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Genetic resistance to infection influences a male's sexual attractiveness and modulation of testosterone

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Abstract

Females may be attracted to males genetically resistant to infectious diseases, and one potential mechanism for this mating bias is that such males may be better able to maintain high testosterone. To test these two hypotheses, we collected scent-marks from male house mice (*Mus domesticus*) genetically resistant and susceptible to *Salmonella* due to a single locus (Nramp 1, also known as Slc11a1). We tested whether females are more attracted to the scent-marks of resistant males, and whether such males are better able to maintain testosterone concentrations during an experimental *Salmonella* infection. We found that females preferred the scent-marks of genetically resistant males compared to susceptible ones; but they showed no preferences 5 d after males were infected. As predicted, genetically resistant males maintained their testosterone concentrations during the experimental infection, whereas susceptible males showed a significant decline 14 d after inoculation. These differences in the males' ability to modulate testosterone, however, do not explain females' attraction to resistant males. Thus, our results indicate that females sometimes prefer males genetically resistant to infection, and they provide the first evidence that males modulate their testosterone depending upon their genetic resistance to infection; however, we found no evidence to link these two findings.

Keywords: Sexual selection; Hamilton–Zuk hypothesis; Immunocompetence handicap hypothesis; Testosterone; Mammals; House mice; *Mus*; *Salmonella*; Nramp; Scent-marking

1. Introduction

It is often suggested that females prefer males that are genetically resistant to infectious diseases (Hamilton and Zuk, 1982), but direct tests of this idea are lacking. Females prefer males with exaggerated secondary sexual traits, and such traits often indicate a male's parasite load or immunocompetence (Hamilton and Poulin, 1997; Møller et al., 1999), but it is unclear whether they reveal genetic resistance to infectious diseases. Most work on parasite-mediated sexual selection has addressed the mechanisms through which secondary sexual traits indicate parasite load or immunocompetence (Hillgarth et al., 1997). The leading idea proposes that genetically susceptible males cannot afford to maintain high concentrations of sex hormones, such as testosterone, necessary for the development and expression of secondary sexual traits due to their immunosuppressive properties (the immunocompetence handicap hypothesis) (Folstad and Karter, 1992). For example, testosterone controls the production of pheromones and scent-marking in male mice (Jemiolo et al., 1992; Novotny et al., 1990; Sam et al., 2001); however, it also inhibits T- and B-cell production, nitric oxide defenses, activates suppressor T cells, and subsequently reduces resistance to pathogens and parasites (Friedl et al., 2000;

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Grossman, 1984, 1985; Mossmann et al., 1997; Tanriverdi et al., 2003). These immunosuppressive effects are not necessarily maladaptive, as testosterone could function to allocate energy and resources between the competing demands of immunity versus reproduction (Wedekind and Folstad, 1994). This functional hypothesis could explain why T-lymphocytes and macrophages have androgen receptors (Benten et al., 2002a,b), and why testosterone acts on the androgen receptors of T lymphocytes to control production of interleukin-10, a cytokine that down-regulates a variety of antiviral responses, including antigen presentation (Liva and Voskuhl, 2001). Our goal in this study was to test whether females prefer males genetically resistant to infectious diseases compared to susceptible ones, and whether genetically resistant males are better able to maintain their testosterone concentrations during infection (the central assumptions of the Hamilton–Zuk and immunocompetence handicap hypotheses, respectively).

The evidence for the immunocompetence handicap hypothesis is mixed (Muehlenbein and Bribiescas, 2005; Roberts et al., 2004); however, conclusions are impossible due to several methodological problems. First, most studies have been conducted with birds, although their secondary sexual traits are not usually testosterone-dependant (Owens and Short, 1996). Second, many studies have manipulated testosterone, but the subsequent immunosuppressive effects might have been an artifact from using high (pharmacological) dosages or disrupting normal fluctuations (Hillgarth and Wingfield, 1997). For these reasons, we use mice to study how normal variations in testosterone affect resistance to pathogens (Zala et al., submitted for publication), and here we examined how males modulate their testosterone during infection. Third, most studies use antibody responses to antigens or other indirect immunocompetence assays to measure resistance to infectious diseases, but they assume that stronger responses are better and ignore immunopathology (Penn and Potts, 1998). Therefore, we examined how mice resolve and cope with an actual infection, using an avirulent strain of *Salmonella enterica* (serovar Typhimurium). Finally, studies are needed that manipulate *genetic resistance* to infection and examine the subsequent effects on males' secondary sexual traits and attractiveness to females (Kurtz and Sauer, 1999). We manipulated genetic immune resistance by using two congenic mouse strains, one that is resistant ('knock-in') and the parental strain which is susceptible to *Salmonella* and a variety of other pathogens (Vassiloyanakopoulos et al., 1998).

