Behav Ecol Sociobiol (1999) 45: 323–329

ORIGINAL ARTICLE

Sabra L. Klein · H. Ray Gamble · Randy J. Nelson

Trichinella spiralis infection in voles alters female odor preference but not partner preference

Received: 23 April 1998 / Accepted after revision: 25 October 1998

Abstract Females may choose mates based on secondary sex traits that reflect disease resistance. Accordingly, females should be able to distinguish between unparasitized and parasitized males, and should prefer to mate with unparasitized individuals. Mate and odor preferences for uninfected males or males infected with the nematode, *Trichinella spiralis*, were examined among prairie voles (*Microtus ochrogaster*) and meadow voles (*M. pennsylvanicus*). In a 15-min odor preference test, only female meadow voles distinguished between bedding from parasitized and unparasitized conspecific males, and preferred to spend time with bedding from unparasitized males. Although *T. spiralis* infection influenced odor preference in female meadow voles, there was no effect of infection status on mate preference among either species. Testosterone and corticosterone concentrations were not different between parasitized and unparasitized males. However, among prairie voles, males that spent an increased amount of time with females during the mate preference test had elevated testosterone concentrations. Taken together, these data suggest that (1) female meadow voles can discriminate between unparasitized and parasitized males, (2) the effects of infection on steroid hormone concentrations may be masked by the effects of social interactions, and (3) parasites may represent a selective constraint on partner preference in voles; however, the life cycle of parasites may influence female preference and should be considered in studies of female preference.

Key words Arvicoline rodents · Corticosterone · Female preference · Parasite infection · Testosterone

R.J. Nelson (✉)
Department of Psychology, Behavioral Neuroendocrinology Group, Department of Neuroscience
Department of Biochemistry
The Johns Hopkins University, Baltimore
MD 21218-2686, USA
e-mail: rnelson@jhu.edu
Tel.: +1-410-5168407, Fax: +1-410-5166205

H.R. Gamble
USDA Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory Beltsville, MD 20705, USA

S.L. Klein
Molecular Biology, Microbiology and immunology, Johns Hopkins School of Public Health
615 North Wolfe Street, Baltimore, MD 21205-2179, USA

Introduction

Parasites can affect the reproductive success of an individual by increasing female choice of resistant or healthy individuals as mating partners, which in turn may reduce the probability that infected individuals gain matings (Edwards and Barnard 1987). Females may discern these parasitized individuals based on secondary sex traits that can be used as general indicators of resistance to disease (Hamilton and Zuk 1982). A dynamic relationship is assumed to exist among hormones, secondary sex traits, and the immune system. Thus, because hormones often modify both the expression of secondary sex traits and immune responsiveness, the condition of secondary sex traits may be used as overt indicators of immunocompetence (Folstad and Karter 1992). A testable prediction that emerges from these hypotheses is that females may distinguish healthy males based on the expression of hormone-dependent traits, and may prefer to mate with males that possess these traits. Several studies suggest that parasite infection alters mate preference, with females generally preferring unparasitized over parasitized males (Hamilton and Zuk 1982; Kavaliers et al. 1997; Zuk et al. 1990).

From a proximate perspective, females may use changes in visual, olfactory, or behavioral traits to discriminate healthy from parasitized males. Unlike female birds that rely heavily on the condition of visual secondary sex traits in mate selection (Zuk et al. 1990), female rodents may discern parasitized males based on odor cues (Kavaliers et al. 1997). Olfaction is the

primary sense used for communication in rodents and has been hypothesized to be important for the detection of parasitized individuals, especially if the parasite is transmissible (Kavaliers et al. 1997). The extent to which *Trichinella spiralis* alters odor stimuli, including urine and feces, was examined in the present study. Because this parasite is not easily transmissible between rodents (Despommier 1983) olfactory cues may not be important for female discrimination between parasitized and unparasitized males. The only known modes of transmission of this parasite are via cannibalism and coprophagia (Despommier 1983). Although voles are coprophagic, the limited social interactions between infected males and uninfected females was hypothesized to reduce the possibility that females would become infected in the present study.

Female rodents may also use behavioral modifications to discriminate between parasitized and unparasitized males. *T. spiralis* can alter reproductive and non-reproductive behaviors of the host. Male mice are less likely to mount and copulate with female mice infected with *T. spiralis* compared to uninfected females (Edwards and Barnard 1987). Additionally, infection with *T. spiralis* reduces activity and exploratory behaviors in male mice (Rau 1983a, 1984) and during aggressive encounters, males infected with *T. spiralis* are more inclined to assume subordinate status, as opposed to dominant status (Rau 1983b). Hormones can modify behavioral and olfactory cues (Nelson 1995). While the effects of steroid hormone manipulation on *T. spiralis* burden have been studied (Mankau and Hamilton 1972; Reddington et al. 1981), endogenous hormonal changes following *T. spiralis* infection are not known. Because parasite infection in vertebrates often suppresses testosterone and elevates corticosterone concentrations, steroid hormone concentrations were hypothesized to be related to behavioral and olfactory changes induced by *T. spiralis* infection (Hillgarth and Wingfield 1997).

