetz 2008). Thus, females could only perceive male vibratory signals. Males generally court once they come in contact with female silk and so females were allowed to lay down silk on the parchment paper the night prior to the trial. Trials were recorded from the moment the males began to court the female chemical cues (Uetz & Denterlein 1979; Stratton &

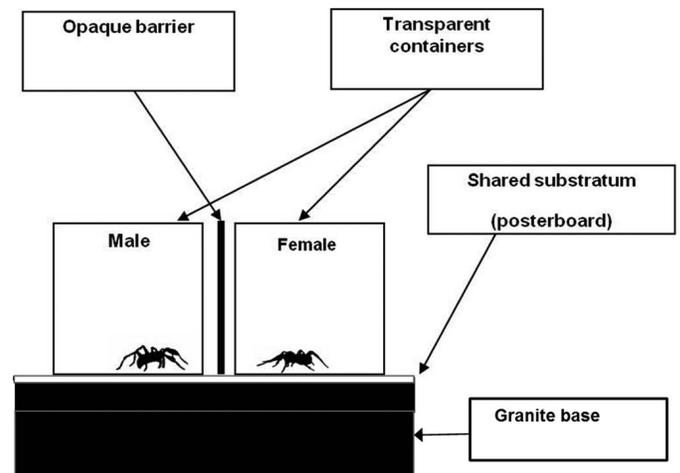


Figure 1.—Diagram of the apparatus used in live male-female courtship trials with isolated male vibratory/seismic courtship signals.

Uetz 1983; Uetz & Roberts 2002; Roberts & Uetz 2005). Trials lasted five minutes and were recorded on a Sony HDRXR260V High-Definition Handycam 8.9 MP Camcorder and scored post-trial for female *S. ocreata* receptive displays.

Female receptivity was scored based on three primary receptivity displays that have been associated with female mate choice and subsequent copulation in previous studies (Uetz & Denterlein 1979; Stratton & Uetz 1983; Uetz 2000; Uetz & Roberts 2002): (1) tandem leg extension (forelimbs of female straightened and extended in a deliberate manner resting on the substratum; (2) settle (lowering of cephalothorax to substrate); and (3) slow pivot (a 90°–360° turn in place). A sum of the number of these displays in the five-minute trial was used to produce a comprehensive receptivity score as used in previous studies (Uetz & Norton 2005; Gibson & Uetz 2008; Uetz et al. 2009; Stoffer & Uetz 2016a, b, 2017; Stoffer et al. 2016). In addition to the receptivity score, we measured association time (total time spent at the side of the arena with the male) and total time in receptive postures, which provide additional assessment of the degree of receptivity.

Body condition measurements of males with missing or regenerated legs: Mortality during the study resulted in uneven sample sizes among treatment groups (26 control, 21 autotomized and 19 regenerated). Consequently, a subset of 45 spiders used in experiment 1 was used to collect data on male condition. Individual measurements and weights were taken at 1–2 days after molting to the penultimate (pre-adult) life stage and again 1–2 days after molting to maturity ($n = 15$ each treatment). Spiders were not fed for 24 hours prior to either of these measurements. Spiders were weighed to the nearest milligram. Cephalothorax width (CW) and abdomen width (AW) of both penultimate and adult male spiders were measured (in millimeters) from digital images captured with ScopeImage 9.0®. All body measurements were determined from scaled digital images using ImageJ® software (NIH). Males were measured and weighed prior to autotomy at both stages (i.e., penultimate or maturity). A growth increment was generated for all three treatment groups by subtracting weight at penultimate stage from weight at maturity and dividing the sum by the weight at the penultimate stage.

