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Costs of Mounting an Immune Response during Pregnancy in a Lizard

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ABSTRACT

Immune defenses are of great benefit to hosts, but reducing the impact of infection by mounting an immune response also entails costs. However, the physiological mechanisms that generate the costs of an immune response remain poorly understood. Moreover, the majority of studies investigating the consequences of an immune challenge in vertebrates have been conducted on mammals and birds. The aim of this study is to investigate the physiological costs of mounting an immune response during gestation in an ectothermic species. Indeed, because ectothermic species are unable to internally regulate their body temperature, the apportionment of resources to homeostatic activities in ectothermic species can differ from that in endothermic species. We conducted this study on the common lizard *Zootoca vivipara*. We investigated the costs of mounting an immune response by injecting females with sheep red blood cells and quantified the consequences to reproductive performance (litter mass and success) and physiological performance (standard metabolic rate, endurance, and phytohemagglutinin response). In addition, we measured basking behavior. Our analyses revealed that mounting an immune response affected litter mass, physiological performance, and basking behavior. Moreover, we demonstrated that the modulation of an immune challenge is impacted by intrinsic factors, such as body size and condition.

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Introduction

Parasites and pathogens led host species to evolve sophisticated protection against infection, ranging from behavioral defenses to physical barriers to the immune system. The ability to mount an immune defense in response to infection by a parasite, as evident in animals, enhances survival but is expected to be costly (Schulenburg et al. 2009; Graham et al. 2011). Because organisms have finite energetic resources, the costs should be manifested through the negative effects of immune activation on various components of fitness (Ilmonen et al. 2000; Moret and Schmid-Hempel 2000; Hanssen et al. 2004; French et al. 2009). Immunological defenses against pathogens may compete for a host's resources, which are required for other energetically demanding processes, including maintenance, growth, and reproduction (Sheldon and Verhulst 1996; French et al. 2007, 2009). Currently, there is a growing interest in individual variation in immune performance and the trade-offs between individual immune system performance and fitness-related traits, such as reproductive effort (French et al. 2009; Schulenberg et al. 2009; Graham et al. 2011). However, the physiological mechanisms that generate these ultimate costs of the immune response are largely unexplored.

Several studies have suggested different mechanisms, including immunopathology and oxidative stress, as key explanations for the costs of an immune response (Roitt et al. 1998; Martin et al. 2003; Horak et al. 2006; Sorci and Faivre 2009). However, evidence for these potential sources of energetic costs of immune defenses is predominantly derived from studies involving laboratory mammals or from clinical studies involving humans (Roitt et al. 1998). Thus, these mechanisms may not be applicable to wild animals studied in the natural habitats. Indeed, earlier studies that have investigated the effect of mounting an immune response on energy expenditure have yielded contradictory results. For example, a significant increase in basal metabolic rate (BMR) after an immune challenge has been documented in the great tit *Parus major* and house sparrow *Passer domesticus* (Ots et al. 2001; Martin et al. 2003). In contrast, no change in BMR was observed in other species (e.g., Greenfinch *Carduelis chloris* [Horak et al. 2003] and blue tit *Cyanistes caeruleus* [Svensson et al. 1998]), despite the use of the same immune challenge. The contradictory nature of these results suggests that energetic costs of the immune response are neither ubiquitous nor induced by similar processes in different model systems and may be context dependent (French et al. 2009).

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For example, several studies have demonstrated that seasonal variation in immunocompetence in females may be related to the energetic challenges imposed by reproduction (see French et al. 2009).

The majority of earlier studies that have investigated the consequences of an immune challenge in vertebrates used endotherms as model systems (Ilmonen et al. 2000; Bonneaud et al. 2003), and few studies have been conducted that involve ectotherms (Svensson et al. 2001; Uller et al. 2006; French et al. 2007, 2009). Ectothermic vertebrates have a complex immune system that is similar to that found in mammals and birds (see Zimmerman et al. 2010 for a review), but their response to infection may differ owing to their temperature-dependent metabolism. Because ectothermic species are unable to internally regulate their body temperature, the apportionment of resources to self-maintenance activities, including immune function, can differ between ectothermic and endothermic species. Furthermore, the costs of an immune response may affect fitness as a consequence of shunting energy from reproduction to maintenance.