From an adaptive functional perspective, the extent to which females discriminate between parasitized and unparasitized males may be related to the mating system. Males of polygynous species mate with many females during the breeding season and rarely provide parental care (Andersson 1994). In contrast, males of monogamous species remain with females following copulation and may assist with nest building, food gathering, and parental care (Andersson 1994). Thus, because monogamous females rely on males substantially during the breeding season, their investment in mate selection is significant. As a result, females of monogamous species may use disease status to a greater extent than females of polygynous species (Møller 1994). Alternatively, because females of polygynous species mate with more than one male during the breeding season, avoidance of disease may be more critical and, therefore, disease status may be more important in mate selection for females of polygynous than monogamous species.

The purpose of the present study was to examine whether female voles discriminate between parasitized and unparasitized males, and to determine the extent to which different mating strategies may influence female partner preference. For this assessment monogamous prairie voles (*Microtus ochrogaster*) and polygynous meadow voles (*M. pennysylvanicus*) were infected with the nematode, *T. spiralis*. These host species were selected because individuals of these two *Microtus* species have virtually identical diet, habitat use, and gross morphology, yet differ dramatically in mating strategies (Dewsbury 1990). Additionally, these species have been used successfully to address evolutionary principles about the role of the mating system in behavior, morphology, and physiology (e.g., Gaulin and FitzGerald 1989; Insel and Shapiro 1992; Jacobs et al. 1990; Klein and Nelson 1998a, 1998b; Klein et al. 1997; Ostfeld and Heske 1993). The parasite, *T. spiralis*, was selected because it is a naturally occurring endoparasite in *Microtus* species (Timm 1985). However, the extent to which *T. spiralis* has modified the evolution of *Microtus* species has not been assessed. Although this study examined the ability of females to use parasite infection as a factor in mate preference, this study was not a direct test of the Hamilton-Zuk hypothesis (Hamilton and Zuk 1982) because variation in resistance to *T. spiralis* was not examined. In other words, unparasitized voles cannot be considered "resistant" because they were not exposed to the parasite. This study does, however, test the general assumption that females should be able to discriminate between parasitized and unparasitized males, and should prefer unparasitized conspecific males during partner preference tests. In addition, this study tested the prediction that females should use cues, such as odors, to discriminate between parasitized and unparasitized males.

Methods

Animals

Sexually mature (>60 days of age) male ($n = 40$) and female ($n = 20$) prairie voles (*Microtus ochrogaster*), and male ($n = 40$) and female ($n = 20$) meadow voles (*M. pennsylvanicus*) were used in this experiment. All animals were obtained from our breeding colonies. Animals were individually housed in polypropylene cages ($28 \times 17 \times 12$ cm) in accredited colony rooms with a 24-h 16L:8D light cycle (lights on at 0600 hours EST). Temperature was held constant at 21 ± 2 °C and relative humidity was held constant at 50 ± 5%. Food (Agway, Prolab 2000) and tap water were available ad libitum throughout the experiment. All procedures described below were approved by the Johns Hopkins University Animal Care and Use Committee.

Procedure

At 60–75 days of age, male meadow voles and prairie voles were assigned to either the infection group ($n = 20$/species), in which each male was inoculated by gavage (i.e., through a tube in the esophagus) with 100 larvae of *T. spiralis* suspended in 0.9% sterile saline, or the control group ($n = 20$/species) in which males remained undisturbed in their home cages. After inoculation, animals remained undisturbed, other than routine cage cleaning, in their

home cages for 25 days. Accumulation of larvae in musculature is essentially completed by 21 days post-inoculation (Despommier 1983); however, live larvae persist in muscle cells for several months as a chronic infection. At 25 days post-inoculation, behavioral testing began, and at 30 days post-inoculation, all animals were killed as described below.

