

Fitness Implications of Nonlethal Injuries in Scorpions: Females, but Not Males, Pay Reproductive Costs

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ABSTRACT: The ability to detach a body part in response to a predation attempt is known as autotomy, and it is perhaps the most intensively studied form of nonlethal injury in animals. Although autotomy enhances survival, it may impose reproductive costs on both males and females. We experimentally investigated how autotomy affects the reproductive success of males and females of a scorpion species. Individuals of *Ananteris balzani* autotomize the last abdominal segments (the tail), losing the anus and leading to lifelong constipation, since regeneration does not occur. Although the male tail is used during courtship and sperm transfer, autotomy has no effect on male mating success. The combined effect of increased mortality and reduced fecundity resulted in autotomized females producing nearly 35% fewer offspring than intact females. In conclusion, the negative effects of tail autotomy are clearly sex dependent, probably because the factors that influence reproductive success in males and females are markedly different.

Keywords: autotomy, courtship, fecundity, maternal effects, mating success, sperm transfer.

Introduction

Nonlethal injuries resulting from unsuccessful predation attempts occur in both plants and animals (Harris 1989). The ability to detach a body part and avoid predation, known as autotomy, is one of the most intensively studied forms of nonlethal injury, being reported for numerous invertebrate and vertebrate taxa (Maginnis 2006; Fleming et al. 2007; Emberts et al. 2019). Autotomy clearly enhances fitness, but the nonlethal injury may impose costs, decreasing future survival and reproductive performance. The costs of autotomy on survival include, for instance,

increased exposure to future predation attempts and reduced foraging performance because of impairments to locomotion, increased mortality risk due to reduced growth and loss of energy storage, and increased susceptibility to infections during the healing period (Maginnis 2006; Fleming et al. 2007; Emberts et al. 2019). The costs of autotomy for reproduction, in turn, are expected to be sex dependent, probably because the factors that influence the reproductive success of males and females are markedly different. For males, the reproductive costs may include changes in social status and resource-holding potential, and also reductions in mating success (Fleming et al. 2007; Emberts et al. 2019). For females, the costs of autotomy on reproduction involve mainly reductions in fecundity (Bernardo and Agosta 2005). There are also largely unexplored negative effects on the offspring of autotomized females. These include potential reductions in per-propagule investment, brooding behaviors, and post-hatching provisioning (Bernardo and Agosta 2005).

Empirical evidence showing that nonlethal injuries related to autotomy have a negative effect on the reproductive success of males and females is scarce. There are only a few studies on the subject among invertebrates (crustaceans: Juanes and Smith 1995; sea stars: Pomory and Lawrence 1999) and vertebrates (salamanders and lizards: Bernardo and Agosta 2005). For males, appendage loss rather than regeneration is likely to be the most direct cost of reproduction, because in many species the autotomized body part is used in female courtship (Arnold 1977; Uetz et al. 1996) and intrasexual contests for mates and territories (Smith 1992; Dodson and Beck 1993). For females, in turn, the reproductive costs seem to be imposed mainly by appendage regeneration, which is an energetically expensive process (Maginnis 2006). The energy allocated to appendage regeneration may reduce both current and future fecundity, and in the most extreme cases it may completely inhibit reproduction (Bernardo and Agosta 2005; Maginnis 2006; Fleming et al. 2007). Finally, nonlethal injuries may impose reproductive costs via maternal effects, defined as

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the effect of the parental phenotype (in this case, autotomy) on the offspring phenotype (Bernardo and Agosta 2005). Nonetheless, there is only one study showing that maternal autotomy negatively affects per-propagule investment: the higher the relative length of the tail loss in a salamander, the smaller are the eggs produced (Bernardo and Agosta 2005).

In this study, we explore the implications of a nonlethal injury related to autotomy on the reproductive success of males and females of the scorpion *Ananteris balzani* (Buthidae). The genus *Ananteris* is the only one among scorpions in which individuals have the ability to autotomize the last abdominal segments, known as the tail (Mattoni et al. 2015). This is an extreme form of nonlethal injury with no parallel in the animal kingdom because the tail bears the telson, which contains a pair of venom glands and the stinger organ, and also parts of the nervous, circulatory, and digestive systems, including the anus (Hjelle 1990). Thus, tail autotomy in scorpions implies the loss of vital body parts, contrary to what occurs with salamanders and lizards. Moreover, contrary to salamanders and lizards, the tail in scorpions does not regenerate and, given that individuals are unable to defecate for the rest of their lives, they eventually die from constipation some months after autotomy, sooner than would be expected if they were intact. This study system is interesting because tail loss alone may impair courtship behavior and mating success in males, providing a comparison with previous, nonhomologous cases of tail loss in vertebrates (Arnold 1977; Houck 1982). For females, we have the opportunity to explore whether the fecundity costs of autotomy also apply to a species in which the lost appendage is not regenerated.

Our hypothesis was that tail autotomy in scorpions will have differential negative costs to the reproduction of males and females and also to the phenotype (i.e., mass and size) of the offspring produced by autotomized females (via maternal effects). Given that *A. balzani* males use the tail to perform a very conspicuous behavior called tail wagging during the courtship, and also to support the body during spermatophore deposition (see video S1, available online), we predict that autotomized males will have a long courtship dance mostly due to high female resistance, low mating success due to failure in spermatophore deposition, and reduced success in sperm transfer. For females, we predict that autotomy will cause high mortality before parturition and low fecundity owing to the negative effects of severe constipation, which causes great accumulation of feces and toxins inside the females during the 5 months of pregnancy. Female autotomy and the subsequent constipation may also induce maternal effects, reducing per-propagule investment, including offspring mass and/or size.