The aim of this study is to investigate the reproductive and physiological costs of mounting an immune response during gestation in an ectothermic species, the European common lizard *Zootoca* (*Lacerta*) *vivipara*, in the family Lacertidae. In this study, we experimentally induced an immune challenge to half of the females via the injection of a solution of sheep red blood cells (SRBCs). In vertebrates, the injection of an antigen elicits the humoral immune response and the production of antibodies (Svensson et al. 2001; Meylan et al. 2010). We assessed the costs of mounting an immune response using the following four physiological traits: endurance capacity, thermoregulatory performance, standard metabolic rate (SMR), and cellular immune response. We also assessed variation in the investment in reproduction using the following two reproductive traits: litter mass and litter success.

Material and Methods

Species

The European common lizard (*Zootoca vivipara*) is a small-bodied (~5 g) species in the family Lacertidae that is widely distributed across Europe and Asia. We studied populations from field sites located on Mont-Lozère (Massif Central, southeastern France, 44°30′N, 3°45′E). Males emerge from hibernation in mid-April, followed by yearlings and females in mid-May. Mating occurs soon after the emergence of females. Parturition occurs after a gestation period of 2 mo, when young are fully formed. The mean clutch size is five eggs (range, 1–12 eggs), and the offspring hatch within 1 h of oviposition. The young are independent of their mother immediately after birth. In this species, trade-offs in reproductive traits and maternal effects on reproductive effort are well described (Sorci et al. 1994; Lorenzon et al. 2001; Meylan and Clobert 2005).

Capture and Rearing Conditions

In mid-June 2009, 62 gravid females were captured at Mont-Lozère and kept in the laboratory until parturition (at the end of July). We measured the snout-vent length (SVL) and body mass of each female. Females were maintained in individual terraria (18 cm × 12 cm × 12 cm) with damp soil, a shelter, and opportunities for thermoregulation. We used incandescent light bulbs (25 W) for 6 h/d from 0900 hours to 1200 hours and from 1400 hours to 1700 hours local time, which created a thermal gradient ranging from 20° to 30°C. We selected these temperatures because they spanned the thermal breadth of the common lizard (Van Damme et al. 1986). In addition, the duration of basking behavior corresponded with activity patterns in the wild (Avery and McArdle 1973; Patterson and Davies 1978). Females were also offered water ad lib. and *Pyralis* species larvae every 5 d. Earlier studies have also shown that one larva every 5 d fulfills the energy demands of this species (Massot and Clobert 1995; Clobert et al. 2000). Immediately after parturition, mothers and juveniles were separated. We weighed females, and we calculated an index of body condition after parturition based on the residuals from a regression of body mass against SVL. Siblings were housed with the same opportunity for thermoregulation and water as adult females. We weighed the juveniles to assess total litter mass. Within 3 d after birth, juveniles and adult females were released. We attest adherence to the National Institutes of Health Guide for Care and Use of Laboratory Animals. Because physiological and behavioral assays have been performed under laboratory conditions, we may have induced a stress component in the response, and this might complicate the interpretation of our results. However, this seems unlikely in our species, because, in the common lizard, captivity has been shown not to induce a chronic stress response. Captivity did not affect corticosterone levels when measured 2 d after the arrival to the laboratory (Meylan et al. 2003) and did not affect the behavior (Meylan et al. 2009).

Inducing an Immune Challenge

We induced an immune challenge 3 d after capture in approximately half of the pregnant females (an average of 3 wk before parturition; fig. 1). We injected 36 females intraperitoneally with 40 μL of a 2% suspension of SRBCs (Meylan et al. 2010; Bleu et al. 2012). The suspension contained, on average, 5×10^8 SRBCs in sterile phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO). SRBCs are a standard complex antigen commonly used in immunological studies to evaluate immunological responses to a novel pathogen (Hudson and Hay 1989). Indeed, Viney et al. (2005) suggest that, even though individual acquired immune responses are highly specific to an antigen, the ability to make a functionally effective immune response might be largely a generic trait, such that the functional measurement of the immune response to one pathogen is likely to be representative of functional responses to other pathogens. We injected 26 females with 40 μL PBS to serve as

