

Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation

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Summary

1. Prey can increase the probability of escaping a predator attack by adopting either behavioural tactics that provide more time or less distance to escape or by increasing their escape performances. It is assumed that the ability to improve escape performances is reserved for species that respond to chronic predation risk by substantially altering morphological traits related to locomotion. This thinking is at odds with fundamental predictions of physiological stress theory that suggest performance should be enhanced both independently of and in addition to morphological change.

2. The purpose of this study was to determine whether and how prey raised under chronic risk of predation can improve their escape performance independent of conspicuous morphological changes.

3. We reared herbivore grasshopper in field mesocosms with and without disarmed hunting spiders and measured their outdoor jumping performance. Grasshoppers were then transferred to the laboratory where we measured the biomechanics of their jumping to elucidate mechanisms that differentiate performance between rearing treatments. We collected second generation nymphal offspring from the field mesocosms and measured their morphology and escape performances to reveal costs associated with antipredator responses.

4. Chronically scared grasshoppers made adjustments in jumping technique that enabled them to take-off 1.2 times faster, which, in combination with additional in-flight behavioural adjustments, led to 2.6 times longer jump distances. These improvements were independent of conspicuous morphological changes but were accompanied by attendant costs: grasshoppers reared in risky environments produced smaller and less athletic offspring than grasshoppers from risk free environments.

5. The results suggest that prey do not need to undergo conspicuous morphological changes in locomotor traits before they can improve escape performance, revealing an adaptive and possibly common aspect of physiological stress reaction to predation.

Key-words: escape behaviour, functional traits, inducible defenses, jumping technique, physiological stress, predation risk, predator–prey interactions, whole-animal performance

Introduction

Prey can increase their probability of escaping a predator attack by using behaviours that enhance response times or shorten distances to the nearest refuge; or by enhancing

their actual escape performances (Lima & Dill 1990; Kats & Dill 1998; Benard 2004; Blumstein 2010). A widely held view in ecology is that enhanced escape performance derives exclusively from conspicuous changes in prey morphological traits such as locomotor appendages (e.g. tail size and shape) or structural defences (e.g. protective shells, spines, larger body sizes) (Tollrian 1995; Relyea 2001; Benard & Fordyce 2003; Benard 2006; Brookes & Rochette 2007; Hoverman & Relyea 2007; Urban 2007).

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Much of the evidence for this response comes from thorough studies of a few aquatic taxa (Tollrian & Harvell 1999; Relyea 2005). Yet, neurophysiologists have long acknowledged that exposure to risk of predation or other environmental stressors should stimulate physiological stress responses in taxa broadly and that stress can have lasting effects on prey escape performances independently of or in addition to morphological changes (Cannon 1915; Selye 1936). Physiological stress is an evolutionary conservative syndrome of neuroendocrine responses that increases survivorship during life threatening situations by allocating resources to vital systems (i.e. brain, sensory and locomotor) that affect animal performances and by maintaining homeostasis (Bonga 1997; Wingfield & Ramenofsky 1999; Sapolsky, Romero & Munck 2000). Persistent risk of predation may induce long-lasting physiological stress responses – ‘emergency life history stage’ (Wingfield *et al.* 1998) that may enduringly enhance prey escape performance but can compromise allocation of resources to growth and reproduction. However, ecological studies to date have exclusively examined predator induced changes in escape performances only in cases in which conspicuous changes in morphological traits related to performance were detected. Consequently, tactics that do not involve conspicuous morphological changes, but which may be common, will be overlooked.

We report on mechanisms underlying a tactic used by *Melanoplus femurrubrum* grasshopper prey to improve their escape performance in response to chronic perceived risk of spider predation. We also provide evidence for fitness costs that are predicted by stress theory to accompany changes in escape performances. This grasshopper is an excellent model species for such an investigation because it displays no evident morphological changes in response to chronic risk of predation by hunting spiders (Schmitz & Suttle 2001). Yet, it shows strong physiological responses to chronic risk of predation including elevated metabolism and changes in body nutrient content (D. Hawlena & O. J. Schmitz, unpublished data). Our results suggest that we may need to revise the conception that enhanced escape performances arise only when prey exhibit conspicuous morphological changes in locomotor or defence traits.

Materials and methods

Our study is comprised of several systematic steps. We initially stocked juvenile grasshoppers in field mesocosms and reared them to adulthood under non-risk (control) or chronic perceived predation risk (treatment) conditions. Adult grasshoppers were then removed and we measured their outdoor jumping performance. Grasshoppers were then transferred to the laboratory where we measured the biomechanics of their jumping to elucidate mechanisms that differentiate performance between rearing treatments. We used this complementary experimental approach because animals often express very different performance levels in laboratory versus field conditions (Irschick 2003). Sample sizes varied between the complementary experimental approaches due to grasshopper limb damage and mortality between the experimental steps. Finally, to reveal fitness costs that are expected to be associated with stress

responses, we collected second generation nymphal offspring from field mesocosms and measured their morphology and escape performances.

