

No risk to scrambling? Mating tactic does not affect the frequency of leg autotomy in a New Zealand harvestman

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Intense sexual selection on males may drive the evolution of exaggerated weaponry, typically used in contests for females or reproductive sites. In some species, males have discontinuous variation in weapon morphology that is accompanied by alternative reproductive tactics (ARTs). Major males with enlarged weapons usually exhibit a mating tactic based on female or resource defence, which makes them relatively sedentary. Minor males with reduced or absent weapons may exhibit a scramble competition mating tactic, which involves increased movement in search of females. Thus, the main costs paid by majors involve the expression/maintenance of exaggerated structures and potential injuries during contests. In turn, the main costs paid by minors are likely related to increased exposure to predation during mate search, yet this cost has rarely been considered for minors. Harvestmen are an arachnid group with diverse male weaponry, including many cases of male polymorphism associated with ARTs. Species of the suborder Eupnoi use leg autotomy as a common antipredator defence. Here we investigated whether leg autotomy (a proxy for predation risk) is more frequent in minors of the weapon polymorphic harvestman *Forsteropsalis pureora*, which has three male morphs with drastically different cheliceral size and morphology. Leg autotomy was very common, with 54% of wild-caught individuals missing at least one leg (mostly leg II), but we found no evidence for differential predation risk between male morphs during adulthood. In a predator simulation experiment, we found no difference in the likelihood or latency to autotomize a leg between male morphs or leg types (legs I, II, III or IV). However, males already missing legs were more reluctant to autotomize an additional leg. Our results suggest that while leg autotomy is a common antipredator strategy, with no difference between male tactics, costs are compounded as additional legs are autotomized and males may strategically decide not to autotomize in future predator encounters.

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Animal weapons, such as exaggerated male tusks, spurs, horns and jaws, are structures used in male–male contests for the acquisition of females or reproductive sites (Emlen, 2008; McCullough, Miller, & Emlen, 2016; Rico-Guevara & Hurme, 2019). Body size, weapon size and even weapon shape often vary significantly within males of a single species, and such intraspecific

variation can generate complex male polymorphisms (e.g. Iguchi, 2013; Kelly & Adams, 2010; Matsumoto & Knell, 2017; Painting, Probert, Townsend, & Holwell, 2015; Powell, Painting, Hickey, & Holwell, 2020; Rowland & Emlen, 2009). The males that invest the most in their weapons may have to trade-off resources or pay costs to maintain their weaponry. For instance, males with the largest weapons may not be able to allocate resources to other fitness-enhancing traits, such as the testes (Somjee, Miller, Tatarnic, & Simmons, 2018), or traits that develop in proximity to weapons, such as the eyes or wings (Emlen, 2001; Nijhout & Emlen, 1998). These males may also have to pay additional metabolic costs to maintain the additional muscle mass associated with their

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weaponry (Bywater, White, & Wilson, 2014; O'Brien et al., 2019; Somjee, Woods, Duell, & Miller, 2018). Large males of some beetles even compensate for large weapons by growing larger locomotory structures, such as wings to support elongated rostra during flight (Painting & Holwell, 2013).

In contrast, smaller-bodied males with reduced or absent weaponry often adopt alternative reproductive tactics (ARTs), which are behaviours that maximize fitness when intrasexual competition for access to females is intense (Gross, 1996). Examples include male behaviours such as sneaking copulations, acting as satellites close to displaying males, scrambling to find unguarded mates, or even mimicking females as a way to invade the harem of large males (see examples in Oliveira et al., 2008). Although these tactics may increase the fitness of small males by allowing them to gain access to females, alternative reproductive behaviours may come with their own costs. In many spider species, where all males use a scrambling mating tactic while females live in webs or burrows, vagrant males encounter more potential predators than females as they are more exposed during mate searching (e.g. Andrade, 2003; Berger-Tal & Lubin, 2011; Kasumovic, Bruce, Herberstein, & Andrade, 2006; Taylor, Cook, & McGraw, 2019). This may also be the case for males that adopt different reproductive tactics, where one morph is stationary (defending territories or displaying in certain spots) and invests in mate monopolization, and the other is more vagile and invests in sequential mate acquisition (Ota, 2020). However, variation in the risks of predation associated with male polymorphisms and their corresponding alternative reproductive tactics have rarely been considered in field studies.