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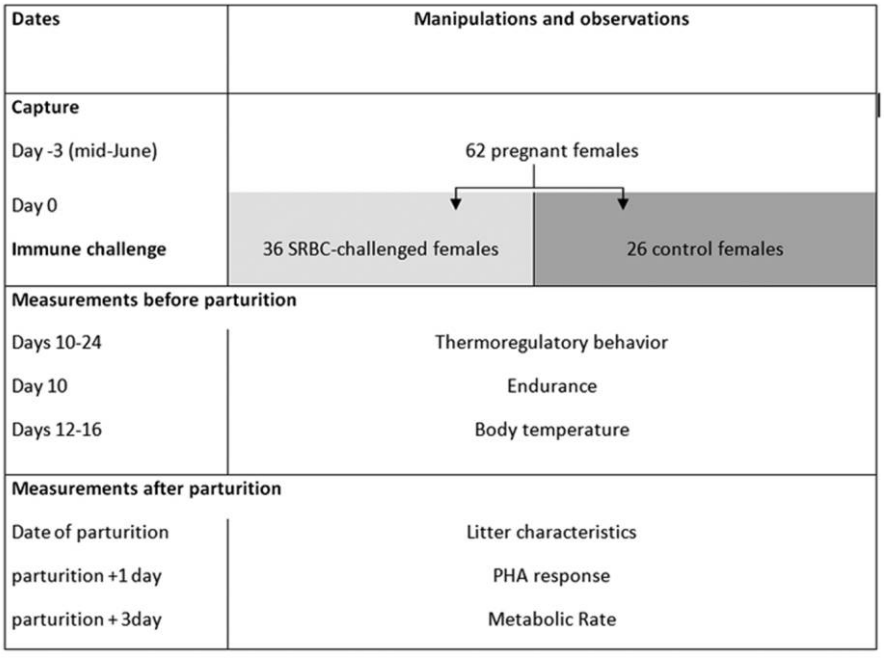

Figure 1. Time line of the experiment. The experiment started 3 d after the capture with the sheep red blood cells (SRBCs) challenge. PHA = phytohemagglutinin.

controls. These two groups did not differ in SVL or weight at the start of the study ($P > 0.05$). An earlier study (Meylan et al. 2010) validated the use of SRBCs to induce an immune response. Because the antibody response to SRBCs is not detectable until 10 d after injection in *Z. vivipara*, all of the following measurements were made more than 10 d after injection (fig. 1).

Assessment of Costs of an Immune Response

Reproductive Performance. Reproductive performance was estimated at parturition by quantifying two life-history traits: litter mass (sum of mass of living and dead neonates) and litter success defined by the proportion of live neonates. We also recorded the date of parturition, because premature neonates can potentially affect the body condition of a female.

Thermoregulatory Behavior and Body Temperature. We investigated thermoregulatory behavior and body temperature of females, because ectotherms are expected to increase body temperature, T_b, in response to an immune challenge (Sherman and Stephens 1998). However, a decrease in T_b can also occur if available energy reserves are insufficient for maintaining a high metabolic rate (Deen and Hutchison 2001). We estimated body temperature using an infrared thermometer with an accuracy of 0.5° (RAYST25XXEU, Raytek, Santa Cruz, CA). We measured the surface temperature of pregnant females two times on 2 d (1000 hours and 1500 hours) and used the mean temperature in the analyses. One of us (D.B.M.) calibrated the dorsal surface temperature of a lizard as determined by an infrared thermometer and cloacal temperature using a quick-reading thermometer (Miller and Weber Cloacal Thermometer). The two estimates of T_b were not statistically different. Moreover, Herczeg et al. (2006) demonstrated that external temperature is very similar to internal temperature in small lizards.

We estimated thermoregulatory behavior of pregnant females using focal observation measurements at different times of the day, between 0930 hours and 1700 hours over a period of approximately 2 wk (e.g., 13–16 observations, depending on the females). We used a blind sampling procedure for obtaining thermoregulatory behavior. An observer noted the location and behavior of each lizard in the terrarium without information regarding the treatment. If a lizard was visible (i.e., if at least a part of the head was out of the shelter), the observer distinguished between two behaviors: basking below the light and/or on the shelter (upright head position and increased respiration; see Carpenter and Ferguson 1977 and Huey 1982*b* for a precise description) and nonbasking (i.e., not under the light; see Lecomte 1993 for a detailed review of thermoregulatory behaviors). Lizards did not appear to change their behavior when an observer entered the room. Therefore, we assume that a lizard's behavior was unaffected by the presence of the observer. We derived an index of thermoregulatory behavior by summing the number of observations for each behavior (e.g., basking and nonbasking) and dividing by the total number of behavioral observations. This method to estimate thermoregulatory behavior has been validated by previous studies (see Meylan et al. 2009; Cote et al. 2010).