The scent-marks and other chemical signals that male mammals produce are functionally analogous to the colourful displays of birds and fish (Penn and Potts, 1998). Male mice increase their scent-marking courtship when they encounter novel females, which makes their scent more attractive to females (Zala et al., 2004). Females are less attracted to the scent of males during *Salmonella* infection (Zala et al., 2004) and other infectious agents (Kavaliers and Colwell, 1995; Penn et al., 1998). It is unclear how infection reduces the attractiveness of a male's scent, but this effect may be due to reductions in testosterone that occur during infection (Hillgarth and Wingfield, 1997; Klein and Nelson, 1998; Kong and Edmonds, 2002; Soudan et al., 1992; Spratt, 2001; Spratt et al., 1993; Willis and Poulin, 2000) or immune activation (Weil et al., 2006). Infected (and genetically susceptible) males may down-regulate the production of major urinary proteins (MUPs) during infection, and some evidence supports this idea (Isserhoff et al., 1986; Litvinova et al., 2005). No study to our knowledge, however, has tested whether females are more attracted to males that are genetically resistant to infection, or whether males modulate their testosterone according to their genetic resistance.

In this study, we found that females were more attracted to the scent-marks of genetically resistant compared to susceptible males before infection, but surprisingly, this preference was abolished during the experimental infection. We also found that genetically resistant males maintained testosterone during infection, whereas susceptible mice significantly reduced testosterone 2 weeks after *Salmonella* inoculation.

2. Materials and methods

2.1. Animals

We used 27 males from two congenic mouse strains, the parental strain genetically susceptible to *Salmonella* due to a single point mutation (11 BALB/c mice, which are Nramp−), and the resistant strain (16 BALB/c.D2 mice), which are Nramp+ knock-ins (Vassiloyanakopoulos et al., 1998). Nramp (natural resistance-associated-macrophage protein), also known as "Slc11a1" (solute carrier family 11 member 1) and previously known as "Ity/Lsh/Bcg," is the most important locus known for controlling resistance to *Salmonella*, and also affects resistance to many other infectious agents (Medina and North, 1998; Sebastiani et al., 1998). It encodes a membrane ion-transport protein exclusively expressed in the phagolysomes of macrophages where it restricts intracellular microbial growth by removing iron, manganese, and other divalent cations (Cannone-Hergaux et al., 1999; Ables et al., 2001). Our colony founders were obtained from different sources, and so we bred a new generation to control for potential confounding differences caused by colony conditions and age. At ca. 2 months of age, all the male mice were housed singly in cages ($30 \times 19 \times 13$ cm) containing pine bedding and paper towels for environmental enrichment. The mice were provided water and food (Harlan Teklad Rodent Chew) *ad libitum* and kept at a constant temperature (22 ± 2 °C) under a 12:12 h light:dark cycle. The treatment and control mice in the experiment were closely age-matched (usually born on the same day). For odor preference assays we used 22 virgin, estrous females of an outbred laboratory strain of mice (Swiss Webster) as smellers. We used an outbred rather than an inbred strain so that our results would be more general, and also because these mice can distinguish the odor of infected versus uninfected males (Zala et al., 2004). Females were kept under a 14:10 light:dark cycle, but otherwise under the same conditions as the males. All the animal experiments were conducted at the University of Utah, and were approved by the local Institutional Animal Care and Use Committee.

2.2. Experimental infection

We used an avirulent strain of *S. enterica* (serovar Typhimurium, 628 strain) (Hormaeche et al., 1985), which invades intestinal mucosa and replicates within host macrophages. We cultured bacteria in 20 ml of heart-