Female odor preference test

Tests of odor preference, for the soiled bedding of conspecific males, were performed in a plastic T-apparatus. The apparatus consisted of one base arm (24 × 5.5 cm) attached by a three-way connector tube (8 × 5.5 cm) to two stimulus arms (each 24 × 5.5 cm). The ends of each stimulus arm (8.5 cm) were partitioned off by a plastic mesh divider. Females were placed in the apparatus at the far end of the base arm and given free access to the base arm and 15.5 cm of each stimulus arm (i.e., up to the plastic mesh divider). Pooled soiled bedding (about 20 g) from either parasitized or unparasitized conspecific males was placed in each stimulus arm. The soiled bedding consisted of fresh excretory matter (both urine and feces) and wood shavings collected from the home cages of parasitized and unparasitized males. Soiled bedding was collected and used in preference tests on the same day. During each trial, females could smell the soiled bedding, but the plastic mesh divider prevented females from coming into direct contact with the soiled bedding. For each preference test, females were given 5 min to acclimate to the apparatus without the soiled bedding present. Females were then removed from the apparatus, soiled bedding from either parasitized or unparasitized males was placed in the ends of the stimulus arms, and females were then released back into the apparatus for a 15-min odor preference test. During the test period, the duration of time a female spent within 15 cm of the plastic mesh divider (i.e., down one stimulus arm), as well as the number of times a female entered a stimulus arm was recorded. If a female failed to enter a stimulus arm during the test period, then the trial was discarded (two female meadow voles failed to exhibit these behaviors and those trials were not included in data analyses). Each T-apparatus was washed thoroughly with hot water and laboratory disinfectant soap between trials. The placement of soiled bedding from parasitized and unparasitized males (i.e., either in the right or left arm) was randomized between trials. All test were conducted between 1300–1500 hours under red lights to avoid aversive effects of light on performance.

Female partner preference test

Female preference tests were run in a three-chamber test apparatus that consisted of three plastic chambers (17 × 20.5 × 22 cm) connected in series by two plastic hollow tubes (8 × 5.5 cm). During each trial, two males (one parasitized male and one control male) were loosely tethered in one of the two end chambers, with each male tethered in a single chamber. A female was released into the center chamber and given access to all three chambers. The males were visible to the female but not to each other. These males were matched in terms of age, size, and reproductive status. Trials lasted for 3 h and were monitored using time-lapse video taping (Panasonic, model no. AG-6124). Trial time was based on previous data that suggested 3 h is enough time for copulation to occur in prairie voles (DeVries et al. 1995). The amount of time (s) that a female spent with either male during a trial and the number of entries a female made into the chamber of a male were recorded. If these behaviors did not occur during a trial, then the trial was discarded (three female meadow voles failed to exhibit these behaviors and those trials were not included in the data analyses). Food and water were available *ad libitum* in each of the three chambers. Immediately after behavioral testing, males were lightly anesthetized with methoxyflurane vapors (Metofane, Schering Plough, Union, N.J.) and bled from the retro-orbital sinus into heparinized tubes (50 µl/tube). Blood plasma samples were stored at −80 °C and used for later analysis of plasma testosterone and corticosterone using the radioimmunoassay (RIA) procedure described below. Following bleeding, animals were killed by CO_2 asphyxiation and cervical dislocation, skinned and eviscerated, and muscle tissue was digested (using the procedure described below). The number of recovered larvae was counted for each vole using appropriate dilutions.

T. spiralis digestion

Digestion of muscle tissue in an acidified pepsin solution releases live trichinae from cysts that develop in muscle tissue (Gamble 1996). Muscle tissue from skinned, eviscerated vole carcasses was ground to expedite digestion. Muscle samples were then digested in artificial gastric fluid containing 1% (w/v) pepsin and 1% (w/v) hydrochloric acid. Ground muscle tissue from each animal was added to the artificial gastric fluid (prewarmed to 37 °C) and stirred on a magnetic stirrer for 3–4 h at 37 °C. The mixture was then allowed to settle for 15–20 min, the upper two-thirds of the mixture was decanted and the remainder poured into conical-bottom pilsner glasses. After settling for 15–20 min, the supernatant was aspirated and the remaining sediment was washed with tap water (37 °C) and allowed to settle for an additional 15–20 min. This washing step was repeated until the supernatant was clear. The remaining washed sediment was transferred to a 50-ml conical tube, allowed to settle, and aspirated down to a final volume of 10 ml. The sediment was then poured into a petri dish and *Trichinella* larvae were counted using a dissecting microscope. Dilutions were made as necessary to facilitate counting.