Methods

Study Species

Ananteris balzani is widely distributed in Brazil, being particularly abundant in areas of savanna in the state of São Paulo (Giupponi et al. 2009). Like all other scorpion species, *A. balzani* is viviparous and, on the basis of our laboratory observations, females can produce no more than two litters in their lives; males, in turn, can mate many times. Gestation takes from 4 to 5 months, during which females remain inside a burrow called a gestational chamber dug in sandy soil. After birth, nymphs stay on the mother's dorsum for 2 weeks, during which time they do not feed. The dispersion of the nymphs from the mother's dorsum and also from the gestational chamber occurs in November–December, that is, at the end of spring and beginning of summer. Development until adulthood takes nearly 6 months for males and nearly 12 months for females. Under field conditions, males probably live no more than 12 months as adults, whereas females probably live from 12 to 18 months. The peak of the mating season occurs in April–May, which corresponds to the middle of fall. During the mating process, the male grasps the female pedipalps with his own pedipalps (fig. 1A, 1B), and the couple walks around for several minutes. When the female is receptive and the male finds a proper substrate, he deposits his spermatophore (fig. 1C), which is taken by the female. The genital opening of males and females is located in the first segment of the mesosoma, away from the metasomal segments that are lost when autotomy occurs. Thus, autotomy results in no damage to the genital opening nor to the reproductive tracts of both males and females.

Collection and Maintenance

The individuals of *A. balzani* used in the experiments (see below) were collected in a savanna area at Santa Bárbara Ecological Station (24°48'S, 49°13'W), São Paulo, Brazil, between November and March in 2017–2018 and 2018–2019. The natural frequency of autotomized individuals in this site was 3.6% for males and 1.1% for females. The individuals (70 adult males and 78 adult females) were transported to the laboratory, where they were maintained individually in plastic containers during a 2-month quarantine period to identify and exclude individuals parasitized by nematomorphs. During the quarantine, individuals were fed every 10 days with cricket nymphs (*Acheta* sp.). Given that female body mass (mean \pm SE = 119.61 \pm 3.15 mg; N = 27) is two times higher than male body mass (mean \pm SE = 51.73 \pm 2.78 mg; N = 21), males received two cricket nymphs (3 mm of total length) and females received four