One defensive behaviour that provides an indication of predation pressure is autotomy, which occurs when body parts, such as limbs or tails, are shed at predetermined breakage planes (Emberts, Escalante, & Bateman, 2019; Fleming, Muller, & Bateman, 2007). Given that autotomy allows an individual to escape a predation attempt, the frequency of autotomy could be used as a proxy of how often individuals are attacked by predators in the field (see Schoener, 1979). Thus, species in which males have weapon polymorphisms and can autotomize limbs are ideal systems to investigate whether the costs associated with predation risk vary between male morphs with alternative reproductive tactics. This intersection between weapon polymorphism and leg autotomy occurs in some long-legged harvestmen of the suborder Eupnoi. In the family Neopilionidae, for instance, males show complex weapon polymorphisms related to cheliceral size and morphology (Painting et al., 2015; Powell et al., 2020). In some species, three male morphs can be recognized: 'gammas' are small-bodied males with small, reduced chelicerae, 'betas' are large-bodied males with long, slender chelicerae, and 'alphas' are large-bodied males with short but robust chelicerae. Gamma males adopt a scrambling tactic, wandering around to find mates and avoiding contests with other males, while alpha and beta males adopt a fighting tactic, using their exaggerated chelicerae as weapons to defend resources (Painting et al., 2015; Powell et al., 2020). Males of the three morphs autotomize legs, and the frequency of leg autotomy in natural populations may be as high as 65% (Powell, Painting, Hickey, Machado, & Holwell, 2021).

To explore how predation risk varies between male tactics, we used the male trimorphic New Zealand harvestman *Forsteropsalis pureora* (Opiliones: Eupnoi: Neopilionidae). The three male morphs in this species co-occur within single populations and show marked differences in overall body size and cheliceral size and shape, with corresponding variation in mating tactics (Powell et al., 2020; Fig. 1). Given this intrapopulational variation in weapon morphology and reproductive behaviour, we tested the hypothesis that males exhibiting different tactics experience differential

predation pressure. Assuming that (1) scrambling gamma males are more vagile and encounter more potential predators than alpha and beta males, (2) exaggerated chelicerae may defend fighting males belonging to the alpha and beta morphs against some predators and reduce the need for leg autotomy and (3) leg loss may make locomotion with exaggerated, cumbersome chelicerae more difficult for alpha and beta males, we predicted that adult gamma males would exhibit a higher frequency of leg autotomy than adult males of the other two morphs. Alternatively, a mutually exclusive prediction would be that gamma males have a lower frequency of leg autotomy than males of the other two morphs because their scrambling reproductive tactic relies mostly on moving around, and thus leg loss would reduce both their locomotor performance and their mating success. To test these predictions, we compared the frequency of leg autotomy between male tactics (fighting versus scrambling) in a natural population of *F. pureora*.

We then experimentally tested the hypothesis that the latency to autotomize legs differs between male tactics, leg type and prior autotomy. We predicted that males exhibiting different tactics would respond differently because they pay different costs for losing different leg types. For instance, fighting alpha and beta males may hesitate longer or not autotomize ambulatory legs (i.e. the first, third and fourth pairs) if they need intact legs to support heavy weaponry (Goyens, Van Wassenbergh, Dirckx, & Aerts, 2015; Painting & Holwell, 2013) or if the total number of intact legs is important for success in male–male contests, as previously reported for other harvestman species (Macías-Ordóñez, 1997). Scrambling gamma males, in turn, may hesitate longer or not autotomize sensory legs (i.e. the first and second pairs) if they need intact legs to navigate on the vegetation or to detect chemical cues from females and/or harems (Willemart, Farine, & Gnaspini, 2009). Finally, given that leg loss is known to be costly in other harvestman species (e.g. disadvantaged in male–male competition: Macías-Ordóñez, 1997; reduced locomotor performance: Domínguez et al., 2016; Escalante, Albin, & Aisenberg, 2013; Escalante, Badger, & Elias, 2020; Escalante, Ellis, & Elias, 2021; Guffey, 1999; Houghton, Townsend, & Proud, 2011; reduced foraging ability: Guffey, 1999; increased oxygen consumption: Escalante et al., 2021), we tested whether the number of legs already missing would affect latency to drop another leg, with the assumption that the cost of leg loss is compounded by losing additional legs, regardless of the male tactic.