FIELD REARING CONDITIONS

We placed seven pairs of 0.25 m² basal area × 1 m high mesocosms over growing vegetation in a meadow in the Yale-Myers Research Forest, USA. During June 2007 we captured, using sweep nets, third instar *M. femurrubrum* grasshopper nymphs and randomly assigned six individuals to each of the 14 mesocosms. One day later, we added one adult *Pisaurina mira* spider to a randomly assigned mesocosm of each pair. The paired spider-free mesocosms served as a control. We rendered spiders ineffective at subduing prey by gluing together their chelicerae with non-toxic, quick drying cement. Gluing mouthparts does not change spider activity and grasshoppers do not seem to distinguish between manipulated and un-manipulated spiders (Schmitz, Beckerman & O'Brien 1997). All spiders survived until the end of the field experiment. In late September 2007, we collected adult grasshoppers from the cages and immediately transferred them to individual containers in the laboratory according to matched spider and spider free treatment pairs.

OUTDOOR JUMPING DISTANCE TRIALS

All jumping distance measurements were conducted on sunny days at 22 ± 3 °C. Grasshoppers from a mesocosm pair were measured consecutively to reduce variation arising from differences in environmental conditions between days. We allowed grasshoppers to acclimate to the outdoor conditions for 30 min prior to experimentation. Grasshoppers were randomly picked and placed in a flat arena. They were then approached from 3 m behind, by walking quickly (114.3 ± 1.8 m min⁻¹) and stimulated by light foot-tapping. We marked the grasshopper's exact location after each jump. We halted the experiment when the grasshopper refused to jump in response to two sequential approaches. We recorded the distance between each two sequential jumps using 10 m measuring tape. Simulated attack by humans on grasshoppers and other small animals was found to be an effective method to test escape behaviour (Stankowich & Blumstein 2005; Cooper 2006).

LABORATORY MEASUREMENTS OF GRASSHOPPER BIOMECHANICS

We placed all containers under natural illumination from daylight spectrum photographer's lamps at constant temperature of 28 ± 1 °C (the room temperature was 20 ± 1 °C). We allowed grasshoppers to acclimate for 1 h and then took individual grasshoppers and placed them on a platform on a laboratory bench. We positioned the grasshopper's main body axis perpendicular to a high speed digital video camera (Photron Fastcam 1024PCI; Photron USA Inc., San Diego, CA, USA) with a 50-mm lens (Nikkor; Nikon Inc., Melville, NY, USA) used to image the jumps at a rate of 2000 frames per second. A typical high speed imaging is presented in Appendix S1. To initiate a jump, we touched a grasshopper using a small paint brush. We approached the grasshopper at an angle of approximately 45° relative to the platform in the focal plane of the camera. We excluded jumps in which the grasshopper jump direction deviated more than 15° from the focal plane of the camera. The uncertainty of 15° in the angle between jump direction and focal plane leads to an uncertainty of 3% in the measurement of the grasshopper position. We used

IMAGEJ software (National Institute of Health, Bethesda, Maryland) to track the positions of the coxa (approximately the centre of mass of the grasshopper) (Bennet-Clark 1975), the knee, the joint between the tarsus and the tibia and the main body axis, from 40 frames (20 ms) before take-off to 60 frames (30 ms) after take-off. Using these data, we calculated the speed, acceleration time, kinetic energy, average force, take-off angle α and the body and leg angles. Figure 1 provides an example for the tracking of the coxa of one of the grasshoppers. Panel 1a shows the vertical (y) displacement against the horizontal (x) displacement of the coxa position. We estimated the uncertainty in the coxa position measurements by tracking multiple jump datasets tens times each. The average standard deviation of the position measurement in x - and y -direction was 0.4 mm. This tracking uncertainty is smaller than the size of the circles indicating the coxa position in Fig. 1a. The take-off angle was determined by fitting a line to the y -displacement as a function of the x -displacement. The example shown in panel 1a has a take-off angle of $\theta = 72.57 \pm 0.06^\circ$. The error in the measurement of the take-off angle was about 0.1%. The total displacement $r(t) = \sqrt{x(t)^2 + y(t)^2}$ as a function of time is shown in panel 1b. The uncertainty for the total displacement r is about 0.6 mm. The take-off speed v , defined as the speed at which a grasshopper jumps away from its resting position, was determined by a linear fit to $r(t)$ in the region from $t = 0$ ms (take-off) to $t = 10$ ms. The example shown in panel 1b has a take-off speed of 3.24 ± 0.03 m s⁻¹. The error in the measurement of the take-off speed was about 1%.

The time point t_s at which the tibia angle α starts to increase was determined for each individual grasshopper jump as follows: Each time series of α started 20 ms before the time point of take-off (defined

to be $t = 0$ ms). At the starting point of the time series, the grasshoppers had not started its motion. The value for α at this time point was defined as the base-line value $\alpha_0 = \alpha(-20$ ms). The time point t_s was then defined as the earliest time point at which α becomes larger than α_0 and stays larger for all subsequent time points. For each grasshopper jump, t_s was then used to determine the corresponding opening angle of the femur-tibia joint $\beta_s = \beta(t_s)$. Using the individual lengths of the tibia l_t and femur l_f of the corresponding grasshopper, the mechanical advantage of the tibia lever dr/dx was then calculated using the equation $\frac{dr}{dx} = \sqrt{l_t^2 + l_f^2} - 2 \cdot l_t \cdot l_f \cdot \cos \beta_s$.