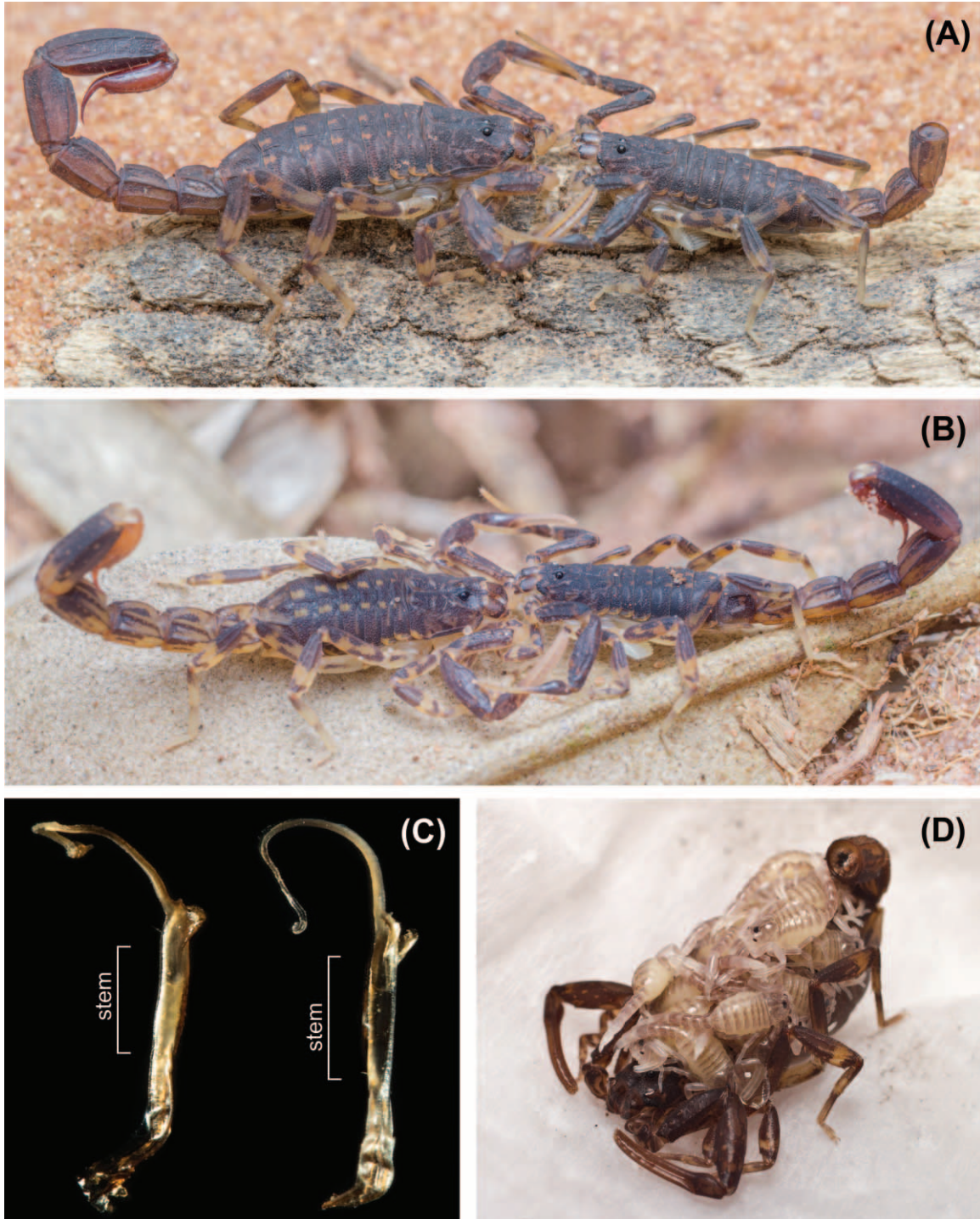


Figure 1: A, B, Mating pair of the scorpion *Ananteris balzani*. In both photos, the female is on the left side and the male is on the right side. During courtship, autotomized (A) and intact (B) males grip the female's pedipalps using their own pedipalps. Note that female coloration is clearly different in A and B. The lighter the tegument, the younger the female is. C, Two flagelliform spermatophores collected after the mating trials. When sperm transfer is incomplete, it is possible to see through the hyaline walls of the spermatophore's trunk the white mass of sperm (left). An empty spermatophore (right) indicates that sperm transfer was complete and that no sperm remained inside the spermatophore. D, Autotomized female with first instar nymphs on her dorsum. Photos provided by John Alexander Uribe (on Twitter at @JohnUribePhotos).

cricket nymphs. We kept the individuals under a 12:12-h light:dark cycle and conducted the experiments in a room with minimal temperature variation (24.5°–26.5°C).

Experiment I: Male Reproductive Success

To assess the possible implications of tail autotomy on the mating success of males, we first measured the cephalothorax length (a proxy of body size) of all individuals and paired males and females assortatively according to their size rank. As the individuals were collected in different years, the size-assortative pairing was done in blocks containing at least 24 individuals each. Then, we split the males into two experimental groups: intact ($n = 40$) and autotomized ($n = 30$). We induced tail autotomy in males of the autotomized group by repeatedly tapping their body with forceps for 1 min and then grasping and pulling their last metasomal segment with the same forceps (fig. 1A). For intact males, we used the same protocol, but we grasped the first metasomal segment, where tail autotomy does not occur (fig. 1B). All males were allowed to rest for at least 3 days to complete the healing of the fracture point.

The mating trials were always performed during the first 6 hours of the dark phase, under dim red illumination and inside a glass box (base: 20 × 10 cm; height: 15 cm) with wet sand as substrate, and with two pieces of flat wooden boards and two pieces of porous tiles to provide a suitable substrate for spermatophore deposition. We placed a male inside the box, and after 30 min of acclimatization, we placed the female inside. We followed and filmed each male-female sexual interaction with two Sony cameras (HDR-CX405), one placed above the mating pair and another placed laterally.

In some mating trials (27.5% of the total) males and females did not engage in courtship within 1 h after the first physical contact between them. This situation usually occurs because the female is pregnant or has already received the maximum number of inseminations (Peretti and Carrera 2005). Thus, whenever a female did not engage in courtship, we followed her for 2 months after the mating trial to identify signs of pregnancy (i.e., an increase in abdominal volume with embryos visible through the ventral intersegmental membranes of the mesosoma). Moreover, within 30 days after an unsuccessful mating trial, we tried to remate the supposedly unreceptive females with up to two other males (either intact or autotomized). We considered as unreceptive all females that were pregnant or did not accept any other male after the first mating trial. In these cases, we discarded the females from the sample and considered all mating trials accomplished with them as invalid. After removing these

females ($n = 19$; 10 were pregnant), we had 28 mating trials with intact males and 23 mating trials with autotomized males.

We estimated male mating success using three measures. The first was spermatophore deposition success, that is, whether a male successfully deposited on the substrate his spermatophore, which is a sclerotized, flagelliform structure attached to a solid substrate at the end of the courtship (fig. 1C). Considering only males that successfully deposited their spermatophore, we estimated sperm transfer success (which is the second measure), that is, whether a male transferred all sperm contained in his spermatophore. Because the walls of the spermatophore are transparent in *A. balzani*, we used a stereomicroscope to score whether some sperm remained inside the spermatophore (fig. 1C). Spermatophores containing any visible amount of remaining sperm were scored as incomplete transferences. The third measure that we estimated was courtship duration of all males that successfully deposited the spermatophore. Courtship duration was the period between pedipalpal grip (i.e., the beginning of the courtship; fig. 1A, 1C) and the moment when both individuals retracted their pedipalps and separated their bodies after sperm transference (see video S1).

