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Trade-offs between reproductive coloration and innate immunity in a natural population of female sagebrush lizards, *Sceloporus graciosus*

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Trade-offs between immune function and reproduction are common to many organisms. Nevertheless, high energetic resources may eliminate the need for these trade-offs. In this study, we consider the effects of food availability on these trade-offs in a wild population of female sagebrush lizards (*Sceloporus graciosus*) during the breeding season. We manipulated food availability by supplementing some lizards but not others. We measured female orange side coloration as an indicator of reproductive state and calculated the bacterial killing capability of collected plasma exposed to *Escherichia coli ex vivo* as a measure of innate immunity. We found that female lizards show a natural trade-off between reproductive effort and immune function; females under high reproductive investment had lower innate immunity than those at a later reproductive state. We did not detect this trade-off with food supplementation. We show that trade-offs depend on the energetic state of the animal, illustrating that trade-offs between immune function and reproduction can be context-dependent.

Key words: context-dependent, energetics, life history, resources

INTRODUCTION

The accessibility of resources determines the amount of energy that is available for the survival and reproduction of an organism (Hill et al., 2008; Owen et al., 1992). In nature, energy resources are often limited, and thus organisms must differentially allocate limited energy budgets to various physiological and behavioural functions (Therrien et al., 2008; Waelti & Reyer, 2007). Reproduction is essential and requires high energetic demands during the breeding season, leaving less energy available for other life-history processes (Ardia, 2005; Martin et al., 2008). Yet, this trade-off may be diminished when energetic resources are relatively abundant. Our goals in this study were 1) to examine a potential trade-off between reproductive and immune processes in female sagebrush lizards (*Sceloporus graciosus*) in the wild, and 2) to test the hypothesis that food supplementation could eliminate this trade-off in females.

Activating an immune response is costly and redirects energy that may be used for other processes (Hanssen et al., 2004; Uller et al., 2006). In captivity, female lizards have been shown to produce a decreased immune response under high reproductive investment only when resources are limited (French et al., 2007). Our study considers whether this differential trade-off is also present in a natural setting. Energy expenditure is typically much higher in the field than in the laboratory (Bennett & Nagy, 1977), and food availability is usually limiting (Gunnarsson et al., 2004). Free-living animals, furthermore, have greater opportunity for variable social interactions and have increased risk of predation. Lab and field settings may thus provide inconsistent results (Calisi & Bentley, 2009). Due to the potential for lower energy available for investment into reproductive and immune function in the field than in the laboratory, we expect trade-offs to be persistent in the field; investment in reproduction may thus come at a cost to immune function. Alternatively, individual quality may vary (Ruiz et al., 2008), allowing some individuals to better balance both reproductive and survival functions, thus removing the presence of a trade-off in the population as a whole (Ahtiainen et al., 2006; Salvador et al., 2007; Tregenza et al., 2006). Male sagebrush lizards do not show a trade-off between reproductive physiology (testosterone levels) and immune function in the field; nevertheless, excess resources increase both reproductive physiology and innate immunity (Ruiz et al., 2010). However, males and females differ in reproductive investment; females are limited by egg production while males are limited by their capacity to attract and compete for mates (McKean & Nunney, 2005). Thus, males and females may differ in trade-offs exhibited during the reproductive season. We therefore expect female sagebrush lizards undergoing high reproductive investment to show decreased innate immune investment. Furthermore, food supplementation should eliminate the need for this trade-off.

MATERIALS AND METHODS

Sceloporus graciosus are small, territorial lizards with overlapping male and female territories (Martins, 1993). Lizards used in this study were adult females (50–61 mm snout–vent length; 5–10g mass). Their robust site fidelity makes this a particularly useful species for conducting field studies and for manipulating resources. Sexually dimorphic ventral coloration allows for quick identification of sex.

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We conducted a field study on female *S. graciosus* in the San Jacinto mountains of southern California (33N, 116W) from May through June 2009. Individual lizards were captured by noose, and each was marked with a unique three-colour code along the dorsal surface using non-toxic paint markers (Painters®, Elmer's Products Inc., Columbus, OH). We marked sites where lizards were captured with a yellow plastic flag and entered locations on a GPS so that we could later find and recapture individuals. Lizards were recaptured 1–2 weeks after initial capture. We photographed lizards and acquired standard measurements of weights and snout–vent length (SVL) upon initial capture and recapture. Lizards were released where they had been previously recaptured.

