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Eliciting an immune response reduces sprint speed in a lizard

Francisco Javier Zamora-Camacho, Senda Reguera, María Virtudes Rubiño-Hispán, and Gregorio Moreno-Rueda

Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, 18071, Granada, Spain

Predators and pathogens can diminish organisms' fitness, and consequently natural selection favors antipredatory and antiparasitic strategies. Nevertheless, as both kinds of strategies consume resources, animals cannot maximize investment in both strategies simultaneously, which should lead to trade-offs between the two. Accordingly, we hypothesize that there is a trade-off between sprint capacity and immune response, which are among the most important antipredatory and antiparasitic strategies, respectively, of many animals. Consequently, we predict that animals eliciting an immune response should reduce their sprint capacity. We experimentally tested this prediction in the lizard *Psammodromus algirus*. A group of lizards was inoculated with the antigen LPS (lipopolysaccharide of the cell wall of *Escherichia coli*), which activates the immune system, whereas the other group (control) was inoculated with phosphate buffer, which has no physiological effect. Before and after the inoculations, we filmed sprint capacity of lizards, estimating the maximal sprint speed. Males were faster than females before the activation of their immune system. Nonetheless, males' sprint speed diminished after the inoculation of LPS, whereas females' did not. Therefore, our results support the hypothesis of a trade-off between sprint speed and immune response in males. Consequently, male lizards combating a pathogen may suffer either higher risk of predation, or reduced fitness if they avoid this risk by spending more time in shelter.

Key words: **elevation, immune system, *Psammodromus algirus*, sprint speed.**

INTRODUCTION

Predators and pathogens can exert a major impact on the fitness of organisms (Caro 2005; Schmid-Hempel 2011). Predators may affect fitness directly by means of predation, reducing the future fitness of the prey to zero. Predators also affect prey fitness indirectly, by altering prey behavior and physiology in order to adopt antipredatory strategies, which may be costly in terms of reduced growth, foraging success, or breeding success (review in Lima 1998). Pathogens and parasites take resources from the hosts, reducing their fitness, and ultimately their survival. Consequently, hosts show a set of antiparasite strategies, of which the immune system is the most sophisticated (Wakelin and Apanius 1997). In sum, there is a strong selection favoring the evolution of antipredatory and antiparasitic strategies.

Quick fleeing when a predator is detected is one of the main antipredatory strategies in lizards (Martín and López 2000), and consequently, survival increases the higher the sprint speed (Irschick and Meyers 2007). In fact, locomotor capacity is under strong natural selection in lizards (Calsbeek and Irschick 2007). As an example of the importance of predators as a selective pressure,
lizards show higher sprint speed in populations where they are more exposed to predators (Vervust et al. 2007). High-speed sprint is energetically costly (Christian et al. 1997). The fact that lizards reduce their sprint speed when they evolve in an environment without predators (Vervust et al. 2007) suggests that morphological and physiological adaptations necessary to maintain a high sprint speed are costly to develop or maintain. Moreover, the muscular exertion during running increases the production of pro-oxidant substances, with the consequent risk of increased oxidative stress (Fisher-Wellman and Bloomer 2009). Oxidative stress is the excess production of pro-oxidant substances relative to antioxidant defenses, with negative effects on organism health (review in Halliwell and Gutteridge 2007).

Regarding pathogens and parasites, the immune system is one of the most important defenses. The immune system includes a number of white corpuscles that recognize and attack alien elements (antigens) that have invaded the organism (Wakelin and Apanius 1997). Immune response implies a number of mechanisms to kill pathogens, such as antibodies, oxidant agents (NO_2), lysozymes, and phagocytosis. Survival increases with immunocompetence (Møller and Saino 2004), but heavy immune responses may lower fitness by generating autoimmune diseases or increased oxidative stress (Sorci and Faivre 2009). Moreover, the activity of the immune system is

Address correspondence to F.J. Zamora-Camacho. E-mail: zamcam@ugr.es

costly, in terms of using energy (e.g. to generate fever) as well as specific resources such as proteins and amino acids (review in Schmid-Hempel 2011).