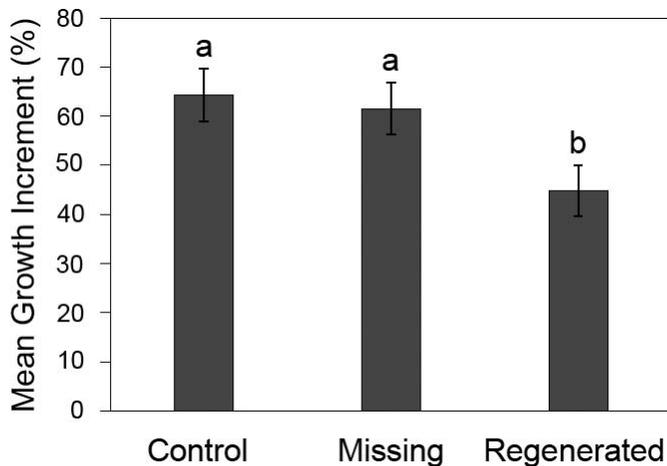


Figure 2.—Mean growth increment (% change in weight) of male *S. ocreata* from the three different treatment groups. Different letters indicate significant differences (One-way ANOVA and subsequent post-hoc Tukey tests). Error bars indicated +/- one standard error.

We used several methods to create and analyze estimates of male body condition: (1) a ratio index of AW/CW (as in Anderson 1974); (2) a residual body condition index calculated from the regression of weight (g) x CW (as in Jakob et al. 1996); and (3) ANCOVAs of AW and weight with CW as a covariate (Garcia-Berthou 2001; Green 2001). While body condition indices have been criticized, subsequent testing reveals an acceptable level of accuracy for both residual indices and ANCOVA approaches (Schulte-Hostedde et al. 2005).

Experiment 2: Effect of leg autotomy and regeneration on male vibratory/seismic signals.—To assess the effect of leg treatment on properties of male vibration signals, an additional set of males from the spring 2013 cohort was subjected to the same experimental treatments as before (n=13 control males, n=13 autotomized males, n=13 regenerated males) with courtship recorded for comparison of vibratory signals. In addition, male body condition measurements were taken as before.

Signal recording: To elicit male courtship in order to record male vibratory signals, females were placed inside a small plastic box floored with a filter paper substrate (diameter: 15 cm) for approximately 24 hours to deposit silk. The next day, males were placed inside a small plastic arena (diameter: 15 cm) on that silk-laden filter paper, and vibratory signals were recorded. Vibration recordings were made in a sound and vibration isolated room, using a Polytec®PDV-100 Laser Doppler Vibrometer (LDV) and a Dell Latitude computer with a Roland® Quad-Capture external sound card calibrated at 50% of maximum gain (at 1kHz). LDV recordings were made with a max velocity of 100 mm/sec, a sensitivity of 25 mm/sec/volt at a sampling rate of 24 kHz and a Fast Fourier Transform size of 2048, and subsequently analyzed using SpectraPlus® software (see below). A small piece of reflective Mylar® tape was adhered to the center of the bottom of the filter paper (to enhance laser reflection) and males were recorded from below for five minutes once courtship began. Trials were simultaneously recorded with the Sony

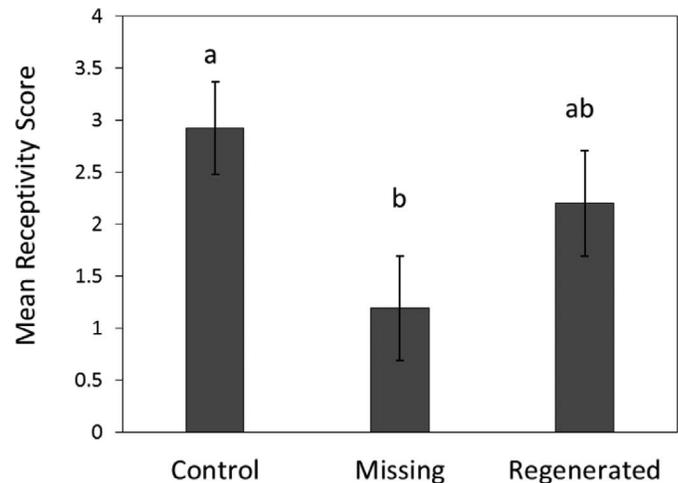


Figure 3.—Mean receptivity score (sum of displays) of female *S. ocreata* in response to males from the three different treatment groups. Different letters indicate significant differences (One-way ANOVA and subsequent post-hoc Tukey tests). Error bars indicated +/- one standard error.