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Table 1: Effect of immune challenge (treatment), female snout-vent length (SVL), and date of parturition on reproductive traits and female physiology

	Treatment			Female SVL			Date of parturition			Treatment × date of parturition			Treatment × female SVL		
Variable	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
A. Reproductive traits:															
Litter mass	.31	1, 40	.57	30.97	1, 40	**.0001**	7.83	1, 40	**.008**	.09	1, 38	.76	.09	1, 39	.72
Litter success	.46	1, 53	.50	.23	1, 52	.63	2.92	1, 54	.09	.15	1, 50	.70	.16	1, 51	.69
B. Physiology:															
PHA response	.54	1, 44	.46	5.31	1, 44	**.02**	3.56	1, 43	.07	.32	1, 42	.57	5.28	1, 44	**.02**
Body temperature	3.04	1, 53	.08	.45	1, 53	.50	.77	1, 52	.38	1.79	1, 51	.57	4.42	1, 53	**.04**
Endurance	1.51	1, 40	.22	.91	1, 40	.34	.02	1, 39	.87	2.66	1, 38	.11	3.94	1, 40	**.05**

Note. Values in boldface type are statistically significant. PHA = phytohemagglutinin.

Immunocompetence Test. We estimated immunocompetence after parturition to avoid affecting reproductive performance. Our methods involved the use of a phytohemagglutinin (PHA)–induced skin-swelling test. PHA swelling response is complex, including both innate and adaptive components of the immune system. Thus, PHA swelling response is not solely based on T cell proliferation but encapsulates the general ability of an individual to mount an inflammatory response (Tella et al. 2008; Vinkler et al. 2010). Although the PHA test has only recently been used in reptiles (Svensson et al. 2001; Tella et al. 2008; Munoz et al. 2009; Martin et al. 2011), and more study is needed on the PHA response and factors that may affect this response, it provides information on a broadscale immune response that is initiated by a T cell response and can allow ecologists to assess an integrated immune response (Zimmerman et al. 2010). In a previous study involving the common lizard, Cote et al. (2010) observed that males with a higher PHA response also had a lower parasite load. Vinkler et al. (2010) demonstrated that the PHA test reliably mirrors the individual general proinflammatory potential, despite the highly complex immunological background. A larger localized swelling indicates a more robust immune response. To assess the PHA response, we obtained an initial measurement of the thickness of the right hind leg (at the midpoint of the thigh) using a spessimeter (Mitutoyo, ID-C112, Kanagawa, Japan) with an accuracy of 0.01 mm. We then immediately administered a subcutaneous injection of 0.04 mL of a solution of PBS containing 2.5 mg/mL of PHA (Sigma-Aldrich; reference L8754) in the same leg. We waited a period of 12 h before taking a second measurement, because previous experiments had found that this coincided with the greatest swelling response (Meylan et al. 2010; Massot et al. 2011; Bleu et al. 2012; Richard et al. 2012). We measured the thickness of the hind leg at the injection site. The reaction to PHA was expressed as the difference in thickness between the preinjection and postinjection measurements.

Metabolism. We measured SMR of female lizards from each treatment group 3 d postparturition. We define SMR as the minimum rate of energy expenditure measured under postabsorptive conditions during scotophase at a temperature within the animal's range of activity (Lewis and Gatten 1985). The estimation of SMR relies on the measurement of the volume of oxygen consumed per mass per unit of time (mL O_2/g/ h; Withers 1992). As in other studies, we assume that energy production related to the consumption of a given volume of oxygen is roughly constant. Oxygen consumption was measured using an open-flow respirometer (Sable Systems, Las Vegas, NV) comprising a two-channel pump, two mass-flow controller electronic units, an eight-channel multiplexer, an oxygen analyzer, and a subsam-

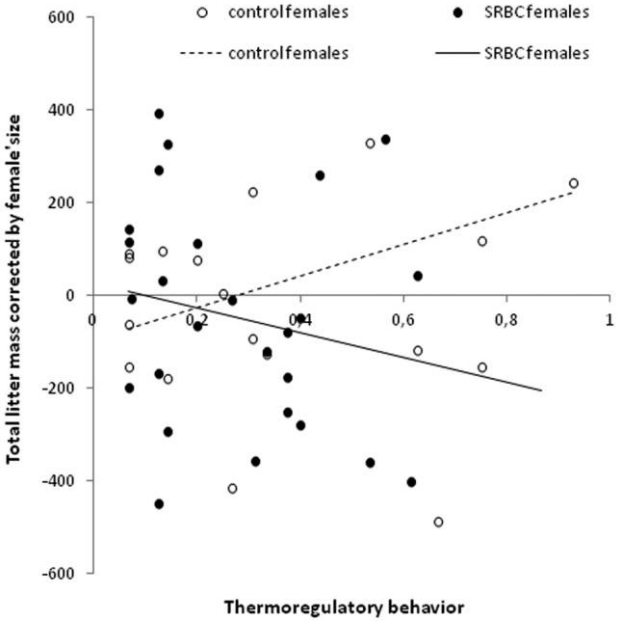

Figure 2. Variation in total mass of the litter (calculated as the sum of the weight of live and dead juveniles) depends on the interaction between immune treatment and thermoregulatory behavior (expressed as the proportion of basking behavior of total behavioral observation). Trendlines are linear regression lines. SRBCs = sheep red blood cells.