METHODS

Study Species and Collection

Forsteropsalis pureora is a long-legged harvestman endemic to New Zealand and distributed throughout the North Island (Taylor, 2013). Individuals are active mainly at night, when they are found out resting and foraging on broad leaves, tree trunks and mossy banks in native forest, most often near streams and other water sources. Like most harvestman species, *F. pureora* is sexually dimorphic, with males and females differing in body size, colour and cheliceral size and morphology (Fig. 1). Intrasexual variation among males is dramatic (Powell et al., 2020). Gamma males have up to seven times lower mass than alpha and beta males (Powell, 2020). Moreover, gamma males possess chelicerae that are up to 4.5 times narrower than those of alpha males and up to 4.5 times shorter than those of beta males (Fig. 1). Individuals of both sexes use the chelicerae, the third segment of which is a pinching claw, for grabbing and manipulating food. Because chelicerae are highly sexually dimorphic and intrasexually polymorphic in males, they primarily function as weapons in alpha and beta males, rather than as antipredator defence (Painting et al., 2015; Powell et al., 2020,



Figure 1. The New Zealand harvestman *Forsteropsalis pureora* has striking sexual and intrasexual variation in body size and also in cheliceral size and shape. Based mostly on cheliceral size and shape, three male morphs can be recognized: (a) alpha males are large-bodied with short but robust chelicerae, (b) beta males are large-bodied with long, slender chelicerae, and (c) gamma males are small-bodied with small chelicerae. (d) Female. Photographs by E. C. Powell.

2021; Taylor, 2004). However, like other harvestman species (Gnaspini & Hara, 2007), individuals of both sexes and all male morphs do use their chelicerae to pinch potential aggressors (Powell et al., 2021). The other defence mechanism commonly exhibited by males and females of *F. pureora* is leg autotomy, for which the breakage point occurs at the femoral–trochanter joint (Powell et al., 2021).

Adult males of *F. pureora* were collected between Waitomo and Te Angra, New Zealand, across four sites: Ruakuri Bushwalk, Waitomo (38°15′53.7″S, 175°04′46.4″E); private land, Te Angra (38°15′41.4″S, 175°00′53.6″E); Tawarau Forest, Te Angra (38°17′24.8″S, 174°56′50.2″E); and Marokopa Falls Track, Te Angra (38°15′33.6″S, 174°50′54.6″E). These four sites are located 5–20 km from each other, and we considered all individuals collected in the field as belonging to the same population. Individuals were hand-collected between 2100 and 0200 hours in January and February 2018. They were then placed in individual large vials and transported to the laboratory. Sexual maturity was confirmed by the presence of a functional genital operculum, which is fused during the juvenile stage, but can be opened upon the adult moult.

This research followed the ASAB/ABS Guidelines for the use of animals in research and the legal requirements under the Animal Welfare Act 1999 for New Zealand. Harvestmen were hand-collected from private land with permission from the landowners and from the New Zealand Department of Conservation (permit no.: 50566-RES). While in captivity, harvestmen were kept in small groups with food and water provided ad libitum. Upon completion of the study, a subset of the harvestmen were humanely euthanized for future study while the rest were released where they were collected.

We used digital callipers (to nearest 0.01 mm) to measure male body size and weapon size. First, we measured the prosoma (carapace) width as a proxy for body size because it is fixed in size, and thus not affected by feeding or desiccation. We measured the

total length of the fused second and third segments of the right chelicera (chelicera length) and measured cheliceral width as the most dilated point of the second cheliceral segment. Powell et al. (2020) conducted geometric morphometric analyses for a subset of the sample and detected a trimorphism (with three discrete morphs) by combining both linear measurements and geometric morphometrics. Following this designation of morphs, we assigned each male to one of three morphs (Fig. 1a–c). We considered alphas and betas to be major males exhibiting a fighting tactic. Thus, we grouped these two morphs together for analyses, because our hypotheses on the risk of predation apply to the tactics used, rather than the individual morphs. In total, we had 63 fighting males and 23 scrambling males.

Observational Data: Frequency of Leg Autotomy in the Field

To estimate the prevalence of autotomy in *F. pureora*, we calculated the overall frequency of leg loss in a subset of wild-collected adult males for which we had preserved specimens ($N = 86$). We compared frequency of leg loss for leg types (I = first pair; II = second pair; III = third pair; IV = fourth pair) because legs in harvestmen may serve different purposes, such as locomotion (legs I, III and IV), chemo- and tactile reception (mainly legs I and II) and intraspecific interactions (mainly legs I and II) (Guffey, 1998, 1999; Sensenig & Shultz, 2006; Willemart et al., 2009). Upon collection, we visually inspected individuals and recorded the legs that were missing, including overall number and type of leg (i.e. I, II, III or IV). Using observational data from field-collected individuals, we first calculated the overall frequency of missing legs in the population. To determine whether the frequency of autotomy differs between leg types, we used a generalized linear mixed model (GLMM). The response variable was presence or absence of autotomy in each leg (with binomial distribution of errors), the

predictor variable was the leg type, and male identity was included as a random factor.

