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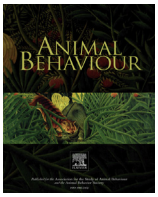

Effect of immune challenge on aggressive behaviour: how to fight two battles at once

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Animals are attacked by pathogens, predators and competitors, sometimes simultaneously. For example, male crickets fight each other for access to females and such challenges may occur when one or both males are also defending themselves against a pathogen. We tested whether males altered their aggressive motivation when faced with both a competitor and a pathogen simultaneously. We found that immune-challenged male crickets (*Gryllus texensis*) were more likely to defeat male competitors when the reproductive payoff (i.e. a female) was nearby. However, when females were not present, males showed a decrease in their aggressive behaviour, and were less likely to win encounters with control males. This plasticity of aggressive motivation did not occur when males were chronically immune challenged. Chronically immune-challenged males were more likely to be defeated by control males regardless of the presence of a female. These results suggest that the costs associated with a chronic immune challenge may prevent terminal reproductive investment. We show that males can benefit from terminal reproductive investment because acutely immune-challenged males were still capable of mating and females did not appear to discriminate against them. Male crickets appear to engage in a context-dependent trade-off between the ability to defeat pathogens and the ability to defeat competitors. This trade-off is mediated, in part, by changes in male aggressive motivation (i.e. the critical threshold for the decision to flee).

Males fight for access to mates in many species (Andersson, 1994). Such fighting behaviour can be energetically expensive (e.g. crickets, Hack, 1997a) as well as carry other costs such as the risk of wounding (Hack, 1997a, 1997b). However, these costs are offset by the increase in reproductive success gained by victorious males (Andersson, 1994; e.g. crickets, Loher & Dambach, 1989), although losing males probably suffer a loss of fitness (Hack, 1997b). Therefore, during an aggressive contest, males that can correctly assess when the benefits outweigh the costs will have a selective advantage. Determining the rules males use for this assessment has been an area of intense research for over 3 decades (Arnott & Elwood, 2009).

Research on a wide range of species from different phyla (Arnott & Elwood, 2009) has helped us understand the rules animals use to determine how heavily to invest in a given contest. Crickets, in particular, provide an excellent model system in which to study these issues. For example, aggressive motivation is easy to assess in crickets because male field crickets use a relatively stereotyped series of aggressive behaviours that escalate in intensity during a fight (Adamo & Hoy, 1995; Loher & Dambach, 1989). Moreover, cricket aggressive behaviour has been well studied. For example, metabolic and behavioural studies have shown that the most intense aggressive behaviour, grappling, is the most energetically expensive and dangerous (Hack, 1997a, 1997b).

The hierarchical organization of aggressive behaviours in crickets allows the use of cumulative assessment to determine how much effort to make during a fight (Rillich, Schildberger, & Stevenson, 2007), although in crickets this method also includes some assessment of the opponent (Arnott & Elwood, 2009; Briffa, 2008; Rillich et al., 2007). Cricket males persist in fighting until the ‘cost’ of the fight exceeds a critical threshold for the decision to flee (Rillich et al., 2007). Males with greater resource-holding potential (e.g. size, strength, condition) win more fights (e.g. Brown, Smith, Moskalik, & Gabriel, 2006; Hall, McLaren, Brooks, & Lailvaux, 2010; Judge, Ting, Schneider, & Fitzpatrick, 2010; however see Fitzsimmons & Bertram, 2013) and have higher mating success than weaker males (see Judge et al., 2010 for review). Factors such as age (Adamo, Schildberger, & Loher, 1994; Dixon & Cade, 1986), a recent win or loss (e.g. Adamo & Hoy, 1995;

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Simmons, 1986) and population density (e.g. Simmons, 1986; Stevenson & Rillich, 2013) influence the critical threshold. Social context is also important, as it is in most animals (Dugatkin, 2013). For example, exposure to females can increase aggressive behaviour in the male crickets of *Gryllus bimaculatus* (Simmons, 1986; Stevenson & Rillich, 2013; Tachon, Murray, Gray, & Cade, 1999), *Acheta domesticus* (Brown, Chimenti, & Siebert, 2007; Brown et al., 2006; Killian & Allen, 2008) and *Gryllus veletis* (Fitzsimmons & Bertram, 2013b). These results demonstrate that the critical threshold is updated for each encounter, taking into account the animal's present ability (i.e. resource-holding potential, e.g. recent win), the risks involved (e.g. population density) and the value of the potential payoff (e.g. presence of a female).

Attacks by parasites and pathogens are common in animals, but their effects on aggressive behaviour are poorly understood. However, an immune challenge provides an interesting test of how individual males set a critical threshold. An immune challenge induces a physiological shift in an organism, resulting in changes in the availability of resources for other demands, such as flight-or-fight (Adamo, 2014a). For example, an immune challenge in crickets requires both energetic (Ardia, Gantz, Schneider, & Strebel, 2012) and molecular resources (Adamo, Roberts, Easy, & Ross, 2008) and this is likely to reduce the ability of males to outcompete healthy rivals. For example, the effects of immune challenge on lipid transport in crickets (Adamo et al., 2008) will reduce their ability to perform fight-or-flight behaviour. An immune-challenged cricket is less able to escape predators, probably in part due to an increase in their response time (Otti, Gantenbein-Ritter, Jacot, & Brinkhof, 2012). In a related insect, the locust, an immune challenge reduces their stamina for flight (Seyoum, Bateman, & Charnley, 2002). The magnitude of an animal's decline in physical ability during an immune challenge will vary depending on the pathogen, the type of host response and the duration of the challenge.