Experiment II: Female Reproductive Success and Maternal Effects

To assess the possible implications of tail autotomy on the reproductive success of females, we used females from the previous experiment that successfully received a spermatophore during courtship (both from intact and autotomized males). We also included nine additional females that were not used in the previous experiment but that were paired with intact males following the same protocol described above. Because the females used in our experiments were collected in the field, we had no control over their previous pregnancy history. However, we reared females in the laboratory, from the last nymphal stage to their deaths, and detected that the tegument coloration of young adult females is lighter than that of older adult females (fig. 1A, 1B). Using tegument coloration, we divided experimental females into two age categories: (a) young ($n = 12$), comprising seven virgin females reared in the laboratory since they were nymphs, and five wild-caught females that were between 1 and 3 months old and were unlikely to have produced a first litter, and (b) old ($n = 37$), comprising 29 females that produced a first litter in the laboratory before the experiment (when they were at least 8–10 months old), and eight wild-caught females that almost certainly produced a first litter in the field and were at least 7–8 months old when

they were used in the experiment. Very old females, that is, those showing the darkest tegument coloration and damages in the stinger, pedipalps, or pectens, were not included in the experiment. We equally split young and old adult females into two experimental groups: intact ($n = 25$) and autotomized ($n = 24$). This procedure ensured that a similar number of females in each experimental group would give birth to the first or second litter.

To induce tail autotomy in females, we followed the same protocol as described in the experiment with males. Contrary to males, however, we autotomized females only after mating because we were interested in the effect of autotomy on offspring development and not on the mating success of females, which are never rejected by the males once the courtship process had begun (Polis and Sissom 1990; see also Peretti and Carrera 2005 and references therein). Moreover, considering that the females used here are the same ones that were used in the previous experiment, the lack of the tail in females during the courtship process could interfere with their receptivity and consequently with males' behavior. Thus, we would be unable to disentangle the effects of male autotomy and other variables that could influence the response variable "courtship duration."

After mating, all females were maintained individually in plastic containers with sand as substrate, wet cotton, and a dry leaf to provide shelter. We inspected the females at 3-day intervals during the entire period of experiment to record whether they were alive and whether they gave birth to the nymphs. We estimated female reproductive success using three measures: (1) female survival until offspring dispersion, that is, the probability of a female remaining alive until her offspring are independent from maternal care, which occurs 4–5 months after mating and 2 weeks after offspring birth, (2) offspring number, which is the mean number of nymphs produced by the females (fig. 1D), and (3) female fitness, which is the mean number of nymphs produced by the females (including those that died before giving birth) and that survived until dispersion. We stress that if a female dies before giving birth, the offspring number produced is zero. If a female dies after giving birth, but before nymphs disperse from her dorsum, the offspring do not survive, probably because the nymphs, which are overly sensitive to dehydration, need to absorb water directly through the mother's cuticle (Vannini et al. 1985). The nymphs produced by these females ($n = 3$) were used to estimate offspring number, but to estimate fitness we considered that they left no offspring because the nymphs did not survive until dispersion.

Finally, we estimated maternal effects using offspring mass, which is the mean weight of all second instar nymphs of each litter, and offspring size, which is the cephalothorax width of all second instar nymphs of each litter. The size

of the nymphs was measured from digital photos using the software ImageJ (Schneider et al. 2012).

Data Analysis

We tested for the implications of tail autotomy on the reproductive success of males using a different model for each measure. For all models, the experimental group (intact or autotomized) was the predictor variable. For the response variables "spermatophore deposition success" (no = 0, yes = 1) and "sperm transfer success" (incomplete = 0, complete = 1), we adjusted generalized linear models (GLMs) with binomial error distribution and logit link function. For the response variable "courtship duration," we adjusted a linear model with Gaussian error distribution, and included an error structure to account for heteroscedasticity using the `gls()` function of the package `nlme` (Pinheiro et al. 2019). Given that the variation in mating duration was markedly different between intact and autotomized males, we performed a Levene test using the package `car` (Fox and Weisberg 2019) to compare the variance of the two experimental groups.

To test for the implications of tail autotomy on the reproductive success of females, we used a different model for each measure. In all models, the predictor variable was the experimental group. For the response variable "female survival until offspring dispersion," we first performed a survival analysis using the Cox proportional hazards test. Because data collection ended when a female died or when the offspring dispersed, survival time was right censored (censoring status: 1 = alive, 2 = dead). The result of this survival analysis is a ratio that describes the relative probability of dying of one group compared with that of another group. Thus, when the confidence interval includes 1, we can conclude that survival is not affected by the predictor variables (i.e., experimental groups or age classes). Second, we adjusted a GLM with binomial error distribution and logit link function to estimate the survival probability until offspring dispersion (no = 0, yes = 1). In both analyses, we included female age (young or old) as a categorical covariate. Finally, for the response variables "offspring number" and "female fitness," we adjusted GLMs with Poisson error distribution. In the analysis of offspring number, we also included female age and female body size as covariates. The data set used in the analysis of offspring number included 13 intact and 12 autotomized females that survived until giving birth, and the data set used in the analysis of female fitness included all females of both experimental groups.

To test for the implications of tail autotomy on offspring phenotype, we used a different model to each measure. For the response variable "offspring mass," we adjusted a

GLM with Gaussian error distribution. For the response variable “offspring size,” we adjusted a generalized linear mixed model (GLMM) with Gaussian error distribution and female identity as a random factor because each female has several nymphs in her litter. Given that offspring size may also be influenced by offspring number, we included the total number of nymphs in each litter as a continuous covariable. In the models of offspring mass and offspring size, we also included female age and female body size as covariates. We did not include the experimental group of the father as a covariable because exploratory analyses showed no effect of this variable on offspring phenotype (sec. S2 of the supplemental PDF, available online).