To consider only autotomy that occurred during the adult life stage, we examined the remaining trochanter and coxa of preserved specimens. Harvestmen are unable to regenerate legs and develop scars where legs are lost. Based on the scars, it was possible to tell whether legs were autotomized during the adult stage or during a prior life stage (before the final moult). Fresh scars representing autotomy during adulthood are characterized by a fully intact trochanter. In autotomy that occurs during development, the trochanter is reduced or even absent (R. Macías-Ordóñez, personal communication). We labelled scars that occurred during adulthood as 'recent' and scars that occurred before adulthood as 'old'. For the 50 (of 86 total) individuals that experienced autotomy, we classified the autotomy as recent or old for each leg missing upon collection.

To analyse the data, we used a model selection approach (Burnham & Anderson, 2002) using GLMMs in the R packages *glmm* (Knudson, 2015), *lme4* (Bates et al., 2014) and *bblme* (Bolker & R Core Team, 2010). We compared the frequency of missing legs, taking into account male tactic, leg type and whether males had legs missing during development. The response variable was presence or absence of autotomy in each leg (with binomial distribution of errors), the predictor variables were male tactic (fighting or scrambling), leg type (I, II, III or IV) and occurrence of missing legs during development (yes or no), and male identity was included as a random factor. We constructed a full model that included the interaction between the three predictor variables and all simpler versions of this full model. We compared the fit of all alternative GLMMs using Akaike's information criterion for small samples (AICc), and all models with $\Delta\text{AICc} < 2.0$ were considered equally plausible.

Experimental Data: Latency to Drop a Leg

Using 130 adult males (gamma: $N = 39$; beta: $N = 79$; alpha: $N = 12$), we performed a predator simulation experiment to test whether males of the two tactics (fighting versus scrambling) differed in latency to autotomize legs, signifying potential intra-sexual differences in the costs individuals may incur after autotomy of legs. In this analysis, we also considered whether the type of leg targeted by a predator may affect latency to drop, because it is known that leg types have different functional roles (Escalante et al., 2013; Escalante et al., 2020; Escalante et al., 2021; Houghton et al., 2011) and the morphs may pay different costs for losing different legs. Lastly, we considered whether missing legs prior to a predator simulation experiment (i.e. the presence of previous autotomy) affected the latency to autotomize further legs.

Individuals ($N = 130$) were placed in a $14 \times 23 \times 15$ cm (width \times length \times height) clear plastic chamber and allowed to acclimate for 1 min. The bottom of the chamber was lined with white felt fabric to provide traction. After acclimation, we used metal forceps to grasp the middle of the femur of the targeted leg. For the first part of the sample, we focused on sensory leg II ($N = 42$). We then randomly selected a locomotory leg for the remainder of the sample, alternating between left and right (leg I, $N = 32$; leg III, $N = 28$; leg IV, $N = 27$). If that leg type was already autotomized on the individual (on either side), we moved to the next locomotory leg. Thus, no individual had autotomy induced where they would be missing two legs of the same type as this combination is rarely found in nature (E. C. Powell, personal observation).

We only grasped legs of individuals that were motionless, although it was not possible to always grasp the leg on the first attempt as individuals often attempted to flee. Once grasped, we held the leg consistently without pulling it for 10 min or until the

leg was autotomized. If the leg was autotomized, we recorded the time at which the leg was dropped. Although we were not able to be blind to male phenotype or the predictions being tested during this experiment, we stress that the same forceps was used for every trial to aid in consistency and that care was taken to apply the same pressure on the grasped leg. Although we cannot rule out the possibility that some human variation might have affected the latency to drop, we argue that any possible between-trial variation was evenly distributed among males exhibiting the two mating tactics and had little (if any) effect on our results.

We first examined the predator simulation data by exploring the number of individuals that autotomized the targeted leg during the test and examined the proportion of the overall sample that autotomized it within 10 s, within 1 min, and within the total 10 min period. This information was important to explore whether autotomy is always an immediate response, or whether costs and benefits may be weighed and based on a decision to autotomize or not. We considered three variables that may influence latency to autotomize a leg with the following predictions. (1) Variation between male morphology and behaviour may cause the two male tactics to experience variable costs after losing a leg. (2) Different leg types have different functions in harvestmen, with legs I and mainly II serving a sensory function and legs I, III and IV serving for locomotion. Thus, costs of autotomy could differ between leg types, affecting leg retention in a predator attack. (3) Individuals with missing legs, which had already autotomized before the predator simulation, may be less likely to autotomize or have higher latency to autotomize because costs should rise with more missing legs, regardless of the male tactic.