Steroid hormone RIA

Plasma testosterone concentrations in males were assayed by RIA using ^{125}I kits purchased from ICN Biochemicals (Carson, Calif.). The testosterone assay is highly specific; cross-reaction with other steroids is $< 0.1\%$. Testosterone values were determined in a single RIA, with a 4.4% coefficient of variation. Corticosterone was measured using a ^{125}I RIA kit (ICN Biochemicals) that had previously been validated for use in voles (Taymans et al. 1997). The only deviation from the manufacturer's protocol was the dilution factor for the plasma. The plasma samples in both species were diluted 1:2121 in assay buffer (Taymans et al. 1997). All plasma samples were assayed at the same time, and the intra-assay coefficient of variation was 4.8%.

Statistical analyses

Mate partner preference was determined by examining the percentage of time a female spent with either male during a trial and the number of entries a female made into the chamber of a parasitized or unparasitized male. Odor preference was determined by examining the percentage of time a female spent in the stimulus arm containing soiled bedding from parasitized or unparasitized males and the number of entries a female made into the stimulus arm containing soiled bedding from parasitized or unparasitized males. Arcsine transformations were performed on percentage-of-time data from both mate and odor preference tests. Mate and odor preference were assessed using two-way ANOVAs with two between-subject variables (species and infection status). Plasma testosterone and corticosterone concentrations were compared using two-way ANOVAs with two between-subject variables (species and infection status). Numbers of *T. spiralis* larvae recovered from each animal approximately 30 days post-infection were compared between species using an independent two-tailed *t*-test. Correlations among female preferences, body mass, steroid hormone concentrations, and worm burden were examined using Pearson product-moment correlations. All mean differences were considered statistically significant if $P < 0.05$.

Results

Female mate preference data

Within each species, females spent an equivalent amount of time in the chambers of parasitized and unparasitized conspecific males ($P > 0.05$; Fig. 1A). Additionally, female voles made an equal number of entries into the chambers of parasitized (meadow voles = 21.33 ± 8.06; prairie voles = 39.50 ± 5.24) and unparasitized (meadow voles = 30.25 ± 9.92; prairie voles = 38.81 ± 5.74) conspecific males ($P > 0.05$). Overall, female prairie voles spent more time with males (regardless of infection status) than did female meadow voles ($F_{1,52} = 34.941$, $P < 0.001$).

Female odor preference data

Female meadow voles spent significantly more time with soiled bedding from unparasitized as compared to parasitized conspecific males; conversely, female prairie voles spent an equivalent amount of time with soiled bedding from parasitized and unparasitized conspecific males ($F_{1,48} = 9.339$, $P < 0.01$; Fig. 1B). Female meadow voles and prairie voles made an equal number of entries into the stimulus arms containing soiled bedding of parasitized (meadow voles = 3.92 ± 0.99; prairie voles = 10.15 ± 1.18) and unparasitized (meadow voles = 4.46 ± 1.02; prairie voles = 9.76 ± 1.18) conspecific males ($P > 0.05$).

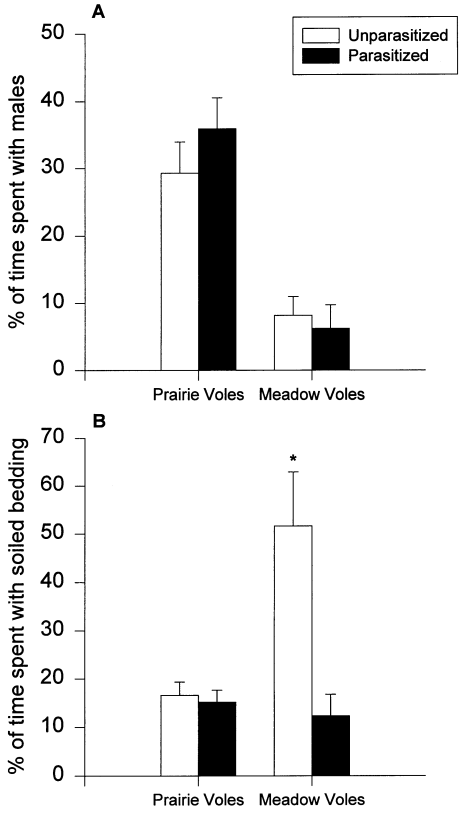

Fig. 1 Mean (±SEM) proportion of time female prairie voles and meadow voles spent with conspecific males, either infected with *Trichinella spiralis* or uninfected, during a 3-h mate preference test (**A**) and mean (±SEM) proportion of time female prairie voles and meadow voles spent with soiled bedding from conspecific males, either infected with *T. spiralis* or uninfected, during a 15-min olfactory discrimination test (**B**). *Asterisk* indicates that female meadow voles spent significantly more time with soiled bedding from unparasitized than parasitized males

T. spiralis infection data

Following inoculation with 100 larvae, more larvae were recovered from prairie vole as compared to meadow vole males approximately 30 days post-inoculation ($t = -2.351$, $df = 27$, $P < 0.05$; Fig. 2). Among prairie voles, heavier males had lower worm burdens ($r^2 = -0.57$, $P < 0.05$). No such relationship existed for meadow vole males ($P > 0.05$).