We used a simple numerical simulation to predict how jump distance is influenced by risk. The simulation calculated the ballistic flight trajectory of a grasshopper using only take-off speed, take-off angle and size dependent drag as input parameters. The drag was approximated by the Rayleigh equation $F_d = \frac{1}{2} \rho v^2 C_d A$, where ρ is the density of air, and v is the grasshopper's speed. Following Vogel (2005), we used a drag coefficient, $C_d = 0.5$, and an area, $A = (3m/4\rho_g)^{2/3} \pi^{1/3}$. The area was calculated for each grasshopper using its mass, m , and an average density, $\rho_g = 500$ kg m⁻³.

Grasshoppers were weighed using a digital laboratory scale (Ohaus: Adventure Pro AV313; ± 1 mg), were photographed using a dissecting microscope ($\times 20$) and their standard morphology (body length, tibia length, femur length, wing length and pronotum length) were measured using the software IMAGEJ. To test whether size differences of the extensor tibiae muscle could account for the observed differences in escape performances we measured the area, length and depth of the extensor tibiae muscle compartment using a three-dimensional image produced by Keyence 3D digital microscope 600E ($\times 30$).

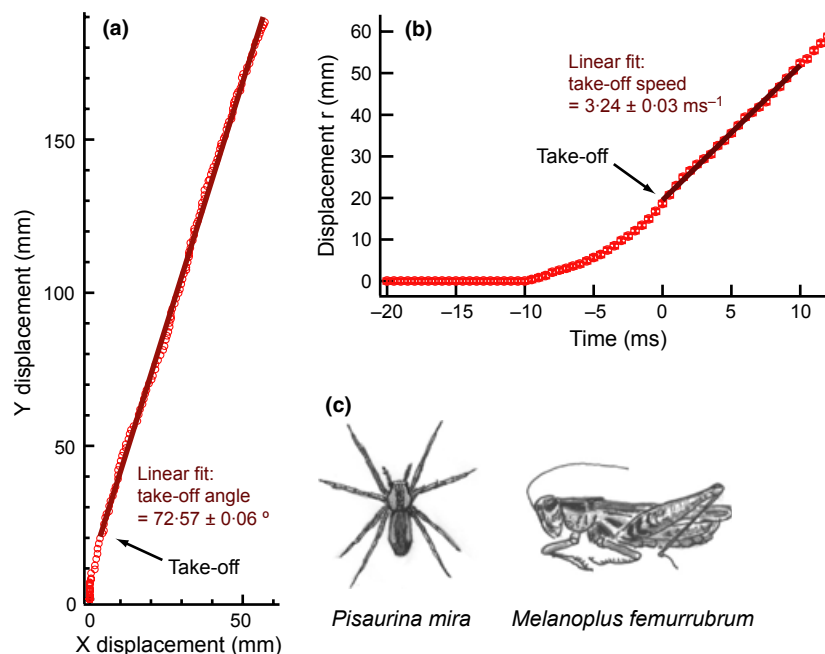


Fig. 1. An example for the tracking of the grasshopper position and illustrated images of the study organisms. (a) Vertical (y) displacement against the horizontal (x) displacement of the coxa position (approximately center of mass of the grasshopper). The uncertainty in the coxa position measurements in x - and y -direction was about 0.4 mm. This tracking uncertainty is smaller than the size of the circles. The take-off angle of the grasshopper was determined by a linear fit. The error in the measurement of the take-off angle was about 0.1%. (b) Total displacement of the grasshopper $r(t) = \sqrt{x(t)^2 + y(t)^2}$ as a function of time. The uncertainty for the total displacement r is about 0.6 mm (see error bars). The take-off speed was defined as the speed at which a grasshopper jumps away from its resting position and it was determined by a linear fit to $r(t)$ in the region from $t = 0$ ms (take-off) to $t = 10$ ms. The error in the measurement of the take-off speed was about 1%. (c) Illustrated images of the study organisms: the herbivore grasshopper *Melanoplus femurrubrum* and its predator the nursery-web spider *Pisaurina mira*.

BETWEEN GENERATION EXPERIMENT

We evaluated implications to first generation offspring of behavioural performance differences by parents reared in different risk environments. We placed six pairs of circular mesocosms, 1.6 m in diameter and 1.5 m high on naturally growing vegetation among the mesocosms of the main field experiment. During June 2007 we captured, using sweep nets, third instar *M. femurrubrum* grasshopper nymphs and randomly assigned 12 individuals to each of the 12 mesocosms. One day later, we added two adult *P. mira* spiders to a randomly assigned mesocosms of each pair. We rendered spiders ineffective at subduing prey by gluing together their chelicerae with non-toxic, quick drying cement. The paired spider-free mesocosms served as a control. We allowed grasshoppers to grow and reproduce within those mesocosms. During July 2008 we captured the second instars hatchling from all mesocosms weighed them using a digital laboratory scale (Ohaus: Adventure Pro AV313; ± 1 mg), and measured their morphology (body length, femur length and pronotum length) under a dissection microscope with reticles ($\times 30$). Then, we measured their jumping distance in an outdoor arena on sunny days at 28 ± 4 °C using the same procedure we used for adults. However, hatchling had much higher endurance than adults and hence we arbitrarily halted the experiment after 25 jumps.

DATA ANALYSIS

Outdoor experiment

We compared the number of grasshoppers that survived in spider and spider-free mesocosms using Wilcoxon signed ranks test. We compared all jumping performances recorded in the outdoor experiment between treatments using a mixed model analysis of variance (ANOVA). The risk treatment and the grasshopper sex were treated as fixed factors and the mesocosms pair in which a grasshopper was raised was treated as a random factor. Some variables were \log_{10} transformed to achieve homogeneity of variance and to normalize the variance. When reported, those results were back transformed and the 95% confidence intervals were presented instead of standard error. Two observations in which grasshoppers actively flew were deleted from the analysis.