To test the predictions above, we used the *coxph* function from the 'survival' package in R (Therneau, 2019) to perform a Cox proportional hazards model of survival to analyse the effect of the variables male tactic, leg type and missing legs on the time it took for an individual to autotomize a leg. We first employed a model with predictive variables of male tactic (fighting or scrambling), leg type (I, II, III or IV) targeted in the experiment and number of legs missing prior to the experiment (0, 1, 2 or 3), along with interactive effects of these variables. Upon finding no support for interactive effects, we performed a reduced model, with additive variables of male tactic, leg type and number of missing legs. We then employed post hoc tests using the function 'pairwise_survdiff', which calculates multiple pairwise comparisons and corrects for multiple testing, in order to compare the leg retention for the significant variables in the models.

All statistical analyses were completed in R 3.5.2 (R Core Team, 2018).

RESULTS

Observational Data: Frequency of Autotomy in the Field

When all autotomy that occurred was examined (including autotomy during both adulthood and juvenile development), we found that 52% of 86 adult males were missing at least one leg upon collection. Among the autotomized males ($N = 44$), 28 were missing one leg, 15 were missing two legs, one was missing three legs, and one was missing four legs. We found that leg types were missing at unequal frequencies in field-collected individuals, and leg II was missing more frequently than the other legs ($\beta \pm \text{SE} = 1.37 \pm 0.42$; $z = 3.24$; $P = 0.001$; Fig. 2).

Among the 86 males included in our sample, 13 lost legs during adulthood. The best-fitted model to explain leg autotomy during adulthood included only leg type as a predictor variable (Table 1). However, there were two other concurrent models with $\Delta\text{AICc} < 2.0$, both with more parameters than the best-fitted

model: one including the additive effect of leg type and tactic, and another including the additive effect of leg type and past autotomies (i.e. legs lost during development) (Table 1). The Akaike weight of each predictor variable was as follows: leg type = 0.756; tactic = 0.471; past autotomies = 0.353.

Experimental Data: Latency to Drop a Leg

Of the 130 individuals we tested, 45 did not autotomize a leg during the predator simulation experiment. Of the 85 individuals that autotomized, 48 (56.47% of 85) autotomized within 1 min, and 25 of those (29.41% of 85) took 10 s or less to autotomize. The remaining 37 individuals (43.53% of 85) took over 1 min to autotomize, and three of those (3.53% of 85) took over 9 min before autotomizing the leg (Fig. 3).

Upon finding no interactive effects between the variables (i.e. male tactic, leg type and previous missing legs), we performed a model including only additive effects. The model was significant (likelihood ratio = $\chi^2_3 = 30.25$, $P = 0.01$). Male tactic was not significant ($z = -1.35$, $P = 0.18$; Fig. 3a) nor was the type of leg targeted during the predator simulation ($z = 1.42$, $P = 0.16$; Fig. 3b). However, the number of missing legs was a significant predictor of the latency to autotomize a leg ($z = -3.73$, $P = 0.0001$; Fig. 3c). In a log-rank comparison, the difference between missing no legs and missing a single leg was not significant ($P = 0.180$), nor was there a significant difference in individuals missing one leg and individuals missing two legs ($P = 0.085$). However, individuals missing two legs had higher latency to autotomize a leg than individuals missing no legs ($P = 0.002$; Fig. 3c).

DISCUSSION

Here we explored how predation risk and responses to such risk vary between male tactics of the male trimorphic harvestman *F. pureora*. As a proxy of predation risk, we used the frequency of leg autotomy (Schoener, 1979), a commonly employed antipredator defence in the study species (Powell et al., 2021). We hypothesized that males would experience differential predation pressure because of the drastic variation in weapon morphology and behaviours associated with their different reproductive tactics. However, when we compared leg loss during the adult stage, we found no difference in the frequency of autotomy for the two male tactics. Thus, we found no evidence to support our hypothesis of

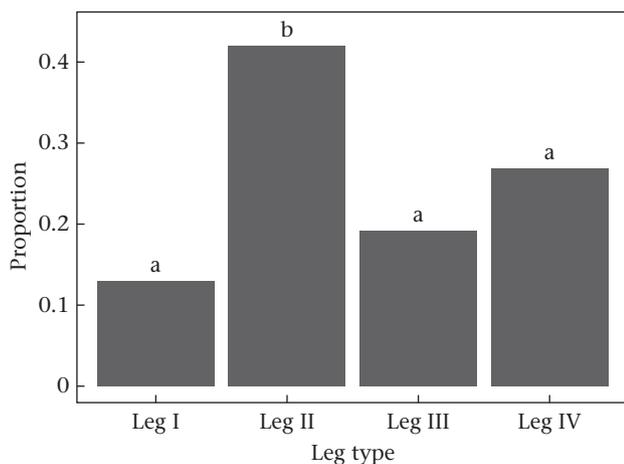


Figure 2. Proportion of leg types autotomized in field-collected individuals of the New Zealand harvestman *Forsteropsalis pureora* across all life stages ($N = 86$). Different letters above the bars indicate significant difference in a post hoc test.