Field supplementation of food resources has previously been shown to be an effective method of manipulating resource availability in males of this population (Ruiz et al., 2010). We placed a Petri dish on the territory of each marked individual after initial capture; this individual was then placed into either the food supplemented or control group, alternately. We placed an average of three mealworms and three crickets in the territories of individuals in the food-supplemented group (*n*=10) every day until the day of recapture (1–2 weeks), directing food sources towards marked individuals when possible (see Ruiz et al., 2010). Lizards were often seen in their territories during feedings and readily consumed the crickets and mealworms provided. We similarly visited the Petri dishes of lizards in the control group (*n*=11) daily without feeding.

We used photographs of lizards at the time of recapture to look for the presence of distinct orange coloration on the sides, which is a good indicator of the reproductive state of female lizards. Orange coloration is an indicator of gravidity in other species of lizards (Vaclav et al., 2007; Zani et al., 2008), with lack of orange coloration suggesting females are undergoing vitellogenesis. In *S. graciosus*, males have been shown to preferentially court females with less orange coloration (Ruiz et al., 2008), suggesting that colour may be an indicator of gravidity. We sorted females into two categories based on the presence or absence of distinct orange coloration on the sides of the lizard (orange females, *n*=10; non-orange females, *n*=11). On average, orange females were presumed to be gravid, whereas non-orange females were likely to be vitellogenic. Coloration remained constant within the 7–14 days of resource manipulation.

Blood was acquired from each lizard immediately upon recapture by rupturing the post-orbital sinus with a heparin-coated microcapillary tube. Blood was stored with ice until centrifuged in a VWR Model V microcentrifuge at 4000 rpm (equivalent to 537 g) for 10 minutes and plasma separated within eight hours of collection. We acquired 20–50 µl plasma from each lizard and kept plasma frozen at –20°C until immune assays were conducted.

Innate immune function is a non-specific, continuous immune defence used by organisms. The bacteria killing assay is emerging as a valuable tool to measure overall intrinsic immune investment across a variety of organisms (Chester et al., 2010; Matson et al., 2006; Zimmerman et al., 2010) We thus determined immune response by calculating the *ex vivo* bacterial killing capability of collected plasma exposed to *Escherichia coli*. To do this, we prepared a bacterial stock solution by dissolving one pellet of *E. coli* (ATCC#8739, Microbiologics, St Cloud, MN) in 40 ml 1M sterile PBS and activating this solution at 37 °C for 30 min. A working bacterial solution was prepared with 2 ml of this bacterial stock solution in 8 ml 1M PBS. We diluted individual lizard plasma samples 1:5 in CO_2-independent media (Gibco #18045, Carlsbad, GA) with 2.34 mg L-glutamine (Sigma-Aldrich). We added 10 µl of the working bacterial solution to each sample and incubated these plasma/bacteria cocktails for 30 min at 37 °C in order for bacteria killing to occur. Subsequently, we plated 50 µl of each sample on agar plates, in duplicates, including a positive control (of only bacteria) and a negative control (with no bacteria). Plates were incubated overnight at 37 °C to allow colony growth. We then counted the number of colonies on each plate and compared them to the positive control plate in order to calculate the percentage of bacteria killed by each lizard plasma sample [percent bacteria killed = 100 – (average # of colonies on sample plate/average # of colonies on positive control plates)*100]. No colonies formed on negative control plates.

Our immune data violated the assumptions of normality. To normalize the data, we transformed the percent age of bacteria killed with the following formula: arcsine(square-root(percent bacteria killed)), typical for analysis of percentage data. We then conducted two-tailed t-tests to determine the effect of orange coloration on innate immunity in both control and food-supplemented females.