All GLMs were performed using the package *stats* (R Core Team 2019). The GLMM for offspring size was performed using the package *lme4* (Bates et al. 2015). Because the results of the models adjusted with binomial and Poisson error distributions are in logit and log scale, respectively, we used the package *emmeans* (Lenth 2019) to transform them into probabilities and number of individuals. Using *emmeans*, we also calculated effect size (Cohen’s *d*) for all analyses. The fit of the survival curve and the Cox proportional hazard model were performed in the package *survival* (Therneau 2020). All analyses were performed using R version 3.6.2. (R Core Team 2019). Data underlying all analyses have been deposited in the Dryad Data Repository (<https://doi.org/10.5061/dryad.vq83bk3r8>; García-Hernández and Machado 2020b).

Results

Male Reproductive Success

Two males, one autotomized and one intact, did not engage in courtship behavior. All 49 males that engaged in courtship were accepted by the females, and the spermatophore deposition success of intact and autotomized males was similar (table 1). Among the males that successfully deposited the spermatophore, both the sperm transfer success and the courtship duration were also similar between intact and autotomized males (table 1; fig. 2A). The only difference that we detected was that the courtship duration varied almost four times more in autotomized males (SD = 92.4 min, CV = 1.25) than in intact males (SD = 25.6 min, CV = 0.56; Levene test: $F = 11.503$, $P = .002$; fig. 2A).

Female Reproductive Success

Old females, whether intact or autotomized, had a higher probability of dying before offspring dispersion when compared with young females (hazard ratio_(old/young) =

6.153; 95% CI: 1.325 to 28.578). Although autotomized females belonging to the same age (young or old) had a higher probability of dying than intact females (hazard ratio_(autotomized/intact) = 2.301; 95% CI: 0.915 to 5.789; fig. 3), the confidence intervals of the estimate overlaps 1. Similarly, female survival probability until offspring dispersion was significantly influenced by female age ($df = 46$, $z = 2.122$, $P = .034$; table 1) but not by the experimental group (table 1). After controlling for age and body size, intact females produced 30.7% more offspring than autotomized females (table 1; fig. 2B). We stress, however, that neither age ($df = 21$, $z = 0.335$, $P = .738$; table 1) nor body size ($df = 21$, $z = -0.546$, $P = .585$) had a significant influence on offspring number. The estimated female fitness was 54% higher for intact than for autotomized females (table 1; fig. 2C).

Maternal Effects

After controlling for female age ($df = 20$, $t = -1.714$, $P = .102$) and body size ($df = 20$, $t = -0.706$, $P = .488$), offspring mass was similar between the experimental groups (table 1). Offspring size also was similar between intact and autotomized females (table 1) after controlling for female age ($df = 29$, $t = 0.289$, $P = .775$), body size ($df = 13$, $t = -1.454$, $P = .169$), and offspring number (intercept = 1.510, slope = -0.005 , $df = 14$, $t = -1.326$, $P = .206$).

Discussion

Our results show that permanent tail loss has no effect on the mating success of *Ananteris balzani* males because spermatophore deposition success, sperm transfer success, and courtship duration were similar between intact and autotomized males. Tail loss, however, impaired the reproduction of females by reducing their fecundity, and ultimately their fitness, when compared with intact females. Regarding the maternal effects, we did not detect any effect of female autotomy on the offspring phenotype because the mass and size of the nymphs were similar between the two experimental groups. In what follows, we discuss the implications of these findings to the evolution and maintenance of one of the most extreme forms of nonlethal injury among animals.

We demonstrate here that the tail of *A. balzani* males is not crucial for sperm deposition and transfer. This is surprising, because the stinger is used to anchor the body immediately before the male pulls the female to uptake the spermatophore. However, our observations show that autotomized males can use only the basal tail segments to perform this anchoring behavior and successfully

Table 1: Summary of results obtained from the adjusted models to test the implications of tail autotomy on the reproductive success of males and females and on the offspring phenotype (maternal effects) of the scorpion *Ananteris balzani*