Therefore, both sprint speed and mounting an immune response appear to be costly and consume a huge quantity of resources. Consequently, it is improbable that sprint speed and immunocompetence could be maximized simultaneously. When organisms cannot simultaneously maximize different life-history functions, optimal trade-offs emerge among life-history traits in favor of fitness (Roff 2002). The genetic architecture of different life-history traits can make them co-vary positively despite their costs (Lailvaux et al. 2010). Nonetheless, even with positive genetic covariation, phenotypic trade-offs may emerge when life-history traits compete for the same resources (Zera and Harshman 2001), experimental manipulation being necessary to ascertain the existence of such phenotypic trade-offs (Garland 2014).

In the case we are dealing here, several facts make us expect a trade-off between immune response and sprint speed. First, both traits need proteins (Christian et al. 1997; Lochmiller and Deerenberg 2000), and thus the reallocation of proteins to the immune system when an antigen should be combated, could weaken muscles used in sprint activity. Alternatively, the reallocation of amino acids to the immune system could depress brain activity (which also needs amino acids), thus increasing torpor (see Stevenson et al. 2000; Otti et al. 2012). Second, both fleeing and an immune response are energetically costly (Christian et al. 1997; Hancock and Gleeson 2002; Schmid-Hempel 2011), therefore immune-activated animals probably reallocate energy to the immune system, and energy demands of musculature involved in sprint capacity could not be fulfilled . Third, mounting an immune response together with locomotion augment the burst of reactive molecules and thus increase oxidative stress (Fisher-Wellman and Bloomer 2009; Sorci and Faivre 2009). Indeed, immunocompetence is traded with several other traits, such as sexual signals, reproduction, growth rate, or even survival (examples from reptiles in Uller et al. 2006; French et al. 2007; López et al. 2009; Meylan et al. 2013).

In sum, given the competition for resources and the fact that both sprint and immune responses increase oxidative stress, here we propose that there is a trade-off between immune response and sprint capacity. On the one hand, this hypothesis predicts that animals under increased predation risk should reduce their immune response in order to increase their sprint capacity. This part of the hypothesis is well supported by studies showing that animals usually reduce their immune response when exposed to predators (Rigby and Jokela 2000; Zhang et al. 2003; Navarro et al. 2004; Hõrak et al. 2006; Stoks et al. 2006; Mikolajewski et al. 2008). On the other hand, the hypothesis also predicts that immune-challenged animals should reduce their escape capacity, an implication which has received little attention. There is some evidence that eliciting an immune response increases the probability of predation. In the damselfly *Calopteryx splendens*, immune-challenged males lose their territories and are more susceptible to bird predation (Rantala et al. 2010). In field crickets (*Gryllus campestris*), immune-challenged males spend more time outside their shelters and show less reaction to predators, but its top crawling speed is not affected by the immune challenge (Otti et al. 2012).

In this study, after the aforementioned reasoning, we test the prediction that an immune challenge should diminish the sprint capacity of animals. We used the lizard *Psammodromus algirus* as model system. In a group of lizards, we stimulated the immune system by the inoculation of an innocuous antigen (lipopolysaccharide of the cell wall of *Escherichia coli*, LPS), whereas another group of lizards served as a control. Afterwards, we analyzed the change in sprint speed as a consequence of the immune challenge, under the prediction that lizards inoculated with LPS would reduce their sprint speed, while control lizards would show no change.

In addition, we also tested gender-based differences in this trade-off between immune response and sprint speed. On the one hand, testosterone has an immunosuppressant effect that provokes males to have lower immune response than females (Møller et al. 1998), which may also occur in the lizard studied here (Belliure et al. 2004). Therefore, we would expect a higher immune response in females, and thus the trade-off will be more evident in females. On the other hand, male lizards sprint faster than females in different species (Lailvaux et al. 2003) such as *P. algirus* (Zamora-Camacho et al. 2014). Accordingly, diverting resources for immune response might be more appellant in males than in females, conducing to a more evident trade-off in males than in females.

