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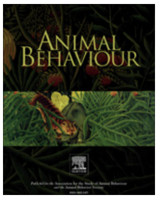

No experimental effects of parasite load on male mating behaviour and reproductive success

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Parasites can negatively affect their host's physiology and morphology and render host individuals less attractive as mating partners. The energetic requirements of defending against parasites have to be traded off against other needs such as feeding activity, territoriality, thermoregulation or reproduction. Parasites can affect mating patterns, with females preferentially mating with parasite-resistant or parasite-free partners. We tested experimentally whether removal of both ectoparasites and endoparasites on free-living, male Columbian ground squirrels, *Urocitellus columbianus*, affected male mating behaviour, reproductive success and seasonal and posthibernation weight gain compared to control males. We predicted that experimental males would lose less body mass and mate more often than control males. In addition, we predicted experimental males would copulate earlier than control males in the mating sequences of receptive females and sire more offspring, because this species exhibits a strong first-male paternity advantage. Parasite treatment significantly reduced the parasite loads of experimental males. None of these males had ectoparasites at the end of the season, compared to 70% infestation of the control males. However, contrary to our expectations, the experimental treatment did not affect male reproductive behaviour (mating frequency, mating position, consort duration and mate-guarding duration), did not increase male reproductive success, and did not influence male body mass. We conclude that parasite infestation plays a minor role in affecting male reproductive behaviour, maybe because of the overall low infestation rates. Alternatively, males may be able to compensate for any costs associated with moderate loads of parasites.

Parasites may have detrimental effects on their hosts (Thompson & Kavaliers 1994; Sheldon & Verhulst 1996; Møller et al. 1999). For example, an infection may lead to a reduction in host fertility (Lockhart et al. 1996), alter an animal's relative attractiveness to potential mates (Hamilton & Zuk 1982; Møller et al. 1999; Verhulst et al. 1999) or affect whether and when to start breeding (Buchholz 2004). Studies in several taxa have also shown that parasites may affect mate choice in both sexes (Freeland 1976; Birkhead et al. 1993; Møller et al. 1999; Barber 2002; Moore & Wilson 2002; Altizer et al. 2003).

Frequent contact with conspecifics increases the likelihood of parasite transmission; thus parasites are expected to create a 'cost' of sociality (Alexander 1974; Hoogland & Sherman 1976; Hoogland 1995). In addition, males are usually more parasitized than females (Poulin 1996; Schalk & Forbes 1997; Zuk & Johnsen 2000; Moore & Wilson 2002; Morand et al. 2004; Perez-Orella & Schulte-Hostedde

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2005; Gorrell & Schulte-Hostedde 2008). Larger home ranges (Greenwood 1980; Ims 1987; Brei & Fish 2003; Nunn & Dokey 2006) and androgenic hormones suppressing the immune system (Folstad & Karter 1992; Mougeot et al. 2006) may both increase risk of infection and thus may explain this male-biased parasitism (Ferrari et al. 2004).

Parasites and resistance to parasites play a prominent role in sexual selection theory (Hamilton & Zuk 1982; Clayton 1991; Zuk 1992; Zuk & Johnsen 2000). Females cannot increase their reproductive output simply by increasing their number of mating partners because their output is limited by their egg production (Bateman 1948). However, females can optimize their reproductive success by acquiring resistant genes for their offspring from the sire (Zeh & Zeh 1996; Jennions & Petrie 1997). According to the theory of Hamilton & Zuk (1982), females may discriminate against parasitized males by considering costly secondary sexual traits indicative of parasite burden. This theory has frequently been tested by relating conspicuous visual or acoustic displays in male birds and fish to their parasite load or resistance (Clayton 1991; Zuk 1992). Hence, females can increase their fitness both directly by reducing their own risk of parasite transmission and indirectly by enhancing the parasite and/or disease resistance of their offspring (Hamilton & Zuk 1982; Zuk et al. 1995). Parasite-mediated sexual selection assumes that a genetic advantage is conferred by the 'resistant', uninfected male and that parasite resistance is heritable (Clayton 1991).

In laboratory experiments, avoidance of infected conspecifics has been demonstrated in rodents, fish and birds (Milinski & Bakker 1990; Kavaliers & Colwell 1995; Zuk et al. 1995, 1998; Penn & Potts 1998; Barber 2002; Ehman & Scott 2002; Kavaliers & Colwell 2003; Kavaliers et al. 2003, 2004, 2005b; Deaton 2009). However, few studies have conducted parasite manipulations on free-living mammals and birds, mainly because of the difficulties of manipulation and observation in the field (Richner et al. 1993; Neuhaus 2003; Charmantier et al. 2004; Madden & Clutton-Brock 2009; Hillegass et al. 2010).

