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Costly parasite resistance: a genotype-dependent handicap in sand lizards?

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Male sand lizards (*Lacerta agilis*) with a specific restriction fragment length polymorphism fragment in their major histocompatibility complex (MHC) genotype ('O-males') are more resistant to ectoparasites (a tick, *Ixodes ricinus*) than are males that lack this fragment ('NO-males'). However, emerging evidence suggests that such adaptive immune responses are costly, here manifested by reduced body condition and a compromised defence against secondary infections by haemoprotid parasites that use the ticks as vectors. Subsequent to tick encounter, O-males suffer from a higher leucocyte–erythrocyte ratio, and higher haemoprotid parasitaemia, in particular in relation to vector encounter rate. Furthermore, O-males (i.e. successful tick defenders) with more haemoprotid parasites remaining in their blood stream were in better body condition, whereas this did not apply in NO-males, demonstrating that the adaptive immunoreaction can—in the short term—be energetically even more costly than being moderately parasitized. In agreement with Zahavian handicap theory, O-males had a (marginally) higher reproductive success than males that lacked this fragment.

Keywords: MHC; costly parasite resistance; handicap; sand lizard

1. INTRODUCTION

Within-species alternative genotypes of a parasite and its host may fluctuate in frequency depending on their virulence and resistance (Hamilton & Zuk 1982). In the current paper, we extend this host–single pathogen interaction to a host–multiple pathogen scenario; thus, instead of making the assumption that a host–pathogen arms race is driven by alternative genotypes within a host and a single parasite taxon, we make the assumption that costs from combating one parasite may also compromise host energetic status (body condition) and ability to combat a second pathogen.

The Swedish sand lizard (*Lacerta agilis*), is an ideal model for testing this scenario, since we have shown elsewhere (Olsson *et al.* in press) that males of two different restriction fragment length polymorphism (RFLP) genotypes with respect to the major histocompatibility complex (MHC; with or without the 'O' fragment present in their genotype, hereafter 'O-males', and 'NO-males') differ in capacity to combat ticks, which act as vectors for haemoprotid parasites (Rheichenbach-Klinke & Elkan 1965). Thus, in the light of the emerging evidence that adaptive immune responses can be energetically costly (*sensu* Sheldon & Verhulst 1996; Råberg *et al.* 1998; Svensson *et al.* 1998; Råberg *et al.* 2000; Jacot *et al.* 2004), we make the directional prediction that males with an MHC genotype that causes them to better identify a pathogen that they encounter early in the season, and produce an adaptive immune response against this target, should subsequently suffer more in terms of energetic expenditure and susceptibility to secondary infections.

Previous field experiments demonstrate that ectoparasite resistance in this species is negatively influenced by high steroid levels (Olsson *et al.* 2000). Here we revisit that dataset after having (i) MHC genotyped the males (using RFLP; Madsen *et al.* 2000), and (ii) screened blood smears of a standardized volume for haemoprotid parasites and relative numbers of leucocytes to erythrocytes. This first allowed us to test for independent genotype (O-males versus NO-males) and testosterone treatment effects on parasite resistance (testosterone implants versus controls). We then tested the following predictions to assess if costs associated with pathogen resistance are genotype-dependant: O-males should suffer (i) altered relative blood cell counts (higher leucocyte counts through induced adaptive immunoreactions), (ii) elevated levels of haemoprotid parasites late in the season (suffering the consequences of successful ectoparasite defence early in the season), (iii) greater loss in body condition (as a consequence of higher energetic expenditure early in the season), and (iv) in spite of this, have a higher reproductive success (predicted from handicap theory; *sensu* Zahavi 1975: Folstad & Karter 1992).

2. MATERIAL AND METHODS

Detailed husbandry, field methods and molecular protocols have been published elsewhere (Madsen *et al.* 2000; Olsson *et al.* 2000) and we, therefore, only give a brief account of methods used. Males were captured by noose or by hand within a week of emergence from hibernation (*ca* first week in May), were blood sampled within 30 s of capturing, measured and weighed and, on the day following capture, anaesthetized and given a silastic implant (every second male received an empty control implant or one with a 4 mm crystalline testosterone column; Sigma product no. T 1500; Sigma Aldrich Pty Ltd, Castle Hill, NSW, Australia). They were then individually marked with a numbered cloth adhesive sticker on their backs, released within 24 h at the place of capture, and then monitored with respect to mating success throughout the approximately 3 week mating season every day when weather permitted lizard activity. Male mating success was scored as successful mate guarding of females, which we have shown elsewhere is strongly correlated with male probability of paternity, using mini- and microsatellites (Olsson *et al.* 2000). Since this measure of mating success was correlated with a male's total number of observations ($r_s = 0.53$, $p = 0.0002$, $n = 40$), we divided the number of observations of mate guarding by the total number of observations and used this ratio as an index of male mating success. At the end of the mating season, the lizards were recaptured and blood sampled

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again to assess the effect of the testosterone treatment on the target traits.