Table 1

Model selection for adult leg autotomy in males of the New Zealand harvestman *Forsteropsalis pureora*

Concurrent models	AICc	k	Δ AICc	AICc weight
$N = \text{LT}$	284.46	5	0	0.251
$N = \text{Tactic} + \text{LT}$	284.88	6	0.4	0.205
$N = \text{LT} + P$	286.17	6	1.7	0.107
$N = 1$ (null)	286.52	2	2.1	0.090
$N = \text{Tactic} + \text{LT} + P$	286.67	7	2.2	0.083
$N = \text{Tactic}$	286.93	3	2.5	0.073
$N = \text{LT} \times P$	288.12	9	3.7	0.041
$N = P$	288.21	3	3.7	0.039
$N = \text{Tactic} + \text{LT} \times P$	288.63	10	4.2	0.031
$N = \text{Tactic} + P$	288.72	4	4.3	0.030
$N = \text{Tactic} \times \text{LT}$	288.92	9	4.5	0.027
$N = \text{Tactic} \times P$	290.67	5	6.2	0.011
$N = \text{Tactic} \times \text{LT} + P$	290.73	10	6.3	0.011
$N = \text{Tactic} \times \text{LT} \times P$	297.59	17	13.1	<0.001

N : number of legs lost during adulthood; Tactic: male reproductive tactic (fighting or scrambling); LT: leg type (pair I, II, III or IV); P : past autotomy (legs lost during development). The symbol '+' represents an additive effect and the symbol '×' represents an interaction between the variables. AICc is the Akaike information criterion for small samples, k is the number of parameters of each model, Δ AICc is the difference between the AICc value of each model and the AICc of the best-fitted model (in bold) and AICc weight is the relative weight of evidence of each model.

differential predation pressure among males exhibiting different mating tactics. This finding was surprising as we predicted that scrambling gamma males would experience more predation pressure due to riskier behaviours, such as intense movement across the environment to search for unguarded females. Although a number of vertebrate and invertebrate predators were found to eat neopilionid harvestmen in New Zealand (Powell et al., 2021), it seems that intraspecific variation in size and movement rates is unlikely to affect predation risk. However, it should also be considered that individuals who were captured and eaten by predators were not included in the sample and, thus, using autotomy as a proxy of predation comes with limitations. In fact, according to the mathematical model by Schoener (1979), if there are significant sources of mortality that result in death rather than autotomy, predation risk could not be precisely inferred using the frequency of nonlethal injuries, such as tail or limb autotomy.

In other taxa with exaggerated weaponry, but without autotomy, trade-offs between weapon expression and locomotion have been explored. In some species, males with large weapons pay locomotory costs (e.g. Goyens et al., 2015), but in other species, they do not appear to suffer any biomechanical disadvantages (e.g. McCullough & Emlen, 2013; McCullough & Tobalske, 2013), possibly because the disadvantages have been eliminated by compensatory mechanisms (Painting & Holwell, 2013). While it is not yet known whether the exaggerated chelicerae of alpha and beta males in *F. pureora* compromise locomotion generally, it is possible that gamma males have higher speed and manoeuvrability, as already reported for the minor morph of the dung beetle *Onthophagus taurus* (Moczek & Emlen, 2000). Therefore, gamma males could be better able to escape predator attacks even if they are attacked at disproportionate rates by predators while scrambling for mates. Experiments to investigate the difference in sprint speeds among male morphs would be an important next step to tease out these ideas. Because harvestmen may autotomize legs during juvenile development but never regenerate them, another interesting future direction would be to consider how juvenile autotomy might act as a proximate mechanism underlying male polymorphisms in this group. If losing a leg during early life stages compromises foraging activities (e.g. Guffey, 1999; Sankey & Savory, 1974), individuals missing legs would feed at lower rates