Steroid hormone concentrations

Plasma testosterone concentrations did not differ between parasitized and unparasitized males within either species ($P > 0.05$; Fig. 3A). Additionally, in contrast to previous studies (Klein and Nelson 1998a, 1998b, Klein et al. 1997), testosterone values were not higher in meadow vole than prairie vole males ($P > 0.05$). Among male prairie voles, circulating testosterone concentrations were positively related to the amount of time a female prairie vole spent with a male during the partner preference test ($r^2 = 0.37$, $P < 0.05$; Fig. 3B). There was no correlation between testosterone concentrations and the amount of time a female meadow vole spent with conspecific males ($r^2 = -0.15$, $P > 0.05$).

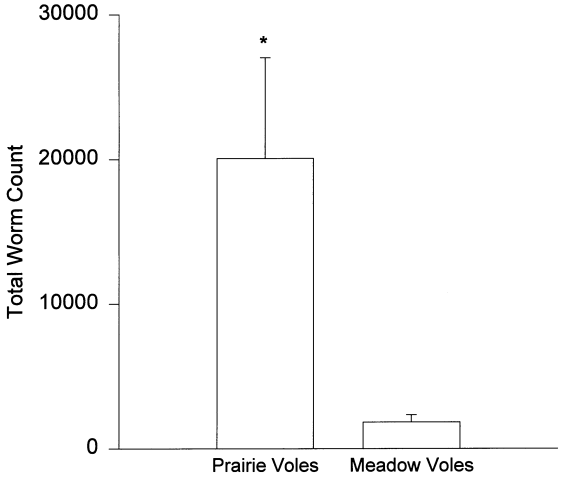

Fig. 2 Mean (±SEM) number of *T. spiralis* larvae recovered from male prairie voles and meadow voles approximately 30 days following inoculation with 100 larvae. *Asterisk* indicates that prairie voles had significantly higher numbers of larvae recovered from muscle tissue than meadow voles

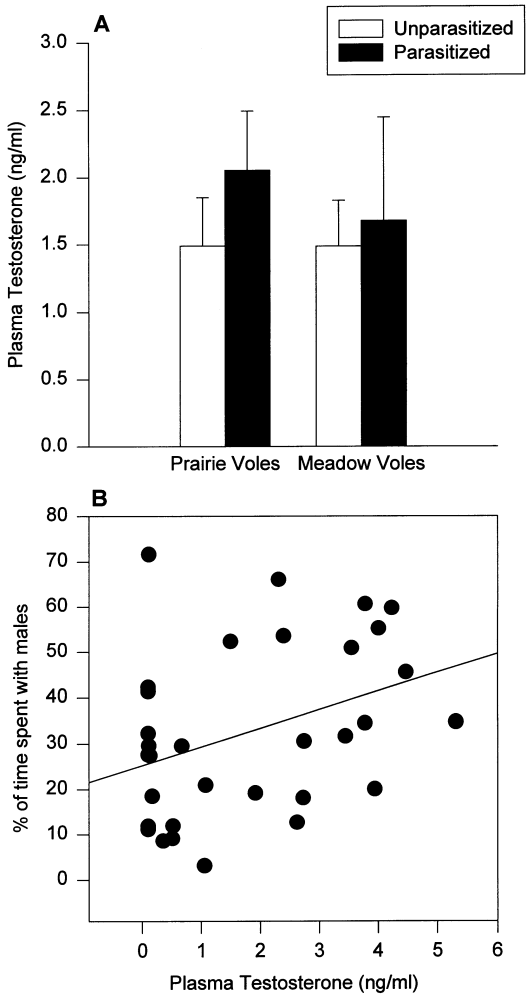

Fig. 3 **A** Mean (±SEM) plasma testosterone concentrations (ng/ml) from male prairie voles and meadow voles that were either uninfected or infected with *T. spiralis*. Blood samples were collected after the 3-h partner preference test. **B** Correlation between the percentage of time female prairie voles spent with both parasitized or unparasitized conspecific males and plasma testosterone concentrations (ng/ml) from parasitized and unparasitized male prairie voles