METHODS

P. algirus is a medium-size lacertid lizard [53–95 mm of snout-vent length (SVL) in our study zone] that inhabits shrubby areas in western Mediterranean climates, from the sea level to more than 2600 m a.s.l. (Salvador 2011). We caught lizards on the southern slope of Sierra Nevada (SE Spain), in 6 plots placed at 300, 700, 1200, 1700, 2200, and 2500 m a.s.l. (Supplementary Figure S1), during the lizards' activity season (April–September), in 2011 and 2012. The escape strategy varies in this species with habitat characteristics (Martín and López 1995; Iraeta et al. 2010), so we chose locations as similar as possible in vegetation and habitat structure (Supplementary Figure S2). In fact, elevation provenance did not affect the findings in this study (Supplementary Table S1).

We brought all captured individuals to the laboratory and registered biometrical measures involved in sprint speed, such as body mass (with a balance model WTB200, accuracy 0.01 g), SVL, and fore- and hind-leg length (with a millimeter ruler). In order to differentiate sexes, we distinguished males for having proportionally larger heads, more abundant and conspicuous femoral pores, and orange spots at the corners of the mouth. We did not include gravid females in the study to avoid confounding effects on sprint speed (Iraeta et al. 2010); gravid females were detected by palpation for the presence of eggs. Also, tailless lizards were not used, because tail loss diminishes sprint speed in this species (Martín and Avery 1998). During their stay in captivity, lizards were kept in individual terrariums ($20 \times 13 \times 9$ cm), and provided ad libitum access to mealworms (*Tenebrio molitor* larvae) and water (nutritious aqueous gel). Lizards were marked by toe clipping (excluding the longest toes) as part of a longstanding ongoing study, 2 days before the trials. This marking method has been demonstrated not to alter lacertids' locomotive capacities (Huey et al. 1990; Dodd 1993).

Sprint speed was measured in a wooden runway ($3.2 \times 0.2 \times 0.4$ m), with a cork base to provide suitable traction (Bauwens et al. 1995). This runway was divided into 10 stretches every 25 cm with a contrasting color strip, considering that this is approximately the distance that lizards run in nature to reach their refuges (Martín and López 2000). Finally, a dark background was placed at the end of the runway to resemble a refuge and thus encourage the lizards to run forward. As an internal control, we recorded sprint speed for all lizards prior to treatment. We released lizards at the

start of the runway, and chased them by hand to encourage escape behavior. After 24 h, we randomly assigned lizards to 1 of 2 treatments. Lizards of 1 treatment were inoculated subcutaneously in the dorsum with 0.1 mg of LPS of bacterial wall of *Escherichia coli* (serotype 055:B5, L-2880, Sigma Aldrich) diluted in 0.01 ml of isotonic phosphate buffer. This substance acts as an antigen mimicking an infection and thus provoking an immune reaction. The remaining lizards were used as manipulation controls, inoculated with the same volume of phosphate buffer (PBS), which has no physiological effects as it is similar to physiological serum. Trials with treated lizards were conducted 4 h after the inoculation of the respective substance, when the immune reaction to LPS is maximal (Parmentier et al. 1998). All trials were repeated 3 times for every lizard (Losos et al. 2002; Adolph and Pickering 2008). No sign of fatigue was observed between trials. All lizards ran at 32ºC of body temperature, in order to avoid confounding effects of body temperature on sprint speed (see Pérez-Tris et al. 2004; Iraeta et al. 2010). This temperature was achieved by placing lizards into an incubator at the trial temperature for 10 min prior to measurements. Before conducting the trials, lizard body temperature was assessed by inserting a 1-mm diameter thermocouple connected to a thermometer (model Hybok 14, accuracy 0.1°C) 8 mm inside the cloaca. We used the same temperature for all lizards in each population, given that lizards in our study zone select the same temperature regardless of elevation (Zamora-Camacho et al. 2013). It is well established that the temperature selected by lizards corresponds to the temperature at which sprint speed is maximal (Bonino et al. 2011), and the temperature at which lizards achieve maximal sprint speed varies little or not at all among lizard populations (Garland and Adolph 1991). Once the experiment was ended, lizards were returned to the same place where they were captured. No lizard died or suffered any damage as a consequence of this study.