We studied the relationships between parasite load, reproductive behaviour and reproductive success of free-ranging male Columbian ground squirrels, *Urocitellus columbianus*, by manipulating male parasite load. Columbian ground squirrels are diurnal, allow reliable observations of mating behaviour, and are tolerant of experimental manipulations in the wild (Murie et al. 1998; Neuhaus 2000; Nesterova 2007). Furthermore, females are in oestrus for only a few hours (<12 h) on a single day each year (Murie 1995), which makes it feasible to obtain complete mating observations on focal females in oestrus. Although mating mainly occurs in underground burrows, copulations or 'consortships' are readily detected using established behavioural criteria (Hanken & Sherman 1981; Hoogland & Foltz 1982; Sherman 1989; Boellstorff et al. 1994; Murie 1995). Females mate with up to eight different males while in oestrus, with mating order predicting siring success, indicating that male–male competition and sperm competition play a major role in generating variation in male reproductive success (Raveh et al. 2010a, b).

In the present study we removed ectoparasites and endoparasites on half of the reproductive males in three different colonies using chemical agents (experimental males). Control males were also caught, and treated with a sham solution. We compared these two groups of males to identify the impact of parasites on male mating behaviour, male reproductive success and changes in male body mass, during the 2–3 weeks of the mating season. This is a critically important period for male reproductive success and perhaps fitness, since males give no parental care to their offspring. We predicted that (1) experimental males should show an increase in reproductive behaviours known to translate into reproductive success, such as a higher mating frequency, a higher likelihood of obtaining the first mating position, longer consorts and increased mate-guarding durations compared to control males. Mate guarding is considered a costly postcopulatory behaviour as a result of increased visibility to predators, energy investment in chasing females, fighting with opponents and missed mating opportunities with other females (Martín & López 1999; Plaistow et al. 2003; Cothran 2004). If parasites have an impact on ejaculate quality or quantity, an increase in time spent mate guarding for parasitized males could be an alternative explanation for differences in mate-guarding duration. We also predicted that (2) experimental parasite-free males should have higher siring success and seasonal reproductive success than control males. Finally, we predicted that (3) experimental males should lose less weight throughout the breeding season and after hibernation than control males.

METHODS

Study Species

We studied Columbian ground squirrels in the Sheep River Provincial Park, Alberta, Canada (110°W, 50°N, and 1500 m elevation). Data on the ground squirrels were obtained from April to mid-July in 2007 and 2008 on three neighbouring colonies ('meadow' A, B, C). Columbian ground squirrels are diurnal, inhabiting subalpine and alpine meadows where they live in groups of a dozen to a few hundred individuals (Dobson & Oli 2001). On our study meadows, adult males emerge first from hibernation around mid-April, followed by females a few days to a week later (Murie & Harris 1982; Raveh et al. 2010a). Females breed on average 4 days after emergence from hibernation (Murie 1995). The mating season lasts about 2–3 weeks, depending on emergence dates of adult females (Murie 1995; Raveh et al. 2010a). About 24 days later, females give birth to a litter averaging three (one to seven) naked, blind juveniles in a specially constructed nest burrow (Murie et al. 1998). The offspring emerge above ground when they are approximately 27 days old (Murie & Harris 1982).

Experimental Procedure

Ground squirrels were caught within the first 2 days of emergence from hibernation with live traps baited with peanut butter (15×15 cm and 48 cm high and 13×13 cm and 40 cm high; National Live Trap Corp., Tomahawk, WI, U.S.A.) and weighed with a Pesola spring scale to the nearest 5 g. This first body mass measurement for each individual male and year combination was entered in the remainder of the analyses. Thereafter, animals were retrapped weekly to obtain body weight. Individually numbered fingerling fish tags (National Band & Tag Company Monel no. 1, Newport, KY, U.S.A.) were attached in both ears for permanent identification. In addition, each ground squirrel was uniquely marked with hair dye on the dorsal fur (Clairol, Hydriance black pearl No. 52, Proctor and Gamble, Stamford, CT, U.S.A.) for visual identification from a distance.

All reproductive males were randomly separated into two treatment groups (experimental or control) in each colony separately. The experimental group (abbreviated with an E) was treated with a spot-on solution (Stronghold, Pfizer Animal Health, Montreal, Canada) and flea powder (Zodiac, Wellmark International, Dallas, TX, U.S.A.) to remove ectoparasites and endoparasites ($N = 33$ males). Stronghold treats against both endoparasites and ectoparasites, and was applied between the shoulders on the skin, using one drop per 100 g of body mass. The flea powder was applied from a shaker, which had several holes on top, and the dosage was three shakes on the back and two shakes on the belly, with the powder applied by rubbing it into the male's fur. To ensure that mate choice by females was not the result of secondary treatment effects (i.e. handling or odour cues), control animals