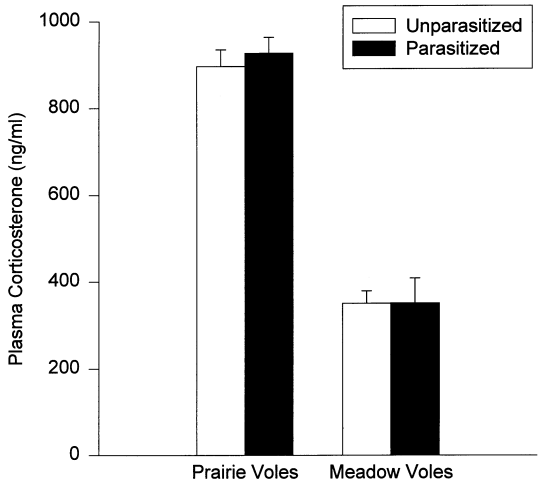

Fig. 4 Mean (±SEM) plasma corticosterone concentrations (ng/ml) from male prairie voles and meadow voles that were either uninfected or infected with *T. spiralis*. Blood samples were collected after the 3-h partner preference test

Corticosterone concentrations did not differ between parasitized and unparasitized males among either prairie voles or meadow voles ($P > 0.05$). Overall, prairie voles had higher corticosterone concentrations than meadow voles, regardless of infection status ($F_{1,59} = 196.052$, $P < 0.001$; Fig. 4). Body mass did not differ between parasitized and unparasitized males of either species ($P > 0.05$). However, among meadow voles, larger males had lower corticosterone concentrations ($r^2 = -0.39$, $P < 0.05$).

Discussion

Several hypotheses suggest that male advertisement of disease resistance represents the dynamic relationship among secondary sex traits, hormones, and the immune system (Folstad and Karter 1992; Wedekind and Folstad 1994; Zuk 1996). The purpose of this study was to examine how male voles advertise infection status and whether females use this information in partner preference tests. In this study, female meadow voles spent less time with soiled bedding from males infected with *T. spiralis* compared to bedding from uninfected males suggesting that males may advertise infection status through odors found in urine and feces. Although infection of males with *T. spiralis* influenced female meadow vole odor preference, there was no effect of infection status on female partner preference in either species.

From a mechanistic perspective, females may use the condition and expression of secondary sex traits to discern parasitized from unparasitized males. The extent to which secondary sex traits are altered by parasites, as well as the extent to which females use this information, may depend on whether the parasite is transmissible. The disease avoidance hypothesis suggests that females should use infection status in mate selection because by mating with unparasitized males, females gain a direct advantage by protecting themselves and current offspring from exposure to potentially contagious diseases (Able 1996; Hamilton 1990; Kirkpatrick 1996; Kirkpatrick and Ryan 1991). *T. spiralis* is not readily transmissible between rodents (Despommier 1983). Therefore, females either may not use cues associated with infection in partner preference or cues important to partner preference may be unaffected by this non-transmissible parasite. Data from American kestrels (*Falco sparverius*) support the latter hypothesis. Specifically, female American kestrels show no preference for uninfected males over males infected with *T. pseudospiralis* (Henderson et al. 1995). Female American kestrels use courtship display rate in mate selection

(Duncan and Bird 1989), and courtship display behaviors in males are unaffected by *T. pseudospiralis* infection (Henderson et al. 1995). Taken together, these data suggest that the effects of parasites on behavior and subsequent partner preference may be related to parasite transmissibility.

An alternative hypothesis is that behaviors associated with infection, including reduced social contact and exploration, are unaffected by *T. spiralis*. Previous data in voles suggest that females attend to behavioral modifications, collectively termed "sickness behaviors," associated with infection (Klein and Nelson, 1999). Specifically, when male voles are exposed to lipopolysaccharide (LPS), which is essentially killed gram-negative bacteria cell walls, they engage in less social contact with conspecific females than saline-injected males (S.L. Klein and R.J. Nelson, unpublished data). As a result, females spend less time with LPS- than saline-injected conspecific males during partner preference tests (S.L. Klein and R.J. Nelson, unpublished data). The extent to which *T. spiralis* alters behavior in *Microtus* species was not examined in the present study, but should be addressed in future studies.