Proxies	Experimental groups		Statistics	Effect size
	Intact	Autotomized		
Males:				
Spermatophore deposition success (probability)	.889 ± .061 (<i>n</i> = 27), 95% CI: .707 to .964	.864 ± .073 (<i>n</i> = 22), 95% CI: .652 to .955	<i>z</i> = .268, <i>p</i> = .789	.266 ± .992 (<i>σ</i> = .880), 95% CI: −1.678 to 2.210
Sperm transfer success (probability)	.708 ± .093 (<i>n</i> = 24), 95% CI: .502 to .854	.842 ± .084 (<i>n</i> = 19), 95% CI: .608 to .948	<i>z</i> = 1.018, <i>p</i> = .308	−.746 ± .738 (<i>σ</i> = 1.054), 95% CI: −2.192 to .700
Courtship duration (min)	41.299 ± 4.914 (<i>n</i> = 23), 95% CI: 31.351 to 51.246	73.796 ± 21.190 (<i>n</i> = 19), 95% CI: 30.900 to 116.692	<i>t</i> = 1.494, <i>p</i> = .143	−1.379 ± .936 (<i>σ</i> = 23.570), 95% CI: −3.273 to .515
Females:				
Female survival (probability):				
Age, young	.866 ± .099 (<i>n</i> = 6), 95% CI: .547 to .972	.801± .131 (<i>n</i> = 6), 95% CI: .447 to .952	<i>z</i> = .769, <i>p</i> = .442	.408 ± .532 (<i>σ</i> = 1.154), 95% CI: −.635 to 1.452
Age, old	.516 ± .111 (<i>n</i> = 19), 95% CI: .310 to .717	.400 ± .111 (<i>n</i> = 18), 95% CI: .212 to .622		
Offspring number: ^a				
Age, young	13.910 ± 1.435 (<i>n</i> = 5), 95% CI: 11.363 to 17.028	10.642 ± 1.253 (<i>n</i> = 5), 95% CI: 8.448 to 13.404	<i>z</i> = 2.207, <i>p</i> = .027	.223 ± .107 (<i>σ</i> = 1.203), 95% CI: .014 to .431
Age, old	13.356 ± 1.240 (<i>n</i> = 8), 95% CI: 11.134 to 16.022	10.218 ± 1.045 (<i>n</i> = 7), 95% CI: 8.361 to 12.487		
Female fitness	7.000 ± .529 (<i>n</i> = 25), 95% CI: 6.036 to 8.118	4.542 ± .435 (<i>n</i> = 24), 95% CI: 3.764 to 5.480	<i>z</i> = 3.545, <i>p</i> < .000	.140 ± .042 (<i>σ</i> = 3.082), 95% CI: .058 to .223
Maternal effects:				
Offspring mass (mg): ^b				
Age, young	3.974 ± .197 (<i>n</i> = 5), 95% CI: 3.587 to 4.361	3.785 ± .207 (<i>n</i> = 5), 95% CI: 3.380 to 4.190	<i>t</i> = .851, <i>p</i> = .405	.362 ± .429 (<i>σ</i> = .524), 95% CI: −.479 to 1.203
Age, old	4.357 ± .174 (<i>n</i> = 8), 95% CI: 4.016 to 4.699	4.168 ± .186 (<i>n</i> = 6), 95% CI: 3.802 to 4.533		
Offspring size (mm): ^c				
Age, young	1.198 ± .023 (<i>n</i> = 40), 95% CI: 1.151 to 1.246	1.154 ± .023 (<i>n</i> = 53), 95% CI: 1.107 to 1.201	<i>t</i> = 1.648, <i>p</i> = .123	.138 ± .084 (<i>σ</i> = .323), 95% CI: −.039 to .316
Age, old	1.192 ± .021 (<i>n</i> = 69), 95% CI: 1.148 to 1.236	1.147 ± .021 (<i>n</i> = 53), 95% CI: 1.103 to 1.192		

Note: For each experimental group (intact or autotomized), we show the estimate value, standard error, sample size (*n*), and the 95% confidence interval (CI). All estimate values from models adjusted with binomial and Poisson error distribution were back-transformed to present them in terms of probability or counts, respectively. For all models, we show the standardized effect size (Cohen's *d*) for the variable "experimental group," the dispersion parameter (*σ*), and the 95% CI. A positive value of effect size indicates that the estimate for the intact group (not back-transformed) is higher than that of the autotomized group.

^aEstimated at the mean of the covariable female body size (i.e., 0.311 cm).

^bEstimated at the mean of the covariable female body size (i.e., 0.311 cm).

^cEstimated at the mean of the covariables offspring number (i.e., 12 nymphs) and female body size (i.e., 0.311 cm).

transfer the spermatophore (see video S1). Moreover, although tail wagging may have a stimulatory role during courtship, we found no difference in courtship duration between intact and autotomized males. In the salamander *Desmognathus ochrophaeus*, males also use their tails to stimulate females, but tail loss does not affect courtship intensity and insemination success (Houck 1982). Thus, it seems that tail loss per se does not necessarily reduce mating or insemination success in species in which males

use this appendage for courtship. Nonetheless, some autotomized males of *A. balzani* needed much more time to transfer the spermatophore than intact males (fig. 2A). This pattern is probably not related to male health because the time between tail loss and the mating trials (10–20 days) is not long enough to make males severely constipated. Instead, we suggest that some autotomized males faced more female resistance due to lack of proper stimulation, which increased courtship duration. A prediction of

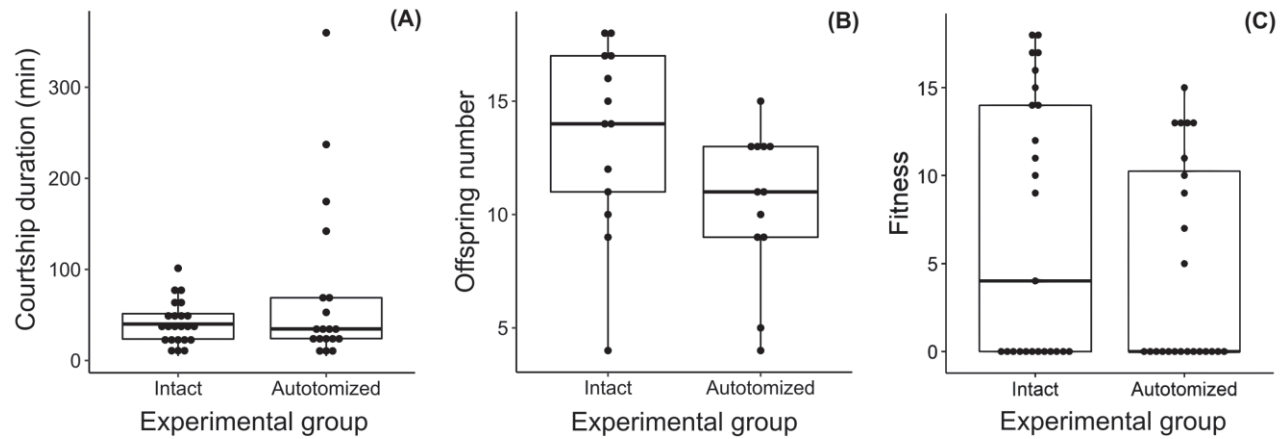


Figure 2: In the three boxplots, shown are the effects of tail autotomy on the reproductive success of males (A) and females (B, C) of the scorpion *Ananteris balzani*. In the boxplots, each point represents an individual. A, The mean courtship duration of intact and autotomized males was similar, but the variation in later was much higher. B, Autotomized females give birth to a lower number of nymphs than intact females. C, The fitness (i.e., mean offspring number produced by the females, including those that died before parturition) of intact females was higher than the fitness of autotomized females.