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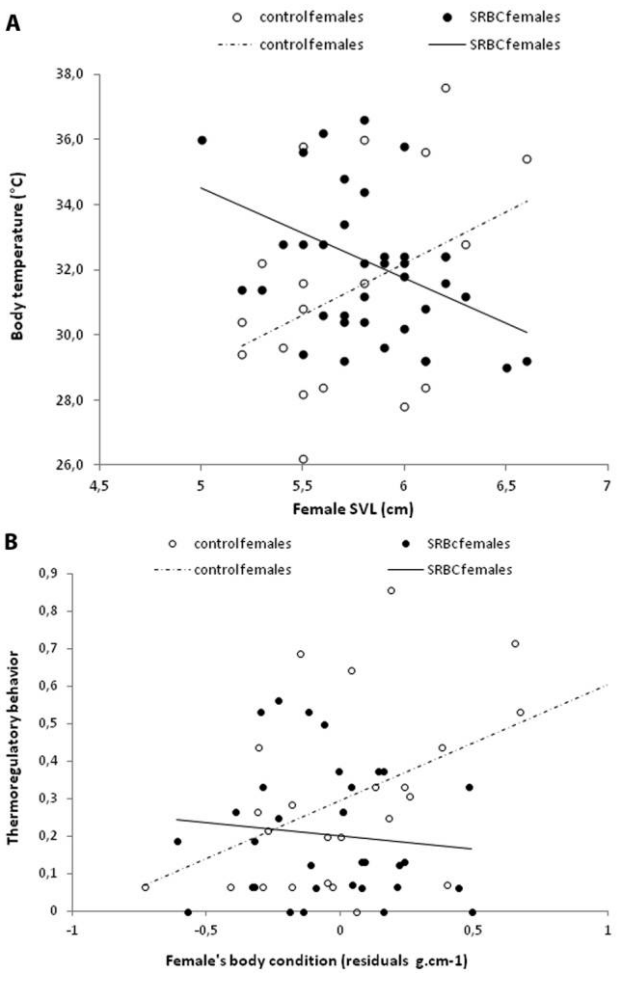

Figure 3. Modulation of the immune treatment by female morphological characteristics on body temperature (*A*) and thermoregulatory behavior (*B*; expressed as the proportion of basking behavior of total behavioral observation). Trend lines represent regression line. SRBCs = sheep red blood cells.

pler/pump/mass-flow meter unit. We removed H_2O and CO_2 by passing the incoming air through columns of Drierite and Ascarite. Animals were placed individually in one of the three 200-mL darkened chambers, which were placed in a room kept at an ambient temperature of 25° ± 0.5°C. We drew air through the system at a rate of 30 mL/min. Outgoing air was also scrubbed of water using Ascarite before entering the O_2 analyzer. We sampled oxygen consumption every 2 min during a 30-min period. Oxygen consumption was calculated as the difference between the oxygen concentration of ambient air and that of excurrent air. All lizards were fasted for 3 d before the measurements, an important criterion for measuring SMR, and were habituated to the metabolic chamber for at least 1 h before the beginning of the observations.

Endurance. We estimated the endurance of female lizards 10 d after the SRBC injection and before parturition. Lizards were warmed to 28°C (which is the mean field active body temperature of *Z. vivipara*; Van Damme et al. 1990) for a period of 30 min. Lizards were fasted for a period of 2 d before quantifying locomotor performance. We measured endurance using a motorized treadmill, which is a standard protocol in studies of physiological performances (Garland and Losos 1994; Miles et al. 2007*a*). We induced the females to run at the pace of the belt (0.5 km/h) by gently tapping on the hind leg. The elapsed time during which lizards maintained their position on the treadmill until exhaustion was our estimate of endurance. A lizard was determined to have been exhausted if it failed to maintain its position on the belt after three attempts.