Laboratory measurements

We used the average of at least two jumps conducted by the same individual to determine the take-off speed, acceleration time, kinetic energy at take-off, average force and take-off angle. We excluded jumps in which grasshoppers did not use both legs in a similar way, kicked the paint brush or jumps that were obviously submaximal. We compared the grasshopper variables calculated from the movies between treatments using mixed model ANOVA, in an identical way to that used to analyse the outdoor jumping performances. We used paired *t*-test to compare the predicted to the observed jumping distances of individual grasshoppers. To compare the jumping techniques between treatments, we used only the first jumps of each individual. Data for five individuals were not included because the quality of the high speed movies was not adequate to accurately track the positions of the knee and the joint between the tarsus and the tibia during acceleration and soon after take-off. We discarded those measurements from analyses. We used two-way ANOVA to compare the time at which the grasshoppers started to lift their tibia (angle α), the corresponding angles β at those time points, and the resulting tibia lever. We used repeated measure mixed model

ANOVA to compare body angles during the 5 ms before and 5 ms after grasshoppers took-off. We used Pearson correlation to test for association between angular velocity and take-off velocity. We used mixed model multivariate analysis of variance (MANOVA) to test for differences in grasshopper standard morphology and to test for differences in the dimensions of the extensor tibia muscle compartment.

Between generation experiment

Hatchling standard morphological variables failed to fulfil the assumption of parametric procedures (normal distribution or homoscedasticity). Thus, we used a nonparametric randomization multivariate analysis of variance (RAMANOVA) test (RAMAN program, Alford 1999), based on algorithms provided by Manly (1991) to compare their morphology between treatments. A Mann-Whitney *U*-test was applied to determine which behavioural index contributes to the significant multivariate effect. We used repeated measure analysis of covariance, with the femur length as covariate, to compare the jumping distances between hatchling of grasshoppers from risk and risk free environments (Fig. 4). For all statistical analysis, we reported the two-tailed *P*-values and used statistical significance value of 0.05. All analyses, except for RAMANOVA, were conducted using the statistical software package spss 13.0 (SPSS Inc., Chicago, IL).

Results

The initial jump by grasshoppers reared with spiders was significantly further (1.36 ± 0.09 m) than individuals from spider free conditions (0.53 ± 0.1 m) (Fig. 2, $F_{1,26} = 33.48$, $P < 0.001$). Grasshoppers reared with spiders jumped on average significantly longer distances (1.20 ± 0.1 m) than grasshoppers from risk free environments (0.77 ± 0.11 m) ($F_{1,26} = 8.07$, $P = 0.009$). Consequently, the cumulative distance covered by grasshoppers raised under chronic perceived risk (6.4 m; 95% CI: 3.8–10.6 m) was 2.7 times greater than grasshoppers in risk-free conditions (2.4 m; 95% CI: 1.4–4.3 m) ($F_{1,26} = 6.05$, $P = 0.021$).

As shown in Fig. 3, grasshoppers that were reared with spiders had significantly higher take-off speed (3.1 ± 0.1 m s⁻¹) than individuals from spider free conditions

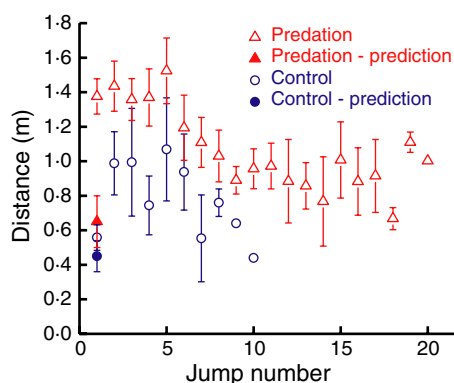


Fig. 2. Comparison of the distance jumped in response to sequential stimuli between grasshoppers reared with (red) or without (blue) spiders. Error bars denote ± 1 SE. For the two predicted values error bars denote 95% confidence intervals.

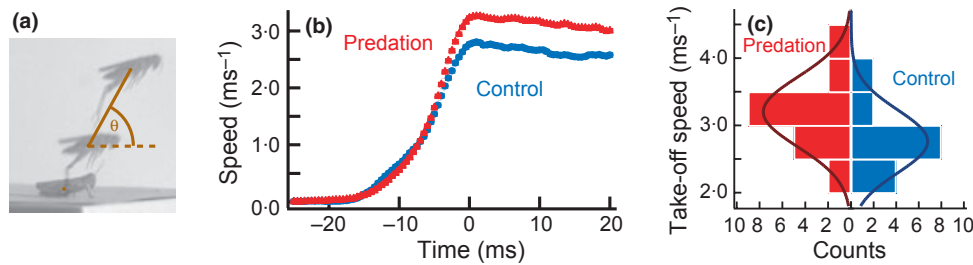


Fig. 3. Grasshopper take-off angle and speed. (a) Superposition of three images of the jump. The angle θ denotes the take-off angle of the jump. (b) The two curves show differences in average velocities between grasshoppers reared in the presence of predatory spiders (red) and grasshoppers from predator free environments (blue). Grasshoppers take-off is at time $t = 0$ ms. The acceleration phase is at times $t < 0$ ms. (c) Distributions of the take-off speed for the predation risk group (red) and the control group (blue). The predation risk group has a significantly higher take-off speed than the control group.