All the runs were recorded with a photo and video camera Canon EOS 550D, at 25 frames per second. Then, the films were analyzed with the software Movavi v. 11, which enables time to be measured in milliseconds (ms) (Chen et al. 2003). For each run, we measured the time interval for every stretch, counting a stretch as completed when the lizard's snout reached the dividing strip (Martín and López 2001). We analyzed speed data of the fastest stretch out of the 3 runs of each lizard in order to determine the maximal sprint speed of the lizards. Laboratory measurements of maximal sprint speed represent an upper limit of field sprint speed, not necessarily correlated with the sprint speed in the field (Irschick and Garland 2001). However, several studies have demonstrated that laboratory measures of locomotive performance are good indicators of field survival in lizards (Le Galliard et al. 2004). Thus, considering the field behavior of this lizard when exposed to a predator (consisting of short runs to the nearest shelter; Martín and López 2000), we consider the maximal sprint speed measured in the laboratory to be a reliable indicator of sprint capacity in this species.

All data were analyzed with parametric statistics, as they fulfilled the criteria of residual normality and homoscedasticity (Quinn and Keough 2002). We first checked differences between experimental groups in morphology and previous run with a 2-way factorial Anova, including treatment and sex as categorical predictors. Then we conducted a similar 2-way factorial Anova to check the effect of the treatment on post-treatment speed and difference in sprint speed between the 2 trials, calculated as sprint speed after treatment minus prior sprint speed.

RESULTS

Since the lizards were randomly assigned to each treatment, there were no significant differences between treatments in body mass, SVL, foreleg length, hind-leg length, or sprint speed prior to the treatment (Tables 1 and 2). There was sexual dimorphism for limb length, and males ran faster than females (Tables 1 and 2). The inoculation of LPS had a significant effect on maximal sprint speed, lizards inoculated with LPS showing significantly lower sprint speed than control lizards, inoculated with PBS (Tables 1 and 2; Figure 1). The effect of the treatment remained significant when the interactions were removed from the model ($F_{1, 69} = 6.39$, $P = 0.014$). Lizards inoculated with LPS significantly decreased their sprint speed in the second trial compared with the first trial, in average −18.68 cm/s, with 95% confidence intervals not including zero (−36.84 and −0.52 cm/s; Nakagawa and Cuthill 2007). There was an almost significant interaction between sex and treatment explaining sprint speed in the second trial ($P = 0.055$; Table 1). When considering only males, post-treatment maximal sprint speed was significantly slower in LPS- than in PBS-inoculated lizards ($F_{1, 33} = 12.09$, $P = 0.001$; Figure 2). However, when only females were considered, the sprint speed did not differ between LPS- and PBS-inoculated lizards ($F_{1, 27} = 0.009$, $P = 0.92$; Figure 2). Discussion Results in this study show that when a lizard elicits an immune response against an antigen, its sprint capacity decreases. This finding has an immediate implication: lizards exposed to pathogens, which are eliciting an immune response, have reduced capacity to flee from predators. This result is consistent with previous studies showing that insects challenged with an antigen show higher predation risk (Rantala et al. 2010; Otti et al. 2012). Nevertheless, the fact that Otti et al. (2012) did not find an effect of immune challenge on maximum speed in field crickets suggests that the trade-off between escape capacity and immune capacity is not general in the animal kingdom. In fact, we found such as trade-off only in male lizards.

Our study suggests that unhealthy animals could suffer more risk of predation, not only as a consequence of pathogen virulence, but also as a collateral effect of immune response. Our findings, therefore, help explain the mechanisms by which unhealthy animals are more frequently depredated (Temple 1987; Hudson et al. 1992; Murray et al. 1997; Møller and Nielsen 2007). In fact, consistent

Table 1

Results of the 2-way factorial Anova examining variation in body mass, snout-vent length (SVL), foreleg length (FLL), hind-leg length (HLL), maximal sprint speed prior the treatment (previous speed), maximal sprint speed post-treatment (post-treatment speed), and the difference in maximal sprint speed, according to treatment, and sex. *F*-values are shown

	Sex	Treatment	Sex*treatment
df	1, 68	1, 68	1, 68
Mass	0.49	0.1	0.11
SVL	1.86	0.01	0.003
FLL	**12.09***	0.12	0.17
HLL	**29.86***	1.35	0.18
Previous speed	**7.88**	0.002	3.31§
Post-treatment speed	**4.24***	**5.68***	3.81§
Difference in speed	0.55	**6.33***	0.03

Symbols indicate: § for $0.10 > P > 0.05$, * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. In bold, significant results. Note that for body mass and SVL, data of 1 lizard were lost, and therefore the df error is 67 for these variables.