Data Analyses

We analyzed total litter mass (sum of the weight of all the juveniles), litter success (proportion of live-born neonates), date of parturition, cellular immune response, SMR, endurance, body temperature, and thermoregulatory behavior using a generalized linear model (MIXED procedure of SAS; SAS Institute, ver. 9.1). All models included (1) immune treatment, (2) female size (SVL) or female body condition, (3) date of parturition, and (4) the interaction of female SVL or body condition with the immune treatment. Immune challenge was defined as a class factor (SRBC and control). We used date of parturition as a covariate to control for variation in the stage of pregnancy. Models were simplified using backward elimination of the nonsignificant terms (marginal *F*-tests). The significance level was set at $P = 0.05$. We checked residuals of the initial models for normality and homoscedasticity.

Results

Reproductive Performance

The injection of SRBCs did not affect litter success (table 1, pt. A). Total litter mass was affected by date of parturition and SVL (table 1, pt. A) and the interaction between immune treatment and thermoregulatory behavior ($F_{1,40} = 4.17$, $P = 0.047$; fig. 2). Litter masses were smaller for females injected with SRBCs that spent more time basking. In contrast, there was no difference between control and SRBC females when females spent less time basking. Immune treatment did not alter the date of parturition ($F_{1,54} = 0.62$, $P = 0.43$) or female body condition after parturition ($F_{1,54} = 0.91$, $P = 0.34$).

Thermoregulatory Behavior

The effects of immune treatment on body temperature depended on female SVL (table 1, pt. B). Body temperature of small females was higher if injected with SRBCs, whereas the body temperature of larger females was higher in the control group (fig. 3*A*). The interaction between thermoregulatory behavior and body condition was also modified by SRBC injection (table 2). Females with low body condition in the SRBC group spent more time basking than did controls, but the females with higher values for body condition basked less (fig. 3*B*).

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Table 2: Effect of immune challenge (treatment), female body condition, and date of parturition on female metabolism and thermoregulatory behavior

	Treatment			Female body condition			Date of parturition			Treatment × date of parturition			Treatment × female body condition		
Variable	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Thermoregulation	3.44	1, 52	.06	1.41	1, 52	.24	9.20	1, 52	**.003**	.83	1, 51	.36	6.93	1, 52	**.01**
Metabolism	.34	1, 23	.56	2.31	1, 23	.14	1.10	1, 22	.30	.11	1, 24	.74	7.49	1, 23	**.01**

Note. Values in boldface type are statistically significant.

Physiological Performance

We found that immune treatment affected all of the physiological traits, endurance, metabolism (SMR), and PHA response. However the effect of SRBCs depended on female body size and condition. The interaction between SRBC treatment and female SVL affected PHA response and endurance (table 1, pt. B). Endurance and the cellular immune response were higher for the control group than for the SRBC group in small females, whereas the opposite pattern characterized larger females (fig. 4*A*, 4*B*). Endurance was also negatively affected by the total litter mass ($F_{1,40} = 5.18$, $P = 0.03$) in the two treatments.

SMR was affected by an interaction of SRBCs and female body condition (table 2). Oxygen consumption was higher for control versus SRBC treatment groups for females with low body condition, but females that had a high value for body condition had higher values of oxygen consumption (fig. 4*C*).

Discussion

In our study, an immune challenge induced by the injection of SRBCs affected reproductive investment and physiology in *Zootoca vivipara*. SRBC females differed from control females in terms of total litter mass but not litter success. Furthermore, immune-challenged females differed in all physiological components relative to control females. However, these responses were modulated by two covariates: female size (SVL) and body condition. We suggest that these patterns are consistent with the allocation principle (Stearns 1992; Roff and Fairbairn 2007) and involve trade-offs between investment in competing energetic functions, such as reproduction and immune response.