Steroid hormone concentrations are often altered by parasite infection (Hillgarth and Wingfield 1997) Specifically, testosterone concentrations are often suppressed and corticosterone concentrations are enhanced by parasite infection. Testosterone concentrations are also typically higher in meadow vole than prairie vole males (Klein and Nelson 1998a, 1998b; Klein et al. 1997). In the present study, steroid hormone concentrations were not altered by *T. spiralis* infection. Additionally, testosterone concentrations were virtually identical between meadow vole and prairie vole males (see Fig. 3A). Testosterone concentrations in prairie vole males were higher than values typically reported for this species (Klein and Nelson 1998a, 1998b; Klein et al. 1997), suggesting that concentrations were enhanced in prairie voles as opposed to suppressed in meadow voles. Steroid hormone concentrations in males were measured after the 3-h female preference test, and brief social interactions can alter steroid hormone values (Alberts et al. 1992; Wingfield et al. 1990). Accordingly, prairie vole males that spent more time with conspecific females had higher testosterone concentrations (see Fig. 3B). Exposure to a conspecific female increases testosterone concentrations in male prairie voles in a similar manner to that reported among birds (Wingfield et al. 1990; S.L. Klein and R.J. Nelson, unpublished data). These results suggest that exposure to conspecific females alters hormonal status in male mammals, and to a greater extent among monogamous than polygynous males. Additionally, these data imply that social interactions may mitigate the effects of infection on endocrine function.

Infection of males with *T. spiralis* resulted in higher numbers of larvae accumulating in muscle tissue of prairie voles compared to meadow voles (Fig. 2; S.L. Klein, H.R. Gamble, R.J. Nelson, unpublished data). Increased parasite infection in male prairie voles did not affect female partner preference in this species; i.e., female prairie voles did not exhibit a preference for unparasitized over parasitized conspecific males. Conversely, female meadow voles were able to discriminate between the odors of parasitized and unparasitized males. This species difference in the ability to discern parasitized males may be related to differential mating strategies. Previous work has illustrated that sex differences in *T. spiralis* infection are more pronounced among meadow voles than prairie voles, with males having higher worm burdens than females (S.L. Klein, H.R. Gamble, R.J. Nelson, unpublished data). Sex differences in parasite infection are not observed among prairie voles. Because female meadow voles have lower worm burdens than conspecific males, female meadow voles may use infection status in mate selection to a greater extent than female prairie voles (Zuk 1990). If selection pressures associated with parasite infection status are increased among polygynous meadow voles, then meadow voles should have lower parasite burden, higher immune function, and an increased ability to discriminate between parasitized and unparasitized mates, all of which have been demonstrated in this species (Klein and Nelson 1998a, 1998b; S.L. Klein, H.R. Gamble, R.J. Nelson, unpublished data; Fig. 1B). Taken together, these data suggest that variation associated with parasite infection may be influenced by evolutionary factors.

In summary, female voles can discriminate between the odors of males based on infection status. Because female voles did not prefer unparasitized over parasitized males during partner preference tests, and because *T. spiralis* is not transmissible, future studies must examine the extent to which transmissible parasites influence partner preferences behavior in voles. Additionally, future studies must discern whether *T. spiralis* is important for the evolution of *Microtus* species. Specifically, if *T. spiralis* infection is not a constraint on selection for individuals of *Microtus* species, then females would not be predicted to use *T. spiralis* infection in partner preference tests. However, these data do support the basic assumption of the Hamilton-Zuk hypothesis (Hamilton and Zuk 1982) that females are able to discriminate between parasitized and unparasitized males and prefer the odors of unparasitized males, suggesting that parasites may play a role in sexual selection among voles.

Acknowledgements We thank Jim McCrary for technical assistance, and Donna Bilu, Marie Bober, Brooke Buckley, Nicholas Falleta, Aliza Katz, J.R. Lee, Drew Maloney, Susan Mozzicato, and Merideth Pasmantier for assistance with the female partner and odor preference tests. We also thank Greg Gurri Glass and Tom Hahn for helpful comments on early drafts of this manuscript and Ed Silverman for expert animal care. This research

was supported by NIMH grant MH57535 and NSF grant IBN 97-23420.

References

Able DJ (1996) The contagion indicator hypothesis for parasite-mediated sexual selection. Proc Natl Acad Sci USA 93:2229–2233

Alberts SC, Sapolsky RM, Altmann J (1992) Behavioral, endocrine, and immunological correlates of immigration by an aggressive male into a natural primate group. Horm Behav 26:167–178

Andersson M (1994) Sexual selection. Princeton University Press, Princeton, NJ

Despommier DD (1983) Biology. In: Campbell WC (ed) *Trichinella* and trichinosis. Plenum, New York, pp 75–151

DeVries AC, DeVries MB, Taymans S, Carter CS (1995) Modulation of pair bonding in female prairie voles (*Microtus ochrogaster*) by corticosterone. Proc Natl Acad Sci USA 92:7744–7748

Dewsbury DA (1990) Individual attributes generate contrasting degrees of sociality in voles. In: Tamarin RH, Ostfeld RS, Bujalska G (eds) Social systems and population cycles in voles. Birkhäuser, Basel, pp 1–10