this hypothesis is that autotomized males compensate for tail loss by increasing the frequency of other stimulatory behaviors (e.g., juddering and cheliceral massage). In fact, studies with other scorpions show that males adjust the intensity of courtship in response to female resistance (Peretti and Carrera 2005). If the same happens in *A. balzani*, compensatory adjustments in the intensity of

courtship could mitigate the reproductive costs to autotomized males.

Contrary to males, autotomized females paid reproductive costs in the form of a 23.5% reduction in offspring number. This value is close to the lowest one reported for autotomized female lizards, in which the reduction in offspring number ranges from 14% to 75% (Bateman and

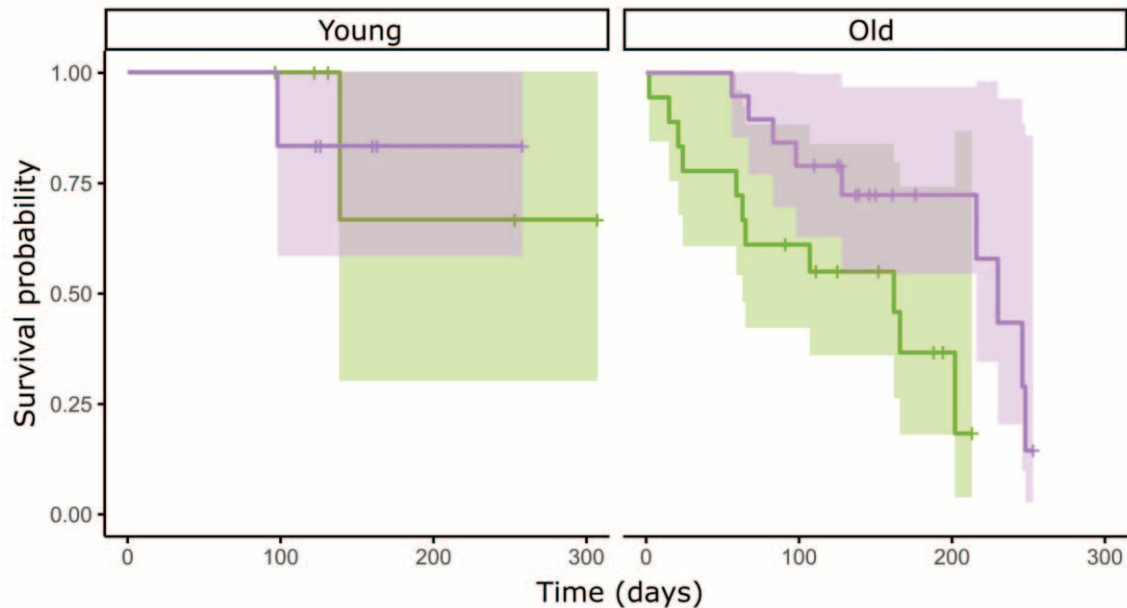


Figure 3: Survival curves of intact (violet) and autotomized females (green) of the scorpion *Ananteris balzani*. Each line represents the probability of young or old females to survive from mating until the moment of offspring dispersion. Vertical tick marks on the curves indicate that an offspring dispersion event occurred on that day. A vertical drop in the curves indicates events of death on that day. Shaded areas show the 95% confidence interval for each survival curve.

Fleming 2009). The greater reduction among lizards is probably related to a double cost of tail autotomy: (1) the loss of energetic resources stored in the tail that are no longer available to egg production and (2) the regeneration of the tail, which diverts energy from egg production (Bernardo and Agosta 2005; Maginnis 2006). In contrast, permanent tail loss in scorpions has no direct effect on energetic reserves because there is no storage organ in the metasoma (Hjelle 1990). Moreover, there is no tail regeneration (Mattoni et al. 2015), and the energetic trade-off between replacement of tissues and offspring production does not exist. The reduction in offspring number reported here may be a consequence of accumulation of toxins during constipation that may impair offspring development and kill some embryos. Alternatively, severe constipation may leave little space for offspring development in the female's reproductive tract. At the moment of parturition, litter mass in scorpions ranges from 22% to 56% of female body mass (Brown 2004; Warburg 2011), which implies that embryos occupy a great volume inside females. Two months after autotomy, the accumulation of feces in the digestive tract causes a 30% increase in the mass of non-pregnant females (sec. S3 of the supplemental PDF). Given that embryos take 4–5 months to develop, some of them may die or be resorbed in the beginning of the gestation due to lack of space inside the females. Thus, although lizards and scorpions suffer a reduction in offspring number after autotomy, it seems that the mechanisms underlying this reduction are completely different.