Reproductive Costs of the Immune Response

The modification in reproductive expenditure was manifested in litter mass but not offspring size, as observed by Uller et al. (2006). The absence of an adjustment in the number of offspring may be a consequence of the limited opportunity for common lizard females to adjust their litter size (Bleu et al. 2011). In contrast, several studies have shown that female *Z. vivipara* can adjust offspring quality during gestation, such as hatchling SVL, mass, and body condition (Massot and Clobert 1995; Lorenzon et al. 2001; Meylan et al. 2002, 2004, 2007; Le Galliard et al. 2006). In this study, we demonstrated that the effect of mounting an immune response on reproductive effort is conditional on two or more traits. Indeed, immune activation decreased the mass of the litter only when females spent more time basking. Because body temperature and metabolic rate are positively correlated, a female who raises her body temperature also incurs a concomitant increase in energetic expenditures. Consequently, immunochallenged females cannot simultaneously maintain a high body temperature and increase litter mass because of energetic constraints (Belliure et al. 2004). Although thermoregulation is costly (Hertz et al. 1982; Blouin-Demers and Weatherhead 2001), maintaining a body temperature at or near a physiological optimum value may be selectively advantageous, because in ectothermic species, many critical physiological activities, such as locomotion, foraging performance, and digestive assimilation, are temperature dependent (Avery 1982; Huey 1982*a*). Because of their small size, lizards are constrained to use behavioral rather than physiological adjustments to regulate their body temperature (Huey 1982*b*; Stevenson 1985).

Thermoregulatory Behavior Consequences of the Immune Response

Our experiment demonstrated that the immune treatment affected thermoregulatory behavior differently depending on the female body condition. We found that females with low body condition responded to an immune challenge by increasing the time spent basking, relative to control females. In contrast, we found the opposite pattern for females with high body condition. We hypothesize that SRBC females have higher energetic demands and can compensate for this by using two different strategies. First, females may rely on intrinsic energy, such as fat stores (as in females with a high body condition), to meet higher energetic demands. Second, they may modify thermoregulatory behavior to be able to forage more and digest food more efficiently (as in females with low body condition). We found a similar effect on the activation of an immune response with body temperature, but it was conditional on female SVL rather than on body condition. Belliure et al. (2004) also found a modulation of an experimental treatment on thermoregulatory behavior by individual size in *Z. vivipara*. By using intrinsic energy reserves to mount a response against infection, SRBC-treated females with high body condition avoid energy-consuming activities.

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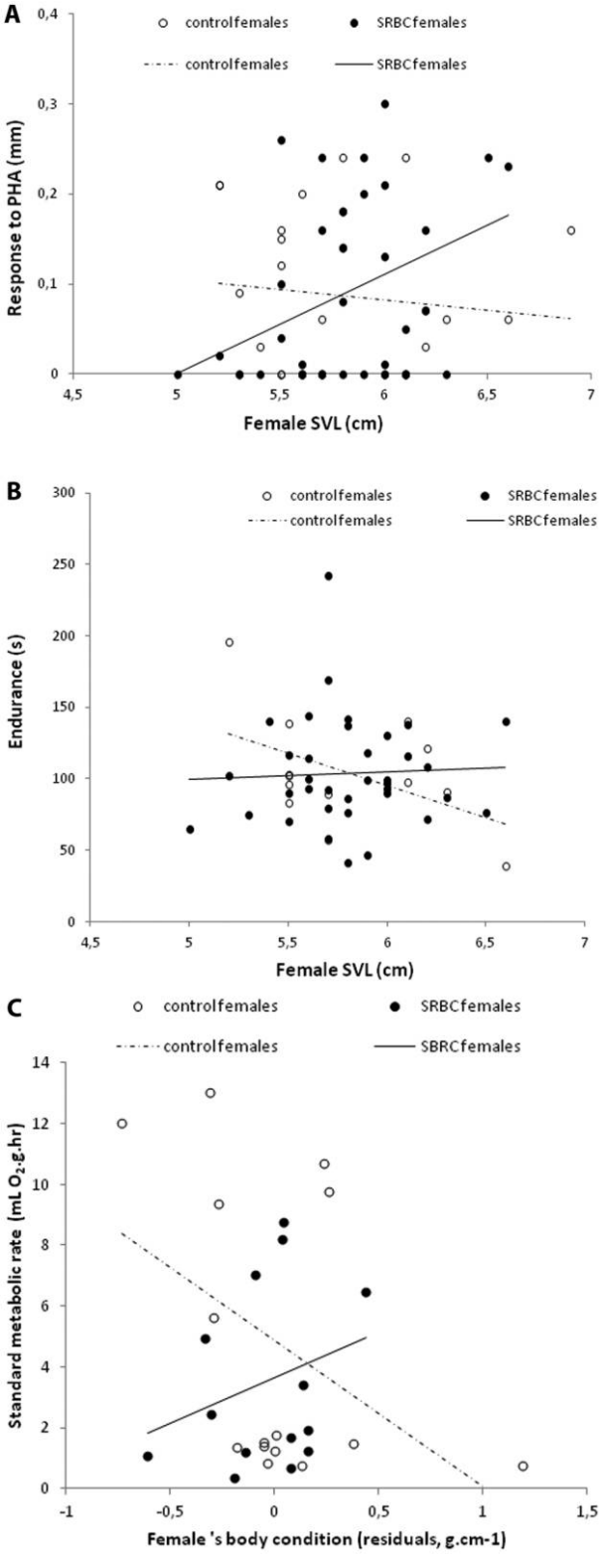

Figure 4. Modulation of the immune treatment on physiological performance by female morphological characteristics. *A*, Phytohemagglutinin (PHA) response; *B*, endurance; and *C*, standard metabolic rate. Trend lines represent regression line. SRBCs = sheep red blood cells; SVL = snout-vent length.