Duncan JR, Bird DM (1989) The influence of relatedness and display effort on the mate choice of captive female American kestrels. Anim Behav 37:112–117

Edwards JC, Barnard CJ (1987) The effects of *Trichinella* infection on intersexual interactions between mice. Anim Behav 35:533–540

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–622

Gamble R (1996) Trichinellosis. In: OIE manual of standards for diagnostic tests and vaccines. Office International des Epizooties France, pp 477–480

Gaulin SJC, FitzGerald RW (1989) Sexual selection for spatial-learning ability. Anim Behav 37:322–331

Hamilton WD (1990) Mate choice near or far. Am Zool 30:341–352

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Henderson D, Bird DM, Rau ME, Negro JJ (1995) Mate choice in captive American kestrels, *Falco sparverius*, parasitized by a nematode, *Trichinella pseudospiralis*. Ethology 101:112–120

Hillgrath N, Wingfield JC (1997) Testosterone and immunosuppression in vertebrates: implications for parasite-mediated sexual selection. In: Beckage NE (ed.) Parasites and pathogens: effects on host hormones and behavior. Chapman & Hall, New York, pp 143–155

Insel TR, Shapiro LE (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygynous voles. Proc Natl Acad Sci USA 89:5981–5985

Jacobs LF, Gaulin SJC, Sherry DF, Hoffman GE (1990) Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. Proc Natl Acad Sci USA 87:6349–6352

Kavaliers M, Colwell DD, Ossenkopp KP, Perrot-Sinal TS (1997) Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. Behav Ecol Sociobiol 40:373–384

Kirkpatrick M (1996) Good genes and direct selection in the evolution of mating preference. Evolution 50:2125–2140

Kirkpatrick M, Ryan MJ (1991) The evolution of mating preference and the paradox of the lek. Nature 350:33–38

Klein SL, Nelson RJ (1998a) Adaptive immune responses are linked to the mating system of arvicoline rodents. Am Nat 151:59–67

Klein SL, Nelson RJ (1998b) Sex and species differences in cell-mediated immune responses in voles. Can J Zool 76:1–5

Klein SL, Nelson RJ (1999) Social factors unmask sex and species differences in humoral immunity in voles. Anim Behav (in press)

Klein SL, Hairston JE, DeVries AC, Nelson RJ (1997) Social environment and steroid hormones affect species and sex differences in immune function among voles. Horm Behav 32:30–39

Mankau SK, Hamilton R (1972) The effect of sex and sex hormones on the infection of rats by *Trichinella spiralis*. Can J Zool 50:597–602

Møller AP (1994) Sexual selection and the barn swallow. Oxford University Press, Oxford.

Nelson RJ (1995) An introduction to behavioral endocrinology. Sinauer, Sunderland, Mass

Ostfeld RS, Heske EJ (1993) Sexual dimorphism and mating system in voles. J Mammal 74:230–233

Rau ME (1983a) The open-field behaviour of mice infected with *Trichinella spiralis*. Parasitology 86:311–318

Rau ME (1983b) Establishment and maintenance of behavioural dominance in male mice infected with *Trichinella spiralis*. Parasitology 86:319–322

Rau ME (1984) Running responses of *Trichinella spiralis*-infected CD-1 mice. Parasitology 89:579–583

Reddington JJ, Stewart GL, Kramar GW, Kramer MA (1981) The effects of host sex and hormones on *Trichinella spiralis* in the mouse. J Parasitol 67:548–555

Taymans SE, DeVries AC, DeVries MB, Nelson RJ, Friedman TC, Castro M, Detera-Wadleigh S, Carter CS, Chrousos GP (1997) The hypothalamic-pituitary-adrenal axis of prairie voles (*Microtus ochrogaster*): evidence for target tissue glucocorticoid resistance. Gen Comp Endocrinol 106:48–61

Timm RM (1985) Parasites. In: Tamarin RH (ed) Biology of new world *Microtus*. American Society of Mammalogists, Shippensburg, Pa, pp 455–534

Wedekind C, Folstad I (1994) Adaptive or nonadaptive immunosuppression by sex hormones? Am Nat 143:936–938

Wingfield JC, Hegner RE, Dufty AM, Ball GF (1990) The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. Am Nat 136:829–845

Zuk M (1990) Reproductive strategies and sex differences in disease susceptibility: an evolutionary viewpoint. Parasitol Today 6:231–233

Zuk M (1996) Disease, endocrine-immune interactions, and sexual selection. Ecology 77:1037–1042

Zuk M, Thornhill R, Ligon JD, Johnson K (1990) Parasites and mate choice in red jungle fowl. Am Zool 30:235–244

Communicated by S. Creel