HDRXR260V High-Definition Handycam 8.9 MP Camcorder (as in Experiment 1 above) and scored post-trial for male behaviors.

Signal Analysis: Male vibratory/seismic signals were analyzed using SpectraPlus® software (Pioneer Hill Software, LLC). A 60 second segment from each recording was analyzed. Segments were chosen at the onset of typical male courtship and from examination of oscillograms of vibration recordings, numbers per 60 sec of each of the following vibratory/seismic signal component elements of complex *S. ocreata* signals (as in Fig. 4, Hebets et al. 2013; see figure 5) were counted: (1) stridulation leading up to a percussive element; (2) percussion; (3) high velocity stridulation following percussion; and (4) low velocity stridulation independent of other elements. In addition, the number of two behaviors observed during vibratory/seismic signal production—cheliceral strikes and pulses of stridulation—was also counted for the same 60-second segment. Measures of signal amplitude (in mm/s velocity) were made for each occurrence of elements 1–4 above and analyzed for each of the segments recorded. Additionally, a 10-second segment within the original 60-second segment was chosen at random, and also analyzed for total amplitude level (all elements combined).

Given the complexity of vibratory/seismic signaling in this species (Hebets et al. 2013) we employed a commonly-used index of species diversity (originally information complexity) as a measure. Index calculations were based upon both the number of vibratory/seismic signal elements recorded and the relative proportion of total signal amplitude in each. The Shannon Diversity Index (Shannon 1948): $[H' = -\sum p_i \log_2 p_i]$, where p_i = proportion of total numbers or amplitude of element i ;] was calculated to measure information content of male vibratory/seismic signals. We compared SDI values between male treatments to determine if signal complexity differed between treatments.

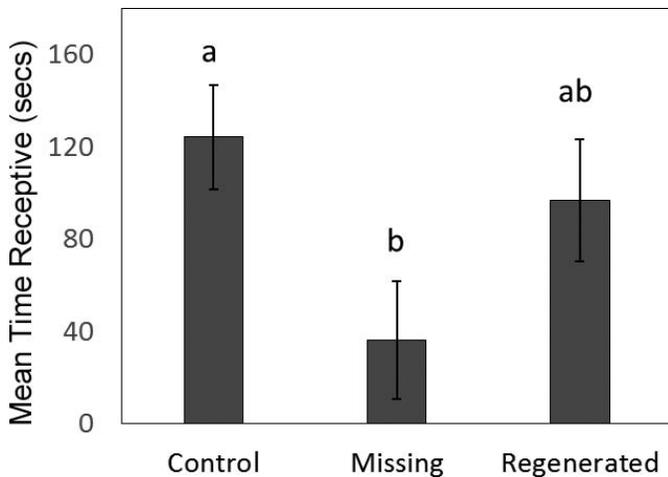


Figure 4.—Mean time spent in receptivity displays of female *S. ocreata* to males from the three different treatment groups. Different letters indicate significant differences (One-way ANOVA and subsequent post-hoc Tukey tests). Error bars indicated \pm one standard error.

RESULTS

Experiment 1: Effect of autotomy and regeneration on male condition and female receptivity.—Although there were no significant differences in any measures of size or condition prior to autotomy and regeneration, analyses of body size and several body condition indices at adulthood showed a different pattern. There were no significant differences among treatments in adult size measured as cephalothorax width (one-way ANOVA: $F_{2,43} = 1.075$; $P = 0.351$). However, all of the body

condition indices measured (Ratio Index [AW/CW], Residual BCI, ANCOVA of weight w/CW as covariate, and ANCOVA of AW w/CW as covariate) showed significant differences among treatments at adulthood (Table 1).