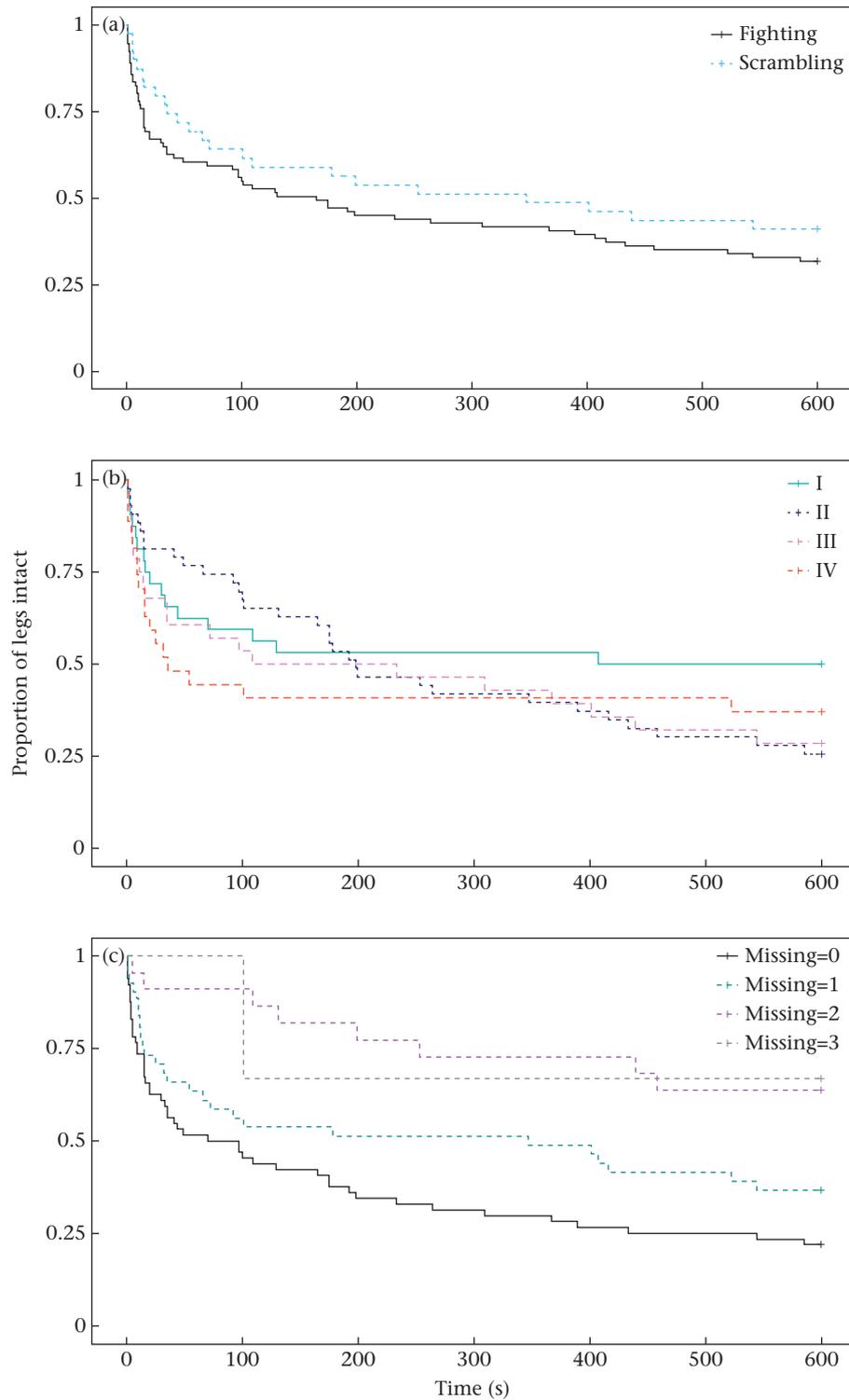


Figure 3. (a) Curve of leg retention among males of the New Zealand harvestman *Forsteropsalis pureora* showing two reproductive tactics (fighting, $N = 91$; scrambling, $N = 39$) during the predator simulation experiment. (b) Curve of leg retention between each leg type targeted in the predator simulation experiment: leg I ($N = 32$), leg II ($N = 43$), leg III ($N = 28$) and IV ($N = 27$). (c) Curve of leg retention for individuals missing legs prior to the predator simulation experiment: missing no leg ($N = 64$), missing one leg ($N = 41$), missing two legs ($N = 22$) and missing three legs ($N = 3$).

and would have poorer body condition when compared with individuals without missing legs. Assuming that body condition is the cue used by the males to define their morphs as adults (Buzatto & Machado, 2014), individuals with missing legs and in poor body

condition would hypothetically have a higher chance of becoming gammas.