Table 2

Average values ± SE of measured variables for both treatments (LPS and PBS), and for both sexes

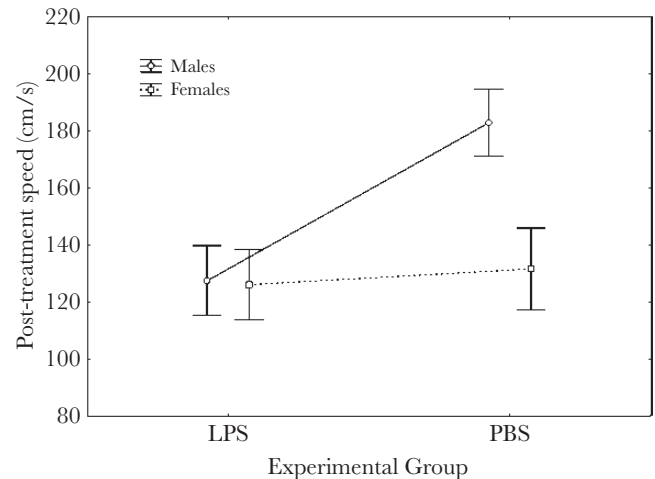| Variable	LPS (n = 38)	PBS (n = 34)	Males (n = 39)	Females (n = 33)
Body mass (g)	7.09 ± 0.34	7.22 ± 0.39	7.64 ± 0.33	6.67 ± 0.40
SVL (mm)	65.98 ± 1.07	65.77 ± 1.21	65.46 ± 1.04	66.29 ± 1.23
Foreleg length (mm)	22.02 ± 0.26	21.83 ± 0.29	22.65 ± 0.25	21.20 ± 0.30
Hind-leg length (mm)	34.89 ± 0.46	35.52 ± 0.53	37.01 ± 0.45	33.40 ± 0.54
Initial speed (cm/s)	143.45 ± 9.19	141.61 ± 10.48	155.79 ± 8.99	129.27 ± 10.65
Post-treatment speed (cm/s)	124.77 ± 9.54	154.48 ± 10.88	151.99 ± 9.33	127.26 ± 11.06
Difference in speed (cm/s)	−18.68 ± 9.08	12.87 ± 10.35	−3.80 ± 8.88	−2.01 ± 10.52
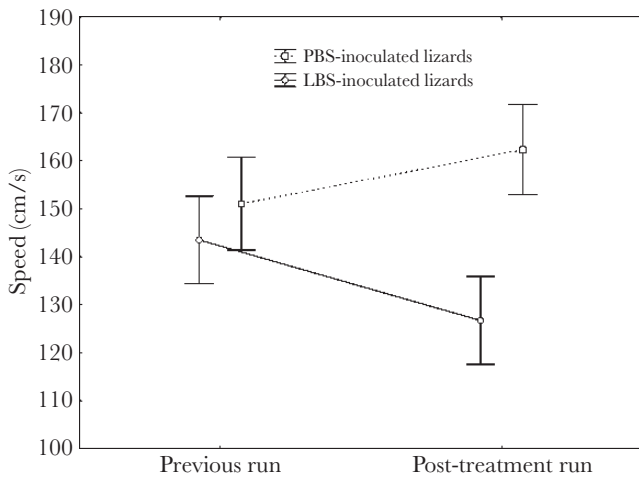
Figure 1

Lizard sprint speeds were similar between groups in the previous run without antigen treatment, as a result of the random assignation of lizards to one or another group. Then, PBS-inoculated lizards showed a trend to increase their speed, whereas LPS-inoculated, immune system-activated lizards ran more slowly, showing a trade-off between immune system and sprint performance. Error bars represent SE. A total of 39 lizards were injected with LPS, and 34 with PBS.