RESULTS AND DISCUSSION

Among lizards with natural levels of food availability, non-orange females had lower immune function than orange females (*t*=2.29, df=9, *P*<0.05; Fig. 1). If non-orange females are indeed vitellogenic, we may expect this to be a more energetically taxing period as they produce

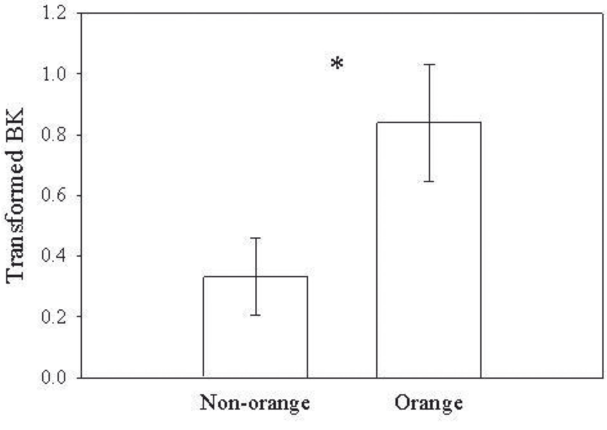

Fig. 1. Mean (± SEM) transformed bacteria killing capability (BK) of non-orange (*n*=7) and orange (*n*=4) control lizards. * = significant difference between treatment groups.

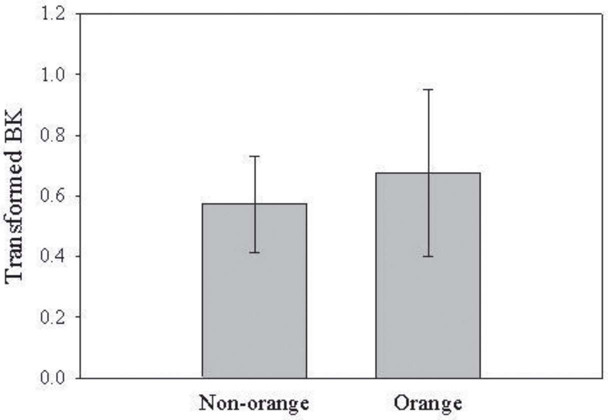

Fig. 2. Mean (± SEM) transformed bacteria killing capability (BK) of non-orange (*n*=4) and orange (*n*=6), food-supplemented lizards.

eggs, explaining the trade-off with immunity noted here. Although we found a trade-off between immune function and reproductive coloration, reproductive state as indicated by coloration did not affect innate immune function after food supplementation (t=0.28, df=8, P=0.79; Fig. 2).

Our results imply a trade-off between reproductive effort and immune function in a wild population of female sagebrush lizards. This suggests that, with limited energy, allocation to reproduction comes at a cost to at least one aspect of innate immunity. A trade-off between reproductive and immune function has been shown in a variety of species in nature (for examples see Ahtiainen et al., 2005; Tomas et al., 2007; Uller et al., 2006). We did not find this trade-off in food-supplemented lizards. Trade-offs may therefore also be a plastic response to limited diet for female lizards in the field as well as in the laboratory (French et al., 2007).

The trade-off seen in females in this study was not detected in a previous study on males (Ruiz et al., 2010). This may be due to a difference in our measure of reproductive investment. Males are continuously engaged in reproductive behaviour throughout the breeding season. In males, reproductive investment, as measured by testosterone levels, is directly related to the quality of the male, and the ability to court and attract females (Gonzalez et al., 2001). On the other hand, female investment in egg production can vary qualitatively with the size and number of offspring. Nevertheless, we only considered the degree of reproductive investment within a female, as indicated by the presence or absence of gravid coloration, and not the quality of this investment. These differential measures may be one factor accounting for the discrepancy in trade-offs between the sexes in our study.

Although we did find differences in trade-offs between reproductive state and our measure of innate immunity in control females, we did not consider all aspects of immunity in this study. Yet trade-offs between different immune responses can occur (Martin et al., 2006). Ovariectimized female anoles have increased immune function, but higher parasite loads, than their reproductive counterparts (Cox et al., 2010). Future studies should consider trade-offs within the immune system during the breeding season in order to determine whether excess energy is preferentially invested in different parts of the immune system.

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