In addition, growth increment (percent change from penultimate to adult, scaled against penultimate values) varied significantly with treatment (One-way ANOVA – $F_{2,43} = 3.978$; $P = 0.0264$), as males of the regenerated treatment gained significantly less weight between penultimate and adult instars (Fig. 2). These differences might be explained by a lower weight gain for males regenerating limbs due to the loss of a leg, i.e., differences between the original intact leg and the regenerated one, since regenerated legs tend to be smaller than those in the other treatments (Uetz et al. 1996). However, since the measurements of spiders in the regeneration treatment were taken before autotomy and after molting to adulthood with a regenerated leg, differences attributable to leg size would likely be minor compared to the lower growth increment incurred as a cost of regeneration.

Female receptivity to males varied significantly with treatment (receptivity score -Kruskal-Wallis test: $X^2 = 6.895$; $df = 2$; $P = 0.032$). Receptivity scores were significantly lower in the missing limb treatments compared to control treatment males, while scores for those with regenerated limbs were intermediate (Fig. 3). Likewise, amount of time spent by females adjacent to males while in receptive postures (Fig. 4) varied similarly with male treatment (total receptive time - Kruskal-Wallis test $X^2 = 10.578$; $df = 2$; $P = 0.005$). Overall association time did not vary among treatments Kruskal-Wallis test – Association time: $X^2 = 1.328$; $df = 2$; $P = 0.515$).

Experiment 2: Effect of autotomy and regeneration on male vibratory/seismic signals.—Analysis of the number of separate component elements present within each male vibratory/

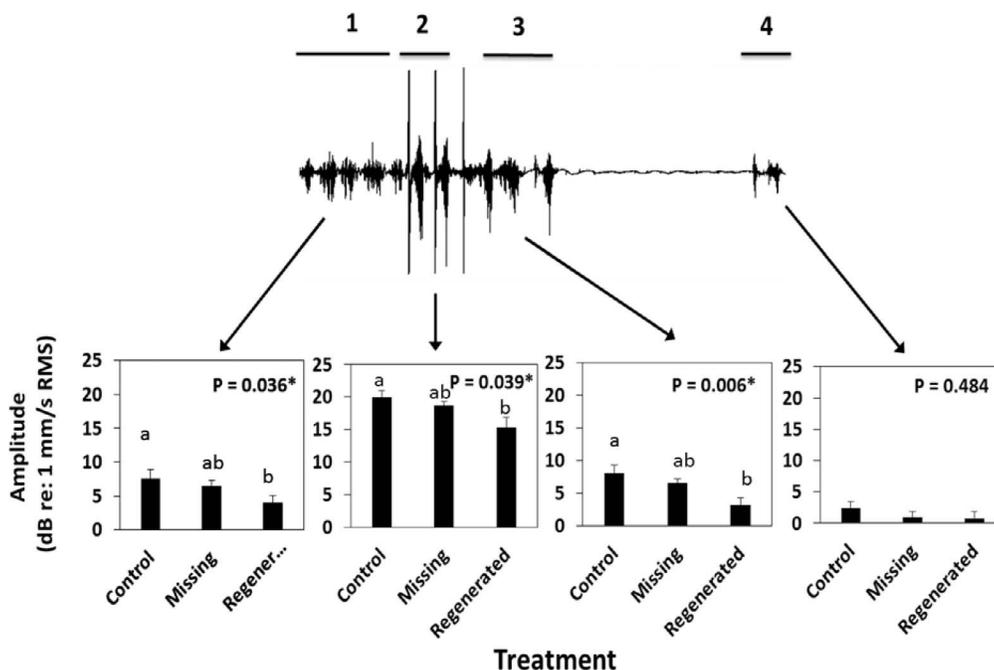


Figure 5.—Oscillogram of a male *S. ocreata* vibratory/seismic signal (from the control treatment), showing four component vibratory/seismic signal elements (based on Hebets et al. 2013). Figures show mean signal amplitude of male vibratory/seismic signals (in dB re: 1 mm/s RMS) of recordings from the three different male treatments. P values represent results of One-way ANOVA (see Table 1 and text for details).