Figure 2

PBS-inoculated males ran faster than females, repeating the trend in the previous run. In turn, LPS had no effect on female running speed, whereas male speed diminished. Male sprint speed underwent a trade-off with the immune system, but female sprint speed did not. Error bars represent SE. Sample sizes were: 19 males and 19 females in the LPS treatment, and 20 males, and 14 females in the PBS treatment.

with our findings, previous studies found that parasitized lizards show reduced take-off capacity (Schall et al. 1982; Oppliger et al. 1996; Main and Bull 2000), which has also been found in amphibians (Chatfield et al. 2013), birds (Lindström et al. 2003), and mammals (Alzaga et al. 2008). Therefore, animals appear to make a trade-off between avoiding predators and combating pathogens .

Several studies have shown that immunity is a costly trait which requires trade-offs with other life-history traits (see Introduction). Those costs of immunity have been confirmed in reptiles (Zimmerman et al. 2010), in which growth rates (Uller et al. 2006), male and female reproduction success (French et al. 2007; López et al. 2009) or even thermoregulation accuracy (Merchant et al. 2007) are negatively affected by immune challenges. The trade-off between immune competence and sprint speed may be achieved by various nonmutually exclusive ways, as explained in the Introduction. Therefore, in our trials, lizards obligated to respond to an antigen may have reduced their sprint speed as a consequence of proteins and/or energy being reallocated to immune response, or in order to avoid excessive oxidative imbalance.

On the other hand, it is noteworthy that the trade-off between sprint speed and immune-competence occurred only in males. We can discard an effect of testosterone, as testosterone, being immunosuppressant (Belliure et al. 2004), would relax the trade-off in males, the reverse that we have found. The reason may be that

locomotion has a higher impact on male than on female fitness (Husak et al. 2006). Males usually spend more time than females defending a territory or searching for mates (for our study species, see Díaz 1993), and consequently they are more exposed to predators (general review in Lima and Dill 1990). Perhaps for this reason, males have longer limbs than females, and consequently run faster (Zamora-Camacho et al. 2014). Actually, the fact that gender differences in sprint speed in this species disappeared when controlling for limb length suggests that they are a consequence of males having longer limbs (Zamora-Camacho et al. 2014). Given that males have longer limbs, they presumably could need more energy during runs (a larger limb needs more energy to be moved, all else being equal), and thus energy depletion by the immune system could have a stronger effect on males than on females.

It should be noted that the trade-off found does not necessarily imply higher actual predation in lizards eliciting an immune response, if they avoid predators by some means or other. Ill lizards could diminish predation risk by other means, for example spending less time outside their shelters. Reduced mobility is characteristic of unhealthy animals (Adelman and Martin 2009), and it may be adaptive in order to reduce predation risk (Caro 2005). Thus, if unhealthy lizards avoid predation by remaining sheltered, lizards mounting an immune response would perhaps not pay a cost in increased mortality by predation, but they would pay a cost in reduced fitness as a consequence of reduced

vigilance of their territories, foraging or mating (Civantos et al. 2010). Matching with these predictions, cane toads (*Rhinella marina*) inoculated with LPS showed diminished feeding and activity rates (Llewellyn et al. 2011) .

In short, male lizards were faster than females before activating their immune system, but, whereas females did not change their sprint speed despite the injection of LPS, male sprint speed diminished when the immune system was challenged by the antigen. This result shows that sprint speed is under a trade-off with immune system in males, as the activation of the latter reduces the performance of the former, but not so in females. These findings imply that male lizards combating a pathogen suffer a higher predation risk, or undergo reduced fitness if they avoid this higher predation risk by staying more time in their shelter, thus suffering a cost in reduced vigilance of their territories, foraging or mating. In addition, according to the trade-off shown in this study, we predict that populations in which predation is reduced, animals should show a stronger immune response, and vice versa in populations with high predation risk. At the same time, in populations with high parasite and pathogen prevalence, animals should show slower sprint speeds.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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