Physiological Adjustments during the Immune Response

We quantified the energy expenditure required when mounting an immune response by measuring SMR. The magnitude of the difference between SRBC treatment and control individuals provides an index to the difference in energy expenditure after an immune challenge. Based on previous results, the energetic cost of an immune response appears to depend on the species (Ots et al. 2001; Horak et al. 2003; Eraud et al. 2005). The effects of immunization on SMR as revealed in our study are complex and depended on an interaction between SRBC treatment and body condition. That is, SRBC-treated females with high values for body condition displayed high values for SMR, whereas, in the control group, females with high values for body condition displayed low values for SMR. We suggest that this pattern also reflects differences among females with respect to current energetic stores. Confronted with an immune challenge, only the largest females that have higher energetic stores can sustain an elevated SMR. This result confirms that individuals that vary in body condition used different strategies to face an immune challenge.

We also investigated different physiological components that were linked with fitness, such as cell-mediated immune system and endurance. Indeed, previous work has verified that cell-mediated immunity provides efficient protection against infections by viruses and intracellular bacteria and therefore may be related to survival (Gonzalez et al. 1999). However, few analyses have examined the effects of immunocompetence and whole-organism performance, such as endurance or sprint speed. Huyghe et al. (2009) noted that bite force and PHA response were correlated, but this was mediated by variation in body size among males in the lizard species *Podarcis melisellensis*. In contrast, we found that endurance in control females decreased with body size but increased with size in SRBC females. This suggests that mounting a response to an immune challenge does not compromise aerobic physiological performance.

Correlations among physiological traits can complicate the interpretation of high PHA responses (Miles et al. 2007*a*). Examining isolated immune components does not provide a comprehensive measure of the immune system and should not be interpreted as such. Indeed, even if individuals with a high cell-mediated immunity can have an advantage in the short term, the immediate costs may compromise response in the long term. In contrast, individuals with low cellular immunity may have a more efficient capacity to respond to antigenic stressors (Lochmiller and Deerenberg 2000; Sorci and Faivre 2009). This hypothesis remains largely untested in wild populations, particularly in reptiles. Few ecological studies have assessed the relations between humoral and cellular immune components (Blount et al. 2003; Ardia 2007). An earlier study, involving the common lizard (Meylan et al. 2010), found a negative correlation between humoral and cellular response. However, including an intrinsic factor such as body size, as in our study, revealed a more complex relationship between the two immune components. The SRBC challenge enhanced the PHA response, but only in females with high body condition. This result sug-

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gests that, in this species, humoral challenge does not result in an altered cutaneous immune response among large individuals. This is in accordance with Alonso-Alvarez and Tella (2001), who found that the PHA response was dependent on body condition. We found exactly the same results for endurance: SRBCs decreased endurance in females with low body condition, whereas the SRBC challenge enhanced endurance for females with high body condition.

Our result suggests that a trade-off between humoral immune response and other physiological components (cell-mediated immune response and endurance) may exist in circumstances when females are energy limited. Size and body condition have been repeatedly associated with individual quality (Partridge and Farquhar 1983; Lefranc and Bundgaard 2000). However, these two morphological traits provide different information regarding female quality and are not sensitive to the same factors. Individuals of *Z. vivipara* are characterized by indeterminate growth. Therefore, female SVL may vary as a result of age as well as success in food acquisition or as a result of allocation of energy to reproduction, rather than growth, in previous years. We suggest that body condition provides an estimate of the current quality of the individuals. Depending on the measured life-history traits and different selective agents, we can expect maternal size or body condition to have significant and variable effects (Meylan et al. 2002). From an adaptive point of view, the modulation of the impact of an immune challenge by intrinsic factors such as body condition and size is not surprising and in accordance with the allocation principle (Sheldon and Verhulst 1996), and it should be better taken into account in future studies on the cost of the immune response.

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