The percentage of females that survived until offspring dispersion depended on the age, but not on the experimental group. Young females showed higher survival probability than old females, regardless of whether they were intact or autotomized. This finding indicates that constipation imposed by autotomy does not promote an increase in female mortality during pregnancy. However, when we combine female survival and fecundity to estimate fitness, the reproductive costs paid by autotomized females are clear; on average, they produced nearly 35% fewer offspring than intact females. This fitness cost is probably an underestimation because it does not consider other impairments that tail autotomy may cause in females. We know, for instance, that the handling time of large prey by females is greatly increased after autotomy (García-Hernández and Machado 2020a). An increase in energy expenditure for subduing large prey items makes them less profitable in terms of energy yield per unit of handling time (Charvov 1976). Among predatory arthropods, such as scorpions, this low energy intake may result in additional reproductive costs to females, such as lower fecundity (e.g., Wise 1979; Sota 1985; Richardson and Baker 1997). Tail loss also impairs males' foraging, decreasing the capture success of large prey and increasing the han-

dling time of small and large prey (García-Hernández and Machado 2020a). The reduction in food intake and the increase in energy expenditure for prey subjugation may negatively affect male condition, and perhaps the search for receptive females, which is important for male mating success (Polis and Sissom 1990). However, we do not expect a marked short-term effect on sperm production and courtship performance because these activities probably represent low costs to the males when compared with female investment in egg production and embryo nourishment. Thus, foraging limitation imposed by tail autotomy has greater potential to magnify the reproductive costs paid by females rather than males.

The fact that females pay higher reproductive costs than males may explain sexual differences in the willingness to autotomize the tail. Under laboratory conditions, *Ananteris* females are more reluctant than males to autotomize the tail: under similar experimental stimulation, 88% of the males released their tail, whereas only 20% of the females did so (Mattoni et al. 2015). Moreover, under natural conditions, the percentage of autotomized *A. balzani* males in different populations ranges from 3.6% to 9.1%, whereas the percentage of autotomized females ranges from 0% to 1.1% (Mattoni et al. 2015; this study). Similar sexual differences in the willingness to autotomize a body part have already been reported for some insects and lizards. In the bush cricket *Scudderia texensis*, for instance, individuals of both sexes autotomize frontal legs, where the tympanum, a structure that assists the location of sexual partners, is located. Females are less prone than males to autotomize frontal legs, possibly because mate search is performed mainly by females, who pay higher reproductive costs if they are unable to find sexual partners (Dixon 1989). Males of the lizard *Uta stansburiana*, in turn, autotomize their tails less readily than females. In this lizard, males and females establish intrasexual dominance hierarchy, and whereas autotomized females manage to reproduce as subordinates, autotomized males completely lose their social status and are deprived of access to sexual partners (Fox et al. 1998). These examples with scorpions, insects, and lizards show that the willingness to autotomize a body part differs between males and females in response to the costs paid by each sex (but see Smith 1992).

Tail autotomy of females did not impose any detectable change in offspring traits (i.e., body size and body mass) via maternal effects. In fact, there is little empirical evidence showing that female autotomy changes offspring traits in lizards (Dial and Fitzpatrick 1981; Wilson and Booth 1998) and salamanders (Bernardo and Agosta 2005). Larger offspring are expected to have higher fitness and thus are favored by natural selection (Stearns 1976). Among arthropods, in particular, the effects of propagule

size on subsequent growth and survival are consistent with the “bigger is better” hypothesis (Fox and Czesak 2000). In scorpions, for instance, mortality in the first instars is strongly size dependent, and larger nymphs have a higher survival rates (Polis 1988). Here we showed that autotomized females of *A. balzani* maintained the same per-propagule investment, so that they give birth to a small number of nymphs of a size similar to that of the nymphs born from intact females. Pregnant female scorpions are known to resorb and transfer nutrients among embryos (Polis and Sissom 1990). Thus, autotomized females could adjust offspring size in response to constipation, as already reported for other animal species that adjust offspring size in response to internal cues, such as age and parental condition (Kuijper and Johnstone 2013). Regardless of the internal cue, the adaptive meaning of the adjustment would be to balance the benefits of producing a few large (and possibly fitter) offspring with the costs of decreased fecundity.

In conclusion, the negative effects of a nonlethal injury related to tail autotomy are clearly sex dependent in scorpions. For males, autotomy does not impair courtship duration, spermatophore deposition, and sperm transfer. Given that males can live several months after tail loss and can potentially mate many times before dying from constipation, autotomy is highly advantageous because it provides the benefit of escaping alive from a predation attempt and imposes no immediate reproductive cost. For females, in turn, autotomy provides an immediate survival benefit but also imposes a reproductive cost in the form of a marked reduction in future fecundity. However, female autotomy imposes no detectable change in offspring traits via maternal effects. Thus, females probably pay no further costs related to giving birth to small nymphs, which are more susceptible to intra- and inter-specific predation. The high cost of autotomy paid by females is a key element in understanding why they are more reluctant than males to perform one of the most extreme forms of defensive behavior among animals.

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Statement of Authorship

S.G.H. and G.M. conceptualized the study and implemented the methodology; S.G.H. collected, curated, and formally analyzed the data; S.G.H. and G.M. wrote and edited the manuscript; S.G.H. and G.M. performed visualization; G.M. supervised and administered the project; and both authors acquired funding.

Data and Code Availability

The analyses reported here can be reproduced using the data and script available through the Dryad Data Repository (<https://doi.org/10.5061/dryad.vq83bk3r8>).

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