Table 1.—ANOVA results for several body condition indices (see text for details). AW – abdomen width; CW – cephalothorax width; BCI – Body Condition Index.

Ratio Index: One-way ANOVA of AW/CW by Treatment					
Source	DF	F Ratio	P		
Treatment	2	3.6119	0.0407		
Error	27	0.14846336	0.005499		
C. Total	29	0.18818478			
Residual BCI: One-way ANOVA of Res wt x CW by Treatment					
Source	DF	Sum of Squares	Mean Square	F Ratio	P
Treatment	2	0.00060878	0.000304	5.4616	0.0102
Error	27	0.00150478	0.000056		
Total	29	0.00211356			
ANCOVA of Weight by Treatment (with CW co-variate)					
Source	DF	Sum of Squares	Mean Square	F Ratio	P
Model	5	0.00424916	0.000850	15.6003	<.0001
Error	24	0.00130741	0.000054		
Total	29	0.00555657			
Source	DF	Sum of Squares	F Ratio	P	
treatment	2	0.00067813	6.2242	0.0066	
CW	1	0.00227570	41.7750	<.0001	
treatment*CW	2	0.00018739	1.7199	0.2004	
ANCOVA of AW by Treatment (with CW co-variate)					
Source	DF	Sum of Squares	Mean Square	F Ratio	P
Model	5	4.1100610	0.822012	10.1781	<.0001
Error	24	1.9383075	0.080763		
Total	29	6.0483685			
Source	DF	Sum of Squares	F Ratio	P	
Treatment	2	0.6924840	4.2871	0.0256	
CW	1	2.5610597	31.7109	<.0001	
Treatment*CW	2	0.3356666	2.0781	0.1471	

seismic signal showed no significant differences among treatments (Table 2). Likewise, there were no behavioral differences in the number of bouts of stridulation (One-way ANOVA: $F_{2,36} = 0.605$; $P = 0.552$) and cheliceral strikes (One-way ANOVA: $F_{2,36} = 1.254$; $P = 0.297$). However, analysis of the amplitude of individual vibratory/seismic signal component elements 1, 2 and 3 (but not element 4) found significant differences among treatments (Table 3, Fig. 5), with significantly lower amplitude for only the regenerated limb treatment (Tukey-Kramer post-hoc tests, $P < 0.05$). To avoid concerns regarding sampling error given the number of seismic signal elements compared, we calculated Bonferroni corrected P values for the above analyses and used a Bonferroni-corrected post-hoc test. Results showed similar results, with a significant difference for signal component element 3 and marginal significance for element 2. Given that the direction of the differences in those seismic signal elements (Fig. 5) reflect the same trend as Element 3, these differences are probably biologically real.

Moreover, measurement of total (10 second) signal amplitude (Fig. 6) also showed significant differences among

treatments (One-way ANOVA: $F_{2, 36} = 3.344$; $P = 0.046$) again with significantly lower amplitude for only the regenerated limb treatment. Analysis of RMS signal amplitude for the 10 second recording segment found a significant correlation with both male weight ($R^2 = 0.697$; $F_{1,7} = 16.167$; $P = 0.005$) and residual body condition index ($R^2 = 0.556$; $F_{1,7} = 7.771$; $P = 0.021$) for control males, but not for males in the missing legs treatment (weight: $R^2 = 0.046$; $F_{1,7} = 0.389$; $P = 0.549$; Residual BCI: $R^2 = 0.134$; $F_{1,7} = 1.24$; $P = 0.297$) or the regenerated legs treatment (weight: $R^2 = 0.273$; $F_{1,7} = 3.38$; $P = 0.099$; Residual BCI: $R^2 = 0.258$; $F_{1,7} = 3.134$; $P = 0.111$).