(2.7 ± 0.1 m s⁻¹) ($F_{1,27} = 5.83$, $P = 0.023$). Due to variation in individual masses within each group, there was no significant difference in the kinetic energy $E = \frac{1}{2} m v^2$ at take off, and therefore no significant difference in muscle energy converted into translational energy (risk-free: 1.06; 95% CI: 0.85–1.33 mJ; risk: 1.38; 95% CI: 1.11–1.72 mJ, $F_{1,27} = 2.67$, $P = 0.114$). Similarly, we found neither a difference between groups in the time taken to accelerate Δt (risk-free: 18 ± 1 ms; risk: 20 ± 1 ms) ($F_{1,27} = 0.95$, $P = 0.338$) nor difference in the average force $F = m v / \Delta t$ used to accelerate (risk-free: 0.042; 95% CI: 0.035–0.049; N:RISK 0.051; 95% CI: 0.043–0.060 N) ($F_{1,27} = 2.91$, $P = 0.10$). Finally, there were no significant differences in take-off angle, θ , defined in Fig. 3a (risk-free: $64 \pm 3^\circ$; risk: $60 \pm 3^\circ$) ($F_{1,27} = 0.66$, $P = 0.423$).

Grasshoppers reared with spiders made further behavioural adjustments during flight to travel larger distances. We used take-off angle θ and speed v to predict ballistic flight trajectories for each grasshopper, including the effects of gravity and air resistance. The simple projectile analysis predicted that grasshoppers raised with spiders should jump longer distances than individuals from spider free conditions ($F_{1,27} = 5.37$, $P = 0.028$). For grasshoppers raised in spider free conditions, predicted jump distances (0.45; 95% CI: 0.36–0.56 m) were in very good agreement with jump distances measured in an outdoor arena (0.53 ± 0.1 m) ($t_{13} = 0.987$, $P = 0.342$). However, the measured jump distances in the outdoor arena were much farther (1.36 ± 0.09 m) than a simple ballistic trajectory would suggest (0.65; 95% CI: 0.52–0.80 m) ($t_{17} = 4.975$, $P < 0.001$; Fig. 2).

Grasshopper reared with disarmed spiders altered their jumping technique (Fig. 4d). Figure 4b,c show the time courses of the average angle α between the tibia and the ground (Fig. 4a), and the average femur-tibia angle β (Fig. 4a) respectively. The red and blue arrows in Fig. 4b indicate the time points when the angle α starts to increase. The red and blue arrows in Fig. 4c show the value for the femur-tibia angle β at these time points. The onset of motion performed by grasshoppers reared with spiders was delayed ($t = -9 \pm 1$ ms) compared to the risk-free grasshoppers ($t = -11 \pm 1$ ms; $F_{1,22} = 5.809$, $P = 0.025$). This delay resulted in significantly larger corresponding femur-tibia angle β at those time points (risk: $28 \pm 2^\circ$; risk-free:

$22 \pm 2^\circ$; $F_{1,22} = 5.274$, $P = 0.032$). These changes in jumping technique result in a mechanical advantage that allows grasshoppers from risky environments to increase take-off speed. For a given change in α , the change of the grasshopper cox position $r = (x, y)$ is

$$\frac{dr}{d\alpha} = \sqrt{\left(\frac{dx}{d\alpha}\right)^2 + \left(\frac{dy}{d\alpha}\right)^2}$$

which depends on the femur-tibia angle β and the lengths of the tibia l_t and femur l_f :

$$\frac{dr}{d\alpha} = \sqrt{l_t^2 + l_f^2 - 2 \cdot l_t \cdot l_f \cdot \cos \beta}$$

This function, which can be considered to be the amount of leverage that the tibia provides (tibia lever) is shown in Fig. 4e. This function depends not on α but only on the femur-tibia angle β and it increases monotonically with β . Therefore, for a given change in α , the resulting change in grasshopper position increases with increasing angle β . The red and blue arrows indicate the angle β at the time points when the spreading of α starts and they also indicate the resulting significantly larger tibia lever for grasshoppers reared under risk of spider predation (risk: 5.3 ± 0.4 mm, risk-free: 4.5 ± 0.4 mm).

In association with these changes, we revealed systematic changes in body angle (Φ) over the time course before (5 ms), during, and after (5 ms) take-off, as shown in Fig. 4f. Grasshoppers from both treatments tilted forward and then upward (Test of within Subjects Effects: $F_{1,202} = 9.41$; $P = 0.003$). But, the jumping technique differed between the groups as a function of time (time \times treatment $F_{1,202} = 6.21$, $P = 0.014$) and also in absolute values (Test of between Subjects Effects treatment; $F_1 = 4.32$, $P = 0.048$). Grasshoppers from the risk treatment leaned forward to a steeper angle and thrust their body upward on average 2.5 ms after take-off. Grasshoppers from risk free conditions kept a flatter profile and thrust upward immediately upon take-off. As a result, grasshoppers reared with spiders tilted at take-off to a more acute angle ($10.0 \pm 3.0^\circ$) than grasshoppers from risk free conditions ($19.6 \pm 3.0^\circ$) ($F_{1,27} = 4.64$, $P = 0.040$). Correspondingly, the angular velocity at take-off differed between