Despite a likely decline in resource-holding potential, some animals such as male crickets (*Gryllus integer*) (Pölkki, Kortet, Hedrick, & Rantala, 2013) and male northwestern song sparrows, *Melospiza melodia morphna*, during the breeding season (Owen-Ashley & Wingfield, 2006), maintain, or even increase, their investment in aggressive behaviour during an immune challenge. This is a surprising result; animals that have lower resource-holding potential would not be expected to win fights and may, in fact, be injured in the attempt. Moreover, the physiological shifts that occur during an immune challenge will alter the relative costs and benefits of fighting (Adamo, 2014a). For example, fighting behaviour reduces disease resistance in the cricket *Gryllus texensis* (Adamo & Parsons, 2006), and, therefore, increased investments in aggressive behaviour will reduce the survival of infected individuals. In other words, fighting now incurs additional costs above the usual ones of energetics and an increased risk of injury. Therefore, immune-challenged males must 'overinvest' in aggressive behaviour in order to defeat a healthy rival. This increased investment may be the result of an increased tolerance for risk and/or an increased value placed on immediate reproductive opportunities.

This phenomenon has been considered an example of terminal reproductive investment (Pölkki et al., 2013), in which animals increase investment in behaviours needed for reproduction at the cost of decreased survival (Clutton-Brock, 1984). For example, female crickets increase the number of eggs they lay in response to a bacterial infection (Adamo, 1999). However, terminal reproductive investment is probably an inexpensive option for female crickets. Females contain fully formed eggs in their lateral oviduct, and, therefore, an increase in egg laying can occur without increased investment in egg production, at least over the short term (Adamo, 1999; Shoemaker, Parsons, & Adamo, 2006). On the other hand, there is no evidence that male crickets show any reproductive compensation when infected with a common parasitic fly (Kolluru, Zuk, & Chappell, 2002; Vincent & Bertram, 2010). In fact, we might expect terminal reproductive investment to occur only rarely in the context of aggressive behaviour. When males have lower resource-holding potential, the number of fights they can hope to win, even if 'overinvesting' in aggressive behaviour, is likely to be limited. This constraint should select for immune-challenged males to be highly context dependent in terms of which fights to escalate. For example, although an immune-challenged male may be able to win a brief fight and mate with an available female, he is unlikely to succeed if a prolonged effort is required (e.g. holding a territory). In a recent study (Pölkki et al., 2013), crickets were tested only in the presence of a female (i.e. only when the reproductive payoff was immediately available).

We predicted that male *G. texensis* crickets presented with an immune challenge would reduce their aggressive behaviour when a reproductive payoff was not immediately available. We further predicted that this reduction would co-occur with the physiological changes that occur during an immune challenge, if these physiological changes are driving changes in aggressive behaviour. From an earlier study (e.g. Adamo et al., 2008), we know that important physiological responses occur 90 min after a standard challenge (i.e. injection of heat-killed bacteria), but that these responses are not measurable 5 min or 24 h after injection. Therefore, we expected that the effects of an immune challenge would occur at the 90 min time point. We also predicted that the decline in aggressive behaviour would be reversed when females were present. When females are present, we predicted that males should 'overinvest' in aggressive behaviour and defeat healthy males (i.e. terminal reproductive investment). However, terminal reproductive investment is an unlikely explanation for increased aggressive behaviour if immune-challenged males rarely mate. Pölkki et al. (2013) found that immune-challenged males were less successful at mating, suggesting that females discriminate against them. If females avoid mating with immune-challenged males, then this raises the question as to whether terminal reproductive investment can explain the observed increase in fighting success. Therefore, we also tested the effect of immune challenge on the ability of males to court and copulate with female *G. texensis*. Finally, we examined the effect of chronic immune activation on male aggressive behaviour to test whether males respond differently to a long-term versus a short-term immune challenge. We predict that chronically immune-challenged males would reduce aggressive behaviour as they are likely to be physically unable to beat a healthy, weight-matched opponent. Under these conditions, terminal reproductive investment may not be a viable option.

METHODS

Animals

Crickets (*G. texensis*) were originally collected near Austin, Texas, and have been maintained in a laboratory colony for several generations (see Stahlschmidt, O'Leary, & Adamo, 2014 for description of colony maintenance). Briefly, animals were maintained at 25 ± 2 °C with 65% relative humidity on a 12:12 h light:dark cycle. Crickets were fed dried cat food pellets and water ad libitum.

All crickets were isolated at least 24 h before treatment, unless otherwise indicated. Crickets were isolated in white opaque plastic tubs (10 cm diameter × 10 cm depth) and stored in the colony room prior to the study. Isolated crickets received food and water ad libitum. The weight of the crickets was recorded at the time of isolation. Crickets were between 1 and 18 days past the moult to the adult stage during the chronic immune-challenge study. For all