All observations in the current paper were made in 1999, with the exception of a tick removal experiment in 2002. Unpublished data show that tick resistance is a heritable trait in sand lizards (at least in females; Olsson *et al.* in preparation). Therefore, in order to assess whether individual differences in tick load were repeatable in males, and potentially heritable, ticks were manually removed the first week of spring activity and were then recounted after the mating season to assess repeatability of tick counts. Although this cannot rule out differences in tick encounter rate among discrete habitats, our previous work shows that males are highly mobile through habitat types, and that mobility (metres moved during the mating season) is uncorrelated with male tick load (Olsson *et al.* 2000). Thus, our removal experiment should be highly indicative of among-individual differences in tick resistance, which we know is partly genetically determined, suggesting that tick resistance is heritable and should evolve in response to selection.

MHC class I genotype was screened using a species-specific probe for RFLP (Madsen *et al.* 2000). On average, males had 11.6 ± 3.3, s.e., RFLP-fragments in their genotype. To estimate prevalence of haemoparasites, a 30 µl blood sample was spread on a microscope slide in a standardized way, air dried, fixed with methanol and Giemsa stained, and stored dry until cell counts were performed. Erythrocytes and leucocytes were counted in 50 standardized areas along four longitudinal transects on each microscope slide at 100×. In total, 226 574 leucocytes and 812 368 erythrocytes were counted and screened for haemoprotid parasites. Haemoprotid parasite counts were made by parasitologist Dr Dan Christensson, Department of Parasitology, the Swedish National Veterinary Institute (SVA), Uppsala, Sweden.

The statistical analyses were based on parametric techniques when the assumptions of normality were met. We consistently tested the directional prediction that O-males suffered higher costs in terms of altered blood cell counts, internal parasite load, and body condition than do NO-males subsequent to an initial tick load accumulated during the first week of spring activities, i.e. when males novel to haemoprotid parasites first become infected and/or already infected males may receive boost infections via tick vectors (Rheichenbach-Klinke & Elkan 1965). The rational for this is the directional prediction that O-males, with an MHC fragment associated with tick resistance, mount a more successful, but energetically costly, immune response than NO-males. Therefore, presented p values for the three t-tests assessing haemocytology and parasitaemia are one-tailed.

3. RESULTS

Our tick removal experiment confirmed that individual tick load early in the season is a repeatable trait as a first and second tick count were positively correlated ($r_s = 0.39$, $p = 0.028$, $n = 37$).

A two-factor ANOVA revealed that both testosterone treatment category (T-implant versus control), and O-fragment category (O present versus absent in genotype) independently contributed to an altered white-to-red blood cell ratio (Model: $F_{3,20} = 4.94$, $p = 0.010$, $R^2 = 0.42$; T-treatment, $F = 4.82$, $p = 0.040$; O-category, $F = 7.16$, $p = 0.015$). We, therefore, standardized our data for testosterone treatment by setting trait means to zero and standard deviations to one before pooling the data for analyses of trait differences between O-fragment categories. Three t-tests between O-categories demonstrated that O-males had on average a significantly higher leucocyte–erythrocyte ratio than NO-males (0.39 ± 0.30, s.e., and -0.46 ± 0.17, respectively; $t_{18.6} = -2.41$, $p = 0.0013$), a higher haemoprotid parasitaemia in absolute terms (0.42 ± 0.33, versus -0.49 ± 0.06, $t_{12.8} = -2.73$, $p = 0.0085$), and a higher infection rate of haemoprotid parasites in relation to tick load (i.e. vector encounter rate) at the end of the mating season (0.43 ± 0.32, versus -0.50 ± 0.09, $t = -2.80$, $p = 0.007$). We then performed two separate analyses of predictors of body condition (residuals from a mass–snout-vent length regression) in O- versus NO-males. In NO-males, our statistical model was not statistically significant (table 1; $p > 0.124$ for all variables). The corresponding analysis for O-males, however, painted a completely different picture. Leucocyte–erythrocyte ratio, haemoprotid parasitaemia, and tick load explained no less than 84% of the variation in body condition, with all three factors being highly significant ($p < 0.009$ for all variables), resulting in a highly significant model ($F_{3,9} = 16.3$, $p = 0.0006$; table 1). Importantly, whereas the leucocyte–erythrocyte ratio impacted negatively on our proxy of energetic expenditure (body condition), tick load and haemoprotid infection rates were positively associated with body condition. Furthermore, O-males had a mean mating success of 0.30 ± 0.26, versus -0.32 ± 0.17 in NO-males ($t_{25.9} = -2.401$, $p = 0.033$).