Overall, leg autotomy was frequent among our entire sample, with 54% of the individuals missing at least one leg. However, only

29% of autotomy occurred during the adult stage. Individuals collected in the field for this study with autotomized legs were most frequently missing one leg, and individuals missing three or four legs were extremely rare. The overall frequency of leg autotomy that we found in *F. pureora* matches that found in studies of other Eupnoi harvestmen in North America and Central America. Thus far, frequency of leg autotomy has only been calculated for members of the family Sclerosomatidae (Eupnoi) that inhabit forests: *Leiobunum nigripes* (47%, Guffey, 1998), *Leiobunum vitattum* (45%, Guffey, 1998), *Leiobunum formosum* (61%, Houghton et al., 2011), *Leiobunum politum* (36%, Houghton et al., 2011) and *Prionostemma* spp. (71%, Domínguez et al., 2016). However, these studies did not consider whether legs were autotomized during the juvenile or adult stages, so we are unable to compare autotomy in different life stages among species. Given that sclerosomatid males do not have exaggerated weaponry of any type and no case of male polymorphism has been reported so far in the family (Buzatto & Machado, 2014), it would be interesting to investigate whether the frequency of autotomy in the adult stage varies between males of sclerosomatids and neopilionids, such as *F. pureora*. A higher frequency of autotomy during the juvenile stages of the neopilionids when compared with the sclerosomatids would suggest that the costs of losing legs during adulthood are higher in species in which the males bear exaggerated, cumbersome weapons.

When considering autotomies that occurred during both the preadult and adult stages, the leg types were lost at unequal frequencies, with leg II showing higher frequency of autotomy than the other pairs of legs. In both the North American species *L. nigripes* and *L. vitattum*, leg II was missing most often in a field study (Guffey, 1998) as was also the case for *Prionostemma* in Costa Rica (Domínguez et al., 2016). Although it seems surprising that the 'antenniform' legs with great sensory importance are missing most often, these legs are likely lost first because they are held away from the body and may be the first part of the body to make contact with the surrounding environment, including potential predators (Guffey, 1999). Moreover, leg II is longer than the other pairs of legs (regardless of the sex or male morph) and may be more easily clasped by a potential predator. Thus, despite its important role in chemo- and tactile perception (Willemart et al., 2009), leg II is probably under higher risk of being singled out during a predatory attack. According to our results, this risk does not differ between male tactics, suggesting that differences in morphology and reproductive behaviours between the morphs do not have a relevant effect on the probability of missing leg II, as well as the other leg types.

In the predator simulation experiment, over one-third (35%) of all individuals ($N = 45$) failed to autotomize a leg during a trial and only 25 of 130 (19%) autotomized a leg within 10 s. Where individuals did not autotomize right away, most used their pinching chelicerae claws for defence, regardless of cheliceral size. These findings suggest that autotomy is a costly behaviour, used only when all other defensive mechanisms have failed (Edmunds, 1974). But what factors determine the latency to autotomize a leg? We predicted that three main factors would affect this latency: (1) male tactic; (2) type of leg targeted; (3) previous legs missing. We found no evidence for differential leg retention among male tactics or among the type of leg targeted in a predator simulation experiment. However, the number of previous missing legs did affect leg retention. While leg retention did not differ between individuals missing no legs and individuals missing one leg, individuals missing two legs autotomized more slowly or not at all. These results reinforce the notion that leg loss is costly for long-legged harvestmen and is compounded by losing additional legs. For instance, walking speed and prey detection were significantly compromised for *Leiobunum* spp. harvestmen only when

individuals lost three legs (Guffey, 1999). Furthermore, in *L. vittatum*, the probability of a male winning a fight for territory possession is positively influenced by the number of legs he has, so that individuals missing two or more legs usually lose contests (Macías-Ordóñez, 1997). Because we found very few individuals missing three or more legs in the field, and individuals missing at least two legs autotomized slowly or not at all in the experiment, it is likely that harvestmen survive with one or two legs missing with some costs, but losing three or more legs is beyond what individuals can cope with. Thus, the willingness to autotomize a leg is mediated by prior experience and the individual's current state.

In summary, leg autotomy is a common defensive strategy for the harvestman *F. pureora*. While leg autotomy allows escape from predators, harvestmen must cope with the costs of leg loss from thereon because, unlike spiders, there is no leg regeneration (Roth & Roth, 1984). We found little evidence for differential predation pressure on the two male tactics or for variation in male behaviour when faced with a simulated predator attack. We found that harvestmen already missing legs took longer to autotomize an additional leg. This finding suggests that the costs of leg loss are compounded and that harvestmen may weigh the costs and benefits of autotomy, but this appears to be independent of male tactic.

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