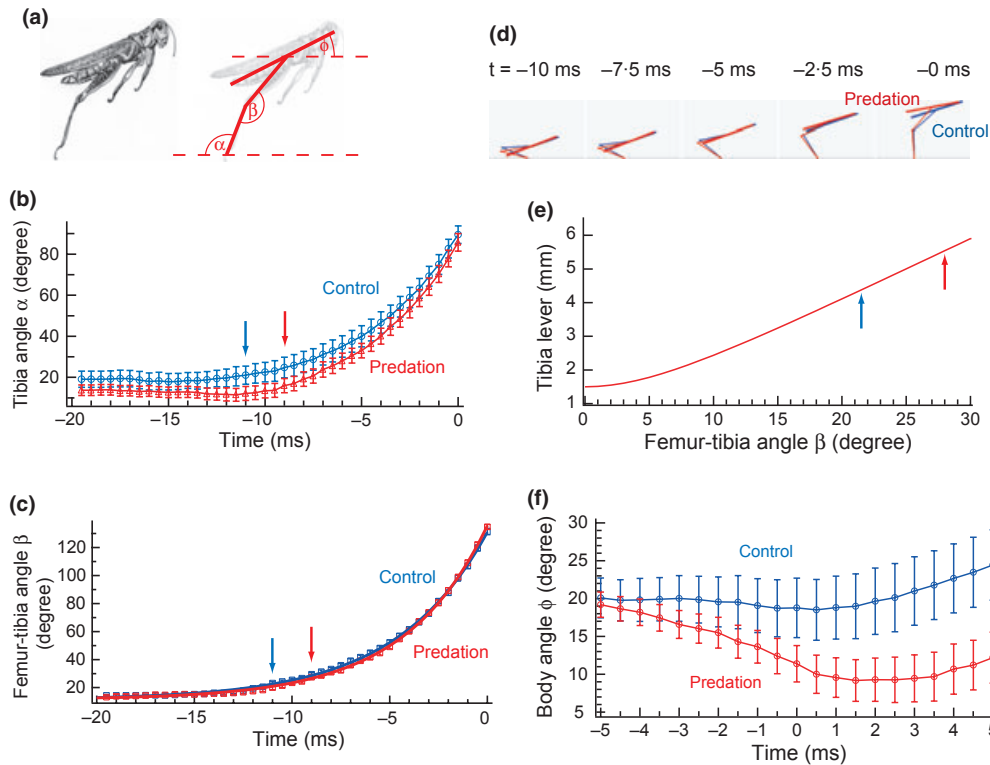


Fig. 4. Comparison of jumping techniques between grasshoppers reared with (red) or without (blue) spiders. $t = 0$ denote the take-off. (a) Scheme of a jumping grasshopper: α is the angle between the tibia and the ground, β is the angle between the tibia and the femur and Φ the angle between the ground and the main body axis. (b) Differences in tibia angle α during 20 milliseconds before take-off. (c) Differences in femur-tibia angle β during 20 milliseconds before take-off. (d) Kinematic of the jump during 10 ms before take-off. The three lines indicate the average orientation values of the main body axis, femur and tibia. (e) Mechanical advantage of the tibia lever as a function of the femur-tibia angle β . (f) Differences in body angle Φ during 5 ms before and 5 ms after takeoff. The error bars show the error of the mean value.

treatments ($F_{1,27} = 7.57$, $P = 0.010$). Grasshoppers reared with spiders had more negative angular velocity (-1.19 ± 0.52 revolutions per second) than individuals from spider-free environments (-1.02 ± 0.61 revolutions per second). We found that the angular velocity of the grasshopper body at take-off and the take-off velocity were negatively correlated ($r = -0.521$, $n = 35$, $P = 0.001$).

We did not find differences in body length, body mass, tibia length, femur length and pronotum length between grasshoppers reared with and without spiders (MANOVA, Pillai's Trace $_{5,23} = 0.16$, $P = 0.975$), nor in the length, depth and area of the exoskeleton compartment of the extensor tibiae muscle (MANOVA, Pillai's Trace $_{3,20} = 1.16$, $P = 0.351$).

The observed changes in jumping technique had no realized costs in terms of parental generation survival; indeed by the end of the field season grasshopper density in risk conditions ($18.8 \pm 1.4 \text{ m}^{-2}$) was even higher than grasshoppers in risk free conditions ($14.3 \pm 1.5 \text{ m}^{-2}$) (Wilcoxon signed ranks test; $z = -2.07$, $P = 0.038$), likely owing to predator-mediated compensatory adjustments in grasshopper competition during the course of the growing season (Ovadia & Schmitz 2002). In sharp contrast, first generation offspring of grasshoppers reared with predators differed morphologically from nymphs of grasshoppers from risk free environments (RAMANOVA, Euclidean dist = 347.26, $P = 0.043$). Offspring

of grasshoppers from risk free environments had longer pronotums (Mann-Whitney U -test, $z = -2.087$; $P = 0.037$) and femurs (Mann-Whitney U -test, $z = -2.068$; $P = 0.038$) than offspring of grasshoppers from risky environments. Moreover, second instar nymphs of grasshopper reared in risky environments had shorter escape jumps than nymphs of grasshoppers from risk free environments (Test of between Subjects Effects treatment; $F_{1,67} = 4.325$, $P = 0.041$), after controlling for differences in femur length ($F_{1,67} = 5.836$, $P = 0.018$) (Fig. 5).