Additional analyses showed that complexity of signal information content (values of the Shannon-Weiner Index) did not differ significantly among treatments for calculations based on either the number of elements (One-way ANOVA: $F_{2, 36} = 0.436$; $P = 0.649$) or their amplitude (One-way ANOVA: $F_{2, 36} = 0.413$; $P = 0.665$).

DISCUSSION

Results of this study suggest female *S. ocreata* can discriminate between intact males and those missing limbs

Table 2.—One-way ANOVA analyses of the number of individual signal component elements (refer to text) (based on Hebets et al. 2013; see Fig. 5) in LDV recordings of male vibratory/seismic signals from the three male treatment groups.

Signal Component	Source	DF	F Ratio	<i>P</i>
Element 1	Treatment	2	0.575	0.568
	Error		36	
Element 2	Treatment	2	0.119	0.314
	Error		36	
Element 3	Treatment	2	0.991	0.381
	Error		36	
Element 4	Treatment	2	0.247	0.782
	Error		36	

based on isolated vibratory/seismic signals alone. Previous studies have found negative effects of limb autotomy and regeneration on prey capture and mating success in *S. ocreata* (Uetz et al. 1996; Taylor et al. 2006, 2008; Wrinn & Uetz 2007, 2008) as well as other lycosid species (Amaya et al. 2001; Brautigam & Persons 2003; Brown & Formanowicz 2012; Steffenson et al. 2014). Additional studies of female mate choice have demonstrated that a female's ability to discriminate among males is based on evaluation of visual or vibratory/seismic signal components alone or in combination (Uetz & Smith 1999; Uetz & Roberts 2002; Gibson & Uetz 2008, 2012; Uetz et al. 2009). Results of this study suggest females prefer signals of males with intact forelimbs over those of males missing a forelimb, based on vibratory/seismic signals alone.

Our data show that females discriminate against males missing legs based on vibratory signals alone, although the cues they use are not so obvious - i.e., there are no behavioral differences among male treatments, and vibration signal amplitude differences are only apparent for males regenerating limbs. While female *S. ocreata* may be able to detect differences in male vibratory/seismic courtship signaling caused by male forelimb autotomy, these findings raise

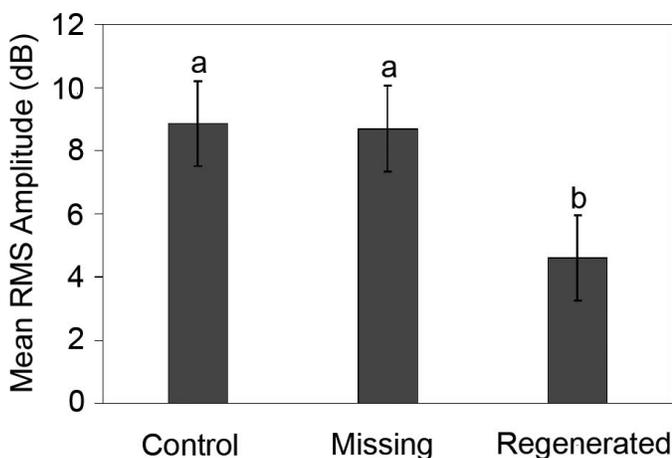


Figure 6.—Mean total overall signal amplitude (dB re: 1 mm/s RMS) of male *S. ocreata* vibratory/seismic signals from the three treatment groups. Different letters indicate significant differences (post-hoc Tukey test). Error bars indicated +/- one standard error.

Table 3.—One-way ANOVA analyses of amplitude (measured in mm/s velocity) of individual signal component elements (refer to text) (based on Hebets et al. 2013; see Fig. 5) from LDV recordings of male vibratory/seismic signals from the three male treatment groups. Bold indicates a significant effect of male treatment on that particular element.

