

Social Environment and Steroid Hormones Affect Species and Sex Differences in Immune Function among Voles

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Testosterone has bipotential effects on male fitness; that is, it both suppresses immune function and maintains characteristics important for reproductive success. Presumably, these effects of testosterone may be more pronounced among polygynous species because testosterone concentrations are generally higher among polygynous than monogamous males. The present study examined sex and species differences in cell-mediated immunity among four arvicoline rodents. The role of mating system and sex steroids in sex differences in immune function was examined in individually housed polygynous meadow (*Microtus pennsylvanicus*) and montane (*M. montanus*) voles and monogamous prairie (*M. ochrogaster*) and pine (*M. pinetorum*) voles in Experiment 1. No sex differences in splenocyte proliferation were observed among the four species and circulating testosterone concentrations did not correlate with immune function of individuals within each species. The contribution of social isolation to these results was examined in Experiment 2, in which meadow and prairie voles were housed individually, or with same- or opposite-sex conspecifics in either pairs or groups of four per cage for 28 days. Overall, prairie voles exhibited more robust immune responses than meadow voles when housed in pairs or in same-sex groups. Sex differences in immune function were also apparent; male meadow voles had higher immune responses than female conspecifics when housed in pairs, whereas female prairie voles had higher responses than male conspecifics when housed in same-sex pairs. Circulating sex steroid hormones and

corticosterone appear to mediate some, but not all, of the changes in immune function evoked by differential housing conditions. Taken together, these results suggest that social factors have significant effects on immunity and should be considered in studies of sex differences in immunity at both proximate and ultimate levels.

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Sex differences in immunocompetence are well established in vertebrates (Alexander and Stimson, 1988; Billingham, 1986; Schuurs and Verheul, 1990). Males typically exhibit reduced cell-mediated (e.g., proliferation of immune cells, inflammatory responses, cytokine production) and humoral (e.g., antibody production) immune responses compared to females (Shuurs and Verheul, 1990). Males and females also differ in susceptibility to various diseases. For example, males are more susceptible than females to a variety of parasitic infections (reviewed in Zuk and McKean, 1996), certain types of cancer (e.g., lymphomas and leukemias) (Billingham, 1986), and viral infections of the central nervous system (Barna, Komatsu, Bi, and Reiss, 1996). Conversely, females of many species more readily produce immune responses against "self" tissue and are therefore more likely to develop autoimmune diseases than males (Olsen and Kovacs, 1996). These sex differences in immune function have generally been reported for polygynous species, in which sex differences in physiology and behavior are often exaggerated. The extent to

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which sex differences in immune function exist in monogamous mammals remains unspecified.

Testosterone has been reported to reduce both cell-mediated and humoral immune responses (reviewed in Schuurs and Verheul, 1990). Among mammals, polygynous males often have higher circulating testosterone concentrations than monogamous males (Klein and Nelson, 1997). High testosterone concentrations may reflect the proximate mechanisms underlying sex differences in immune function (Schuurs and Verheul, 1990; Zuk, 1994). Several hypotheses have been proposed to provide ultimate explanations for sex differences in immune function (Folstad and Karter, 1992; Zuk, 1990). These hypotheses focus on the dual actions of testosterone as both immunosuppressive and necessary for male reproductive success. Accordingly, males with high testosterone concentrations must overcome the immunosuppressive effects of testosterone to maintain testosterone-dependent secondary sex characters (Folstad and Karter, 1992; Wedekind and Folstad, 1994). Because polygynous males generally rely more heavily on testosterone-dependent traits (e.g., competition and aggression) for mating success than monogamous males, it has been hypothesized that sex differences in immune function have evolved to be increased among polygynous species and decreased among monogamous species (Zuk, 1990, 1994).

The goal of this study was to examine species and sex differences in immune function in relation to the mating system and endocrine status of four arvicoline rodent species. Although Hamilton and Zuk's hypotheses (Hamilton and Zuk, 1982; Zuk, 1990) have been central in generating critical experiments in behavioral ecology, the underlying predictions have not been tested directly. In previous studies, hormone concentrations have generally been assumed by the extent of secondary sex character development, and immune function has been judged by parasite load. Our goal was to test the predictions of Hamilton and Zuk's hypotheses directly using four related species of arvicoline rodents. Two species, meadow voles (*Microtus pennsylvanicus*) and montane voles (*M. montanus*), are considered polygynous and two species, prairie voles (*M. ochrogaster*) and pine voles (*M. pinetorum*), are considered monogamous (Dewsbury, Baumgardner, Evans, and Webster, 1980; Getz, Carter, and Gavish, 1981; Jannett, 1982; Madison, 1980; Schadler, 1979). We hypothesized that polygynous males would have higher testosterone concentrations and heavier reproductive organ mass than monogamous males, because selection for higher testosterone concentrations should be greater