Table 1. Multiple regression of predictor variables on male body condition as response variable (residuals from a mass–snout-vent length regression late in the mating season).

predictors	β	T	p	s.e.
NO-males (Model: $F_{3,7} = 1.95$, $p = 0.21$, $R^2 = 0.45$)				
leucocyte : erythrocyte	0.80	0.26	0.802	3.060
infection rate	5.87	1.60	0.151	3.66
tick load	0.02	1.75	0.124	0.01
O-males (Model: $F_{3,9} = 16.3$, $p = 0.0006$, $R^2 = 0.84$)				
leucocyte : erythrocyte	−3.24	−5.27	0.0005	0.64
infection rate	1.80	3.34	0.0087	0.54
tick load	0.04	4.89	0.0009	0.009

4. DISCUSSION

Recent work in ecological immunology has demonstrated costs in terms of energetic expenditure and survival in response to immunoreactions in general, and antibody responses in particular (Sheldon & Verhulst 1996; Råberg *et al.* 1998; Svensson *et al.* 1998; Moret & Schmid-Haempel 2000; Råberg *et al.* 2000; Jacot *et al.* 2004). Immunosuppression has been suggested as one way to counter such costs, depending on context and condition. We demonstrate elsewhere that O-males are more successful at combating ectoparasites under physiological stress early in the season, when their secondary sex trait (green lateral coloration; Olsson *et al.* in press) is under development, than are males that lack this genetic fragment associated with the MHC. However, the more tick-resistant males show an altered blood cell count later in the season with a reduction in erythrocytes and, in spite of this, have higher haemoprotid parasite counts, in particular in relation to vector encounter rate (tick load). Importantly, in O-males, more than 84% of the variation in body condition was explained by leucocyte (increased)–erythrocyte (reduced) ratio, and the 'positive' effects of high tick and haemoprotid counts late in the season. Given that these are independent effects and none of them significant in NO-males, it seems to suggest that

the O-fragment itself is linked to the increased energetic expenditure associated with parasitaemia. However, our current analysis does not admit a separation of the effect of an energetically costly anti-tick defence on body condition versus haemoprotid load; haemoprotid infection rate could increase as a consequence of being in poorer overall health and body condition. Regardless, for the sake of our argument that tick immune defence is associated with subsequent energetic costs and risk of secondary infections, this is less important.

The first of these results agrees with the notion of a more strongly activated immune system (relatively more circulating leucocytes) and associated energetic costs. Furthermore, it shows that males with more haemoprotid parasites remaining in their blood stream also have better body condition and a higher reproductive success. This seems to suggest that, even if tick resistance may contribute to larger badges (Olsson *et al.* in press) and higher reproductive success, i.e. be beneficial in terms of fitness, it may be energetically costly in the short term. Thus, lifetime reproductive success will depend on individual-specific ability to 'suffer costs' (handicaps) in the short term and with sustained competitive ability to acquire partners.

Our data thus suggest that O-males pay for their successful tick defence through elevated infestation rate of haemoprotid parasites later in the season and through a concomitant loss in body condition. Importantly, however, these energetic costs are paid for when O-males have already achieved their relatively higher mating success. Thus, energetic costs carried in the short term are traded against long-term fitness benefits. Furthermore, our sequential analysis of two parasite taxa suggests that differential resistance among hosts to multiple taxa of parasites, depending on host MHC genotype, may maintain variation in heritable parasite resistance on which directional sexual selection may operate. Our work supports recent evidence of costs for mounting adaptive immune reactions, with the added complexity that these arguments need to be assessed at the level of an individual's genotype-specific pathogen resistance, and its long-term effects on host and parasite fitness.

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