Signal Component	Source	DF	F Ratio	<i>P</i>	Bonferroni P
Element 1	Male Treatment	2	3.6156	0.0356	0.0250
	Error		42		
Element 2	Male Treatment	2	3.5509	0.0391	0.0375
	Error		36		
Element 3	Male Treatment	2	5.8052	0.0065	0.0125
	Error		36		
Element 4	Male Treatment	2	0.7399	0.4843	0.0500
	Error		36		

questions about what aspects of male vibratory/seismic signals differ between intact males vs. those missing a forelimb. In other animal species that communicate using sound and/or vibration, females discriminate among males based on signal amplitude and/or information content (Bradbury & Vehrencamp 2011). The analysis of LDV recordings of male signals in this study revealed there are significant differences in amplitude of vibratory/seismic signals among male treatments. Both whole signal amplitude (10 second segments) and the amplitude of several individual signal components showed differences among treatments. In contrast, information content (measured as a Shannon Diversity Index) showed no differences across treatments, suggesting that signal information content or complexity was unlikely to be affected by limb autotomy and regeneration.

Differences in signal amplitude between males with intact forelimbs vs. those regenerating limbs suggest that some aspect of body condition might play a role in male signal generation. Lower growth increment results seen in this study suggest that regenerating a limb is potentially physiologically costly, as affected males grow less than males that do not regenerate a limb. The physiological impact of limb regeneration, evidenced by smaller growth increments, could therefore have some negative effect on vibratory/seismic signal production. Due to the cost associated with regenerating a limb, females may be indirectly discriminating against male condition and/or size, because it has been shown previously that females prefer larger males (Persons & Uetz 2005). In previous studies, it has been shown that vibratory/seismic signals of males in better body condition elicited greater female receptivity, suggesting that vibratory/seismic signals reveal information about male condition (Gibson & Uetz 2008). While some aspects of vibratory/seismic signals (e.g., amplitude) were correlated with male size and mass in control males from this study, neither weight nor residual body condition indices showed a correlation with signal amplitude. It is also possible that limb asymmetry and uneven weight distribution might create a physical imbalance in vibratory/seismic signal production, reflected in the amplitude of the signals. Since males with regenerating limbs have been shown to exhibit foreleg asymmetry (Uetz et al. 1996; Uetz & Smith 1999), this seems a possible explanation as well.

Male vibratory/seismic signals have been shown to be honest indicators of male condition to mature conspecific females (Persons & Uetz 2005; Gibson & Uetz 2012). Females of *S. ocreata* mate only once (Norton & Uetz 2005) and so it is in a female's best interest to discriminate against signals from lower quality males. The results of this study suggest females evaluate male vibratory/seismic signals and can differentiate between males that are missing a limb and those that have intact or regenerated limbs. Presumably the benefit of surviving a predation event or conspecific encounter counteracts the fitness costs of limb loss or regeneration and its effects on vibratory/seismic signaling in *S. ocreata*. It does, however, appear as if females have the ability to evaluate male vibratory/seismic signals based on male condition (i.e., relative mass).

In conclusion, our results support earlier findings that female *S. ocreata* can use vibratory signals alone to evaluate mates (Gibson & Uetz 2008, 2012), but also show that loss of a limb can impact vibratory signals sufficiently to reduce female receptivity, and have the potential to affect male mating success. Interestingly, limb regeneration may allow males to regain levels of female receptivity closer to those of intact males, despite potential physiological impact on growth and reduction in signal amplitude. Evidence from this study as well as others (Gibson & Uetz 2008, 2012) shows that amplitude of vibratory/seismic signals varies with male size and weight, which in turn may reflect condition and feeding history. As a consequence, females can use male vibratory/seismic signals alone to evaluate mates, and males having undergone limb autotomy would be at a potential fitness disadvantage. While results suggest that signal amplitude, and in particular the amplitude of specific elements of the vibratory/seismic signal, is affected by limb loss and regeneration, males with regenerated forelegs appear to be at less of a disadvantage than might be predicted.

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