Discussion

A widely held view in ecology is that enhanced escape performance in response to predation risk derives exclusively from outward changes in prey morphological traits related to locomotion (Tollrian & Harvell 1999). Accordingly, prey species that do not show morphological changes must adopt behavioural tactics to compensate constraints imposed on escape performance due to existing morphology. Using high speed imaging with a biomechanical analysis, we showed that grasshopper reared under chronic risk of spider predation improved their physical escape performances by altering their jumping technique. We also showed that these improvements were consistently evident in different testing arena

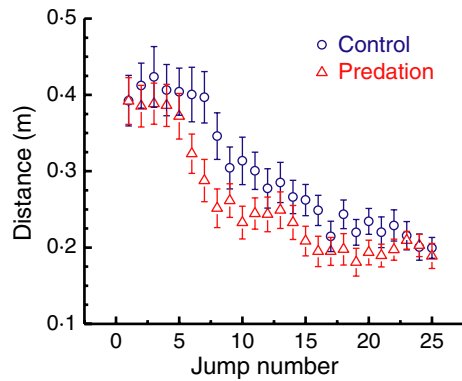


Fig. 5. Comparison of the distance jumped as response to sequential stimuli between second generation nymphs of grasshoppers reared with (red) or without (blue) spiders. Error bars denote \pm 1 SE.

(i.e. outdoor and laboratory) and were associated with fitness costs in the second generation.

Grasshoppers reared in risky environments jumped further when first approached by an investigator than individuals reared in spider free conditions. They also covered on average 2.7 times longer distances when repeatedly approached than grasshoppers reared in risk free conditions. These findings suggest that both the maximal escape performance and endurance were positively affected by chronic exposure to risk of spider predation.

We detected no differences between treatment groups in external morphological traits customarily believed to enhance prey locomotor performance. Thus, we demonstrate that enhanced performance in response to chronic risk of predation is not reserved to a small subset of mainly aquatic prey species that present outward morphological changes.

The observed changes in jumping distance could be partially explained by long-lasting changes in grasshopper jumping technique. Grasshopper reared with disarmed spiders altered the timing and ways they used the jumping legs during acceleration. Specifically, they had greater tibia leverage and they changed the way they tilted their body before and soon after take-off relative to grasshoppers from risk-free conditions. We showed that those modifications in jumping technique lead to substantial differences in biomechanical properties and ultimately to higher take-off speed. These aspects of jumping biomechanics have heretofore not been considered in analyses of grasshopper jumping techniques (e.g. Brown 1967; Bennet-Clark 1975; Burrows & Morris 2001; Santer *et al.* 2005; Sutton & Burrows 2008), emphasizing the benefit biomechanics can gain from adopting an ecological approach (exploring variation within a population).

The spider *P. mira* is a sit-and-wait predator that uses burst attack to ambush its prey. A heightened take-off speed can allow a grasshopper to leave the area in which the predator can pursue and capture it, more quickly. As predicted by our simple ballistic model, higher take-off speed explains significant statistical differences in jumping distances between grasshoppers reared in risky and risk free environments.

Thus, it is possible that the longer distance jumped is just a by-product of the grasshopper attempt to take-off faster. Alternatively, the improved escape performances could be a non-specific anti-predatory response grasshopper use that allows them to leave the danger zone of ambush predators faster and to distance themselves from their pursuing predators more rapidly.

Our results and supplemental evidence (e.g. increased metabolic rate and changes in nutritional requirements; D. Hawlena & O. J. Schmitz, unpublished data) strongly support the hypothesis that improved escape performances due to changes in jumping technique is a consequence of a stress response induced by chronic risk of predation. Physiological stress increases heart rate, respiration, blood flow, and blood pressure, and induce breakdown of stored non-carbohydrate nutrients into glucose (i.e. gluconeogenesis) (Rovero, Hughes & Chelazzi 2000; Beckerman, Wieski & Baird 2007; Slos & Stoks 2008). Those responses aim to supply the brain, sensory and locomotor systems with enough resources to support emergency functions. Thus, stressed animals should present enhanced escape performances independently from morphological changes. The fundamental neuroendocrine processes and the main molecules involved in stress responses are similar and well preserved throughout evolution, leading to remarkable similarity of stress responses across phyla (Ottaviani & Franceschi 1996; Wingfield & Ramenofsky 1999; Stefano *et al.* 2002; Sorensen, Kristensen & Loeschcke 2003; Denver 2009). Accordingly, our findings may represent a common anti-predator response shared by many prey species that can remain unnoticed if one does not specifically search for it in analyses of predator-prey interactions.

Our insights that rearing environment enhanced grasshoppers escape performances when stimulated differently (foot taping in outdoor arena or paint brush in laboratory) complement recent evidence that prey behavioural responses are often correlated (Sih *et al.* 2004; Lopez *et al.* 2005) and are influenced by rearing environment (El Hage, Griebel & Belzung 2006; Bell & Sih 2007). Such avoidance and escape behaviours, together with enhanced escape performances might explain why prey previously exposed to predation risk have a greater likelihood of surviving future encounters with predators (e.g. Brown, Gardner & Braithwaite 2005).