among polygynous males (Zuk, 1990, 1994). Therefore, we predicted that sex differences in immune function should be greater among polygynous than monogamous species and that immune function should be higher among females than males. Sex and species differences in circulating corticosterone were also assessed. We hypothesized that because prairie voles have higher corticosterone concentrations than meadow voles (Taymans, DeVries, DeVries, Nelson, Friedman, Castro, Detera-Wadleigh, Carter, and Chrousos, 1997), prairie voles should have reduced proliferative responses compared to meadow voles (Bateman, Singh, Kral, and Solomon, 1989).

In the present study, the proliferative response of splenocytes to mitogenic stimulation was used to assay cell-mediated immune function because this immune measure does not require specific reagents for *Microtus* species. Additionally, proliferation of lymphocytes *in vitro* is a general indicator of the mitotic ability of lymphocytes following exposure to a pathogen *in vivo* (Borysenko, 1987). Sex differences in immunocompetence were examined in Experiment 1 by assessing the role of sex steroid hormones in cell-mediated immunity among the two polygynous vole species (i.e., meadow voles and montane voles) and the two monogamous vole species (i.e., prairie voles and pine voles). Animals were individually housed in Experiment 1 to mimic most traditional studies of sex differences in immune function. Neither sex nor species differences in immune function were observed among individually housed voles in Experiment 1. Because social environment can affect immune function and disease resistance (e.g., Joasoo and Mackenzie, 1976; Karp, Moynihan, and Ader, 1993; Plaut, Ader, Friedman, and Ritterson, 1969; Rabin, Lyte, Epstein, and Caggiula, 1987; Rabin and Salvin, 1987), the effects of social interactions on steroid hormones and immune function were examined in Experiment 2.

METHODS

Experiment 1

Animals

Adult (>60 days of age) male ($n = 8$) and female ($n = 16$) prairie voles (*M. ochrogaster*) were obtained from our breeding colony. The prairie vole breeding colony was established with animals trapped near Champaign, Illinois. Adult male ($n = 8$) and female ($n = 5$) pine

voles (*M. pinetorum*) were obtained from a breeding colony at Union College, New York, established with animals originally trapped near New Paltz, New York. Adult male ($n = 10$) and female ($n = 8$) meadow voles (*M. pennsylvanicus*) were obtained from a breeding colony at the University of Michigan, originally derived from a colony of animals from northwestern Pennsylvania and south central Michigan. Adult male ($n = 10$) and female ($n = 9$) montane voles (*M. montanus*) were obtained from a stock in Wilmington, North Carolina, originally trapped near Salt Lake City, Utah. All animals were individually housed in polycarbonate cages ($28 \times 17 \times 12$ cm) with food (Agway ProLab 2000) and water available *ad libitum*. Animals were maintained under a 16:8 hr light/dark cycle (lights on from 0600 to 2200 hr) with an ambient temperature of $21 \pm 2^\circ\text{C}$ and relative humidity of $50 \pm 5\%$.

Procedure

All voles were housed with same-sex littermates until 2 weeks prior to testing, at which time all voles were individually housed and acclimated to our laboratory conditions. Following this acclimation period, animals were lightly anesthetized with methoxyflurane vapors (Metofane, Mallinckrodt Veterinary, Mundelein, IL), and a blood sample was obtained from the retroorbital sinus between 0900 and 1000 hr, followed by rapid cervical dislocation. The blood sampling procedure lasted <1.5 min. Blood samples were allowed to clot and were centrifuged at 2000 rev/min for 30 min. Serum was removed and stored at -80°C for later analysis of estradiol concentrations in females and testosterone concentrations in males using the radioimmunoassay (RIA) procedure described below. Immediately after cervical dislocation, the spleen was dissected from each animal under aseptic conditions, and splenocytes were assayed for proliferation using the procedure described below. All procedures were approved by The Johns Hopkins University Animal Care and Use Committee.

Experiment 2

Animals

Adult male ($n = 40$) and female ($n = 38$) prairie voles (*M. ochrogaster*) and male ($n = 41$) and female ($n = 41$) meadow voles (*M. pennsylvanicus*) were obtained from our breeding colonies. All animals were housed in poly-

carbonate cages ($28 \times 17 \times 12$ cm) with same-sex littermates until the start of the experiment.

Procedure

At 90–120 days of age, male and female prairie voles and meadow voles were assigned to one of the following housing conditions: (1) individually housed (I) ($n = 6/\text{group}$), (2) housed in same-sex pairs (SSP) ($n = 6\text{--}10/\text{group}$), (3) housed in mixed-sex pairs (MSP) ($n = 6\text{--}7/\text{group}$), (4) housed in same-sex groups of four (SSG) ($n = 12/\text{group}$), and (5) housed in mixed-sex groups of four (i.e., 2 males and 2 females/cage) (MSG) ($n = 6\text{--}8/\text{group}$). All pairs and groups of four animals consisted of unrelated conspecifics. In mixed-sex pairs and groups, all males were bilaterally vasectomized to avoid impregnation of females. Vasectomized males did not differ from intact males on any of the dependent variables measured. All animals remained undisturbed, other than routine cage cleaning, for the next 28 days. On Day 29, all animals were anesthetized and bled as described above. Serum was collected and stored for later RIA (described below) of testosterone in males, estradiol in females, and corticosterone in both sexes. Following bleeding, animals were killed, and the testes and seminal vesicles were dissected from males, and the ovaries and uteri were dissected from females. All organs were weighed. Spleens were dissected from all animals under aseptic conditions, and splenocytes were assayed for proliferation using the procedure described below.

Proliferation assay. Splenocyte proliferation in response to stimulation with the mitogen concanavalin A (Con A) was determined using a colorimetric assay based on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) (Cory, Owen, Barltrop, and Cory, 1991). Splenocytes were separated from tissue by pressing the whole spleen between sterile frosted-glass slides, and separated cells were suspended in 4 ml of culture medium [RPMI 1640/Hepes supplemented with 1% penicillin (5000 U/ml)/streptomycin (5000 $\mu\text{g}/\text{ml}$), 1% L-glutamine (2 mM/ml), 0.1% 2-mercaptoethanol (5×10^{-2} M/ml), and 10% heat-inactivated fetal bovine serum]. Splenocyte counts and viability were determined using a hemacytometer and trypan blue exclusion (Sigma Chemical Co., St. Louis, MO). Viable cells (which always exceeded 95%) were adjusted to 2×10^6 cells/ml by dilution with culture medium, and 50 μl of each cell suspension (i.e., 100,000 cells) was added to the wells of sterile flat-bottom 96-well tissue

culture plates. Con A (Sigma Chemical Co.) was diluted with culture medium to concentrations of 20, 10, 5, 2.5, 1.25, 0.6, and 0.3 $\mu\text{g}/\text{ml}$, and 50 μl of each mitogen concentration was added to the wells of the plate containing the spleen cell suspensions to yield a final volume of 100 $\mu\text{l}/\text{well}$ (each in duplicate). Unstimulated control cultures (containing 50 μl of adjusted splenocyte suspension plus 50 μl of culture medium) were also included in duplicate. Plates were incubated at 37°C with 5% CO_2 for 48 hr prior to addition of 20 μl of MTS/PMS solution [Promega; 0.92 mg/ml of phenazone methosulfate (PMS) in sterile Dulbecco's phosphate-buffered saline] per well. Plates were then incubated for another 4 hr and the optical density (OD) of each well was determined using a Bio-Rad microplate reader equipped with a 490-nm wavelength filter. Mean OD values for each set of duplicates were used in statistical analyses.

Steroid hormone radioimmunoassays. Blood serum estradiol concentrations in females and testosterone concentrations in males were assayed by RIA using ^{125}I kits purchased from ICN Biochemicals, Inc. (Carson, CA). The estradiol-17 β assay is highly specific; cross-reaction with other steroid is < 0.1%. The estradiol values were determined in a single RIA and the intra-assay coefficient of variation was 5.3%. The testosterone assay is also highly specific; cross-reaction with other steroids is < 0.1%. Testosterone values were also determined in a single RIA, with a 4.4% coefficient of variation. Corticosterone was measured using a specific radioimmunoassay kit (ICN Biomedicals, Inc.) 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 blood. The blood samples in both species were diluted 1:2121 in assay buffer (Taymans *et al.*, 1997). Samples were assayed in duplicate and the standard curve was assayed in triplicate. All blood samples were measured in the same assay, and the intra-assay coefficient of variation was 4.8%.

Statistical Analyses

In Experiment 1, splenocyte proliferative responses from males and females were analyzed separately for each species using mixed overall analyses of variance (ANOVAs) with one between-subjects variable (i.e., sex) and one within-subjects variable (i.e., mitogen concentration). In Experiment 2, only responses to an optimal concentration of mitogen (10 $\mu\text{g}/\text{ml}$) were used in

analyses. Sex and species differences between animals housed in a single condition were analyzed using an ANOVA with two between-subjects variables (i.e., sex and species). Serum estradiol concentrations among females and serum testosterone concentrations among males were analyzed with an ANOVA with two between-subjects variables (i.e., species and housing condition). Serum corticosterone concentrations from animals housed in the same social condition were analyzed using an ANOVA with two between-subjects variables (i.e., sex and species). Reproductive organ masses were analyzed with an ANOVA with two between-subjects variable (i.e., species and housing condition) and body mass data were analyzed with independent two-tailed *t* tests. In cases where the distribution of raw data violated the assumptions of a normal distribution, statistics were performed on log transformed data. Significant interactions were further analyzed using the Student–Newman–Keuls method for pairwise multiple comparisons. All mean differences were considered statistically significant if $P < 0.05$.

RESULTS

Experiment 1

Proliferation Data

Species differences in immunological response to mitogenic stimulation were not observed. Additionally, males and females showed similar responses to mitogenic stimulation among prairie voles, pine voles, meadow voles, and montane voles ($P > 0.05$ in each case) (Fig. 1). A standard dose–response curve to varied concentrations of mitogen was observed in all species, in which proliferative responses were reduced at high (i.e., 20 $\mu\text{g}/\text{ml}$ Con A) and low (i.e., 1.25, 0.6, and 0.3 $\mu\text{g}/\text{ml}$ Con A) mitogen concentrations and elevated at optimal mitogen concentrations (i.e., 10, 5, and 2.5 $\mu\text{g}/\text{ml}$ Con A) ($F(7, 154) = 104.30$, $P < 0.001$ for prairie voles; $F(8, 88) = 85.93$, $P < 0.001$ for pine voles; $F(7, 112) = 54.90$, $P < 0.001$ for meadow voles; and $F(8, 136) = 108.37$, $P < 0.001$ for montane voles).

Steroid Hormone Concentrations

Because serum estradiol concentrations were not normally distributed, log transformed data were analyzed and revealed that female pine voles and montane voles had higher serum estradiol concentrations than

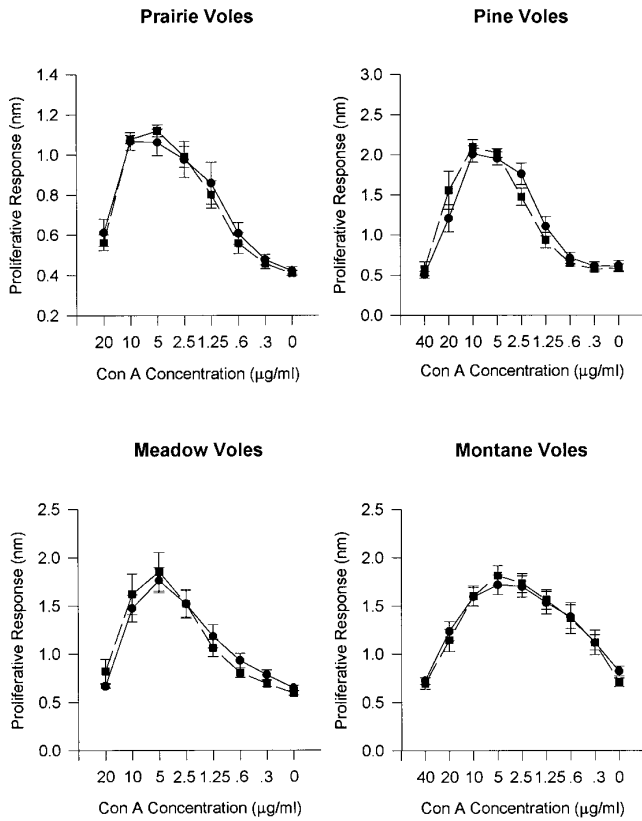


FIG. 1. Mean (\pm SEM) splenocyte proliferation values [absorbance units (nm)] from male (\bullet) and female (\blacksquare) prairie voles, pine voles, meadow voles, and montane voles in response to the mitogen, concanavalin A (Con A). Data are represented as absorbance units based on an enzymatic reaction that positively correlates with amount of cellular division.

prairie voles and meadow voles ($F(3, 36) = 3.10, P < 0.05$) (Fig. 2A). Male montane and pine voles had significantly higher serum testosterone concentrations than prairie voles ($F(3, 32) = 2.95, P < 0.05$). Male meadow voles had marginally higher testosterone concentrations than prairie voles ($P = 0.056$) (Fig. 2B).

Body Mass

Sex differences in body mass were observed in three of the four species. Mean body mass was significantly greater in male ($33.65 \pm 1.40\text{g}$) than in female prairie voles ($29.07 \pm 0.72\text{g}$) ($t = -3.15, df = 20, P < 0.01$, in male ($32.30 \pm 2.65\text{g}$) than in female ($27.18 \pm 2.89\text{g}$) meadow voles ($t = 1.30, df = 16, P < 0.05$), and in male ($40.57 \pm 2.09\text{g}$) than in female ($26.71 \pm 3.29\text{g}$) montane voles ($t = 3.46, df = 17, P < 0.01$). Mean body mass

did not differ between male ($22.15 \pm 1.21\text{g}$) and female ($21.34 \pm 0.85\text{g}$) pine voles ($P > 0.05$).

Experiment 2

Proliferation Data

To test for species differences in immune function, immune responses from meadow and prairie voles were compared. Overall, prairie voles had higher proliferative

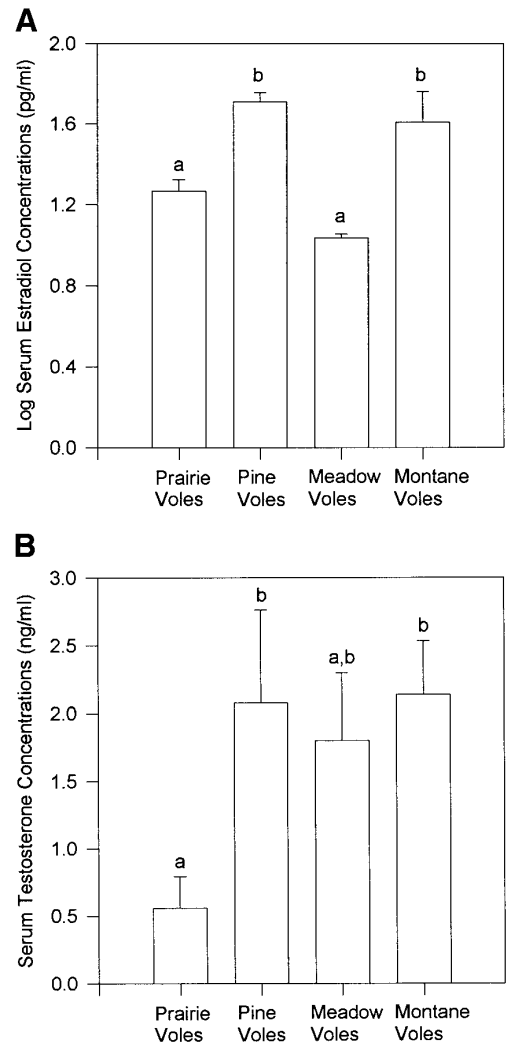


FIG. 2. Mean (\pm SEM) log serum estradiol concentrations (pg/ml) in female prairie voles, pine voles, meadow voles, and montane voles (A). Mean (\pm SEM) serum testosterone concentrations (ng/ml) in male prairie voles, pine voles, meadow voles, and montane voles (B). Bars sharing symbols are statistically equivalent.

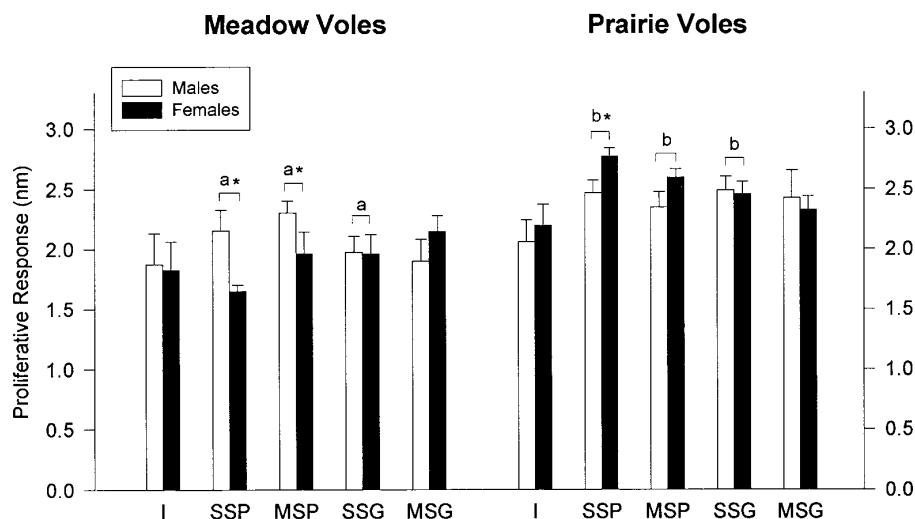


FIG. 3. Mean (\pm SEM) splenocyte proliferation values (nm) in response to an optimal concentration of Con A ($10 \mu\text{g}/\text{ml}$) in both male and female meadow and prairie voles that were housed individually (I), in same-sex pairs (SSP), in mixed-sex pairs (MSP), in same-sex groups of four (SSG), or in mixed-sex groups of four (MSG) for 28 consecutive days. For a single housing condition, bars with different letters (a or b) indicate significant differences between species. For a single housing condition, an asterisk indicates significant sex differences within a species.

immune responses than meadow voles among animals housed in same sex pairs ($F(1, 24) = 40.09, P < 0.0001$), mixed sex pairs ($F(1, 22) = 6.62, P < 0.05$), and same sex groups ($F(1, 41) = 15.02, P < 0.001$) (Fig. 3). There was a trend toward prairie voles exhibiting higher proliferative responses than meadow voles among animals housed in mixed-sex groups ($F(1, 22) = 4.053, P = 0.056$) (Fig. 3). No differences in immune response from individually housed prairie and meadow voles were observed.

Finally, to test whether sex differences in immune function are increased in polygynous compared to monogamous species, immune responses from males and females within each species were compared. Among animals housed in same-sex pairs, male meadow voles exhibited higher proliferative responses than female conspecifics, whereas female prairie voles produced higher proliferative responses than males conspecifics ($F(1, 24) = 12.77, P < 0.01$) (Fig. 3). Among animals housed in mixed-sex pairs, sex differences were apparent only among meadow voles, in which males exhibited higher proliferative responses than female conspecifics ($F(1, 22) = 4.97, P < 0.05$) (Fig. 3). No sex differences in proliferative immune responses were observed among either meadow or prairie voles housed individually or in groups ($P > 0.05$ in each case).

Steroid Hormone Concentrations

Because serum estradiol values were not normally distributed, log transformed data were analyzed and

revealed that female prairie voles in each housing condition had significantly higher serum estradiol concentrations than meadow voles ($F(1, 65) = 80.86, P < 0.0001$). Additionally, among prairie voles, females housed with male conspecifics in either pairs or groups had higher serum estradiol concentrations than females housed individually or with same-sex conspecifics ($F(4, 65) = 4.91, P < 0.01$) (Fig. 4A).

Because serum testosterone values were not normally distributed, log transformed data were analyzed and revealed that male meadow voles in each housing condition had significantly higher serum testosterone concentrations than prairie voles ($F(1, 65) = 37.26, P < 0.0001$) (Fig. 4B).

Prairie voles had higher serum corticosterone concentrations than meadow voles among animals housed individually ($F(1, 20) = 17.09, P < 0.001$), in mixed-sex pairs ($F(1, 21) = 11.4, P < 0.01$), and in same-sex groups ($F(1, 41) = 7.49, P < 0.01$) (Fig. 5). Serum corticosterone concentrations did not differ between prairie and meadow voles housed in either same-sex pairs or mixed-sex groups ($P > 0.05$ in each case).

Sex differences in corticosterone concentrations were also evident; female prairie voles had higher circulating corticosterone concentrations than conspecific males among animals housed individually ($t = -3.92, df = 10, P < 0.01$) (Fig. 5). Serum corticosterone concentrations did not differ among male and female prairie voles housed in same-sex pairs, mixed-sex pairs, same-