Animal jumping performances during natural activities are often lower than their maximal capabilities (Irschick & Losos 1998). Accordingly, the consistent differences in escape performances between grasshoppers from risk-free and risky environments suggest that individuals from the former group perform submaximally, partially due to less optimal energy expenditure during the jump (Vanrenterghem *et al.* 2004; Toro, Herrel & Irschick 2006). Previous attempts to explain why animals jump submaximally suggest that maximal jumping performance may not be important for fitness but coevolves with other locomotion traits that do affect fitness (e.g. sprint speed; Irschick & Losos 1998). In this study, improved jumping performances by grasshopper reared with spiders imply that this is an adaptive cost-effective response. A sub-maximal escape response to an environmental stimulus may

be adequate in low-risk environments but insufficient when the probability of being killed by a predator is higher. Thus, we suggest that transition from performing submaximally to the use of maximal capabilities is an adaptive antipredator response governed by neuroendocrine processes as part of the physiological stress syndrome. An important avenue of future research is to reveal the additional costs involved in performing maximally over submaximal and to uncover the neuromuscular control of those alternative strategies (see also Toro, Herrel & Irschick 2006).

Lasting improvement in escape performances that are not associated with substantial morphological changes may require reevaluation of predator-prey behavioural ecology. For example, escape theory assumes that decisions such as when or to what distance to flee are based on the prey morphology that is assumed to dictate its performances, reproductive assets and environmental conditions (Ydenberg & Dill 1986; Cooper & Frederick 2007). The possibility that prey can enhance performance in response to chronic risk may require the inclusion of predator-prey history as a factor in such models. Moreover, lasting changes in escape performances as a result of changes in escape technique can preclude the need to enhance escape performance via substantial changes in morphology. This possibility may help explain why morphological changes to improve escape performances seem to be uncommon among prey species, especially terrestrial ones (Dewitt, Sih & Wilson 1998; Relyea 2005). In addition, it may help to explain why prey that do not exhibit overt morphological responses exhibit higher levels of physiological stress than those that undergo morphological shifts (Holopainen *et al.* 1997; Steiner & Van Buskirk 2008).

The improved escape performances of grasshoppers reared with disarmed spiders were associated with changes in expression of traits related to fitness. Stress theory predicts that chronically stressed animals should reduce the conversion efficiency of nutrient to body mass (Trussell, Ewanchuk & Matassa 2006), minimize storage of proteins and triglycerides (Stoks, De Block & McPeck 2005), and become susceptible to parasite infestation (Boonstra *et al.* 1998). Thus, chronically stressed grasshoppers are expected to grow slower, to allocate fewer resources to reproduction and to survive less well. We found no trends in adult morphology or survival that would imply an immediate fitness cost of living under chronic perceived risk. However, consequences of living under chronic risk became apparent in the subsequent generation. Nymphs of grasshoppers from risky environments were smaller than grasshoppers from risk free environments. Smaller offspring suffer a competitive disadvantage, delayed final metamorphosis and smaller reproductive output, reducing the overall parental fitness (Abrams & Rowe 1996; Ovadia & Schmitz 2002). Moreover, smaller initial body size can compel individuals to engage in highly risky behaviour needed to enhance resource intake to fully develop to maturity within a growing season (Ovadia & Schmitz 2002). Consequently, smaller nymphs tend to suffer higher predation mortality than larger nymphs (Ovadia & Schmitz 2002). We also found that nymphs of grasshoppers

from risky environments suffer impaired escape abilities. Thus, coping mechanisms in one generation can predispose future generation to higher risks.

These insights complement recent evidence that stress induced by predation risk may reduce the offspring number and quality (Sheriff, Krebs & Boonstra 2009). These authors suggested that maternal stress response may help regulate offspring 'programming' to make them better adapted to the risky maternal environment (i.e. adaptive maternal effect). We instead found that grasshopper nymphs in risky environments showed lower escape performances that makes this explanation unlikely. An alternative explanation is that stress reduces current investment in reproduction to improve future reproduction when the mother experiences less stressful conditions (Love & Williams 2008). This argument cannot be applied to univoltine, semelparous species like the grasshopper *M. femurrubrum*. We instead suggest that stress induced alteration of the parental nutritional balance (especially low nitrogen content) may limit the parental investment in reproduction resulting in overall lower reproductive output and offspring performance. Thus, non-adaptive parental effects that predispose offspring to higher predation risk may be a major cost of physiological stress that was not considered in previous studies of predator-prey interactions.

The integration of ecology and biomechanics is a productive starting point for uncovering the mechanisms and constraints determining prey species' ability to improve escape from predation. Future studies should resolve the mechanisms that allow grasshopper to alter their jumping technique, to reveal the direct costs of using one jumping technique over the other and importantly to consider the intergenerational consequences of switching resource allocation from growth and reproduction to maintenance in the face of elevated stress. To incorporate our novel insights into a broader evolutionary ecological perspective it is important to estimate the rate at which those changes can be acquired and lost, to quantify their adaptive value and to reveal how abundant the ability to boost escape performance is among various prey taxa. Finally, the possibility that a syndrome of physiological stress may underlie the observed improvement in escape performance encourages a research shift back to explore the potentially adaptive aspects of stress reactions that are mostly overlooked.

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Supporting Information

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Appendix S1. A typical example of high speed imaging of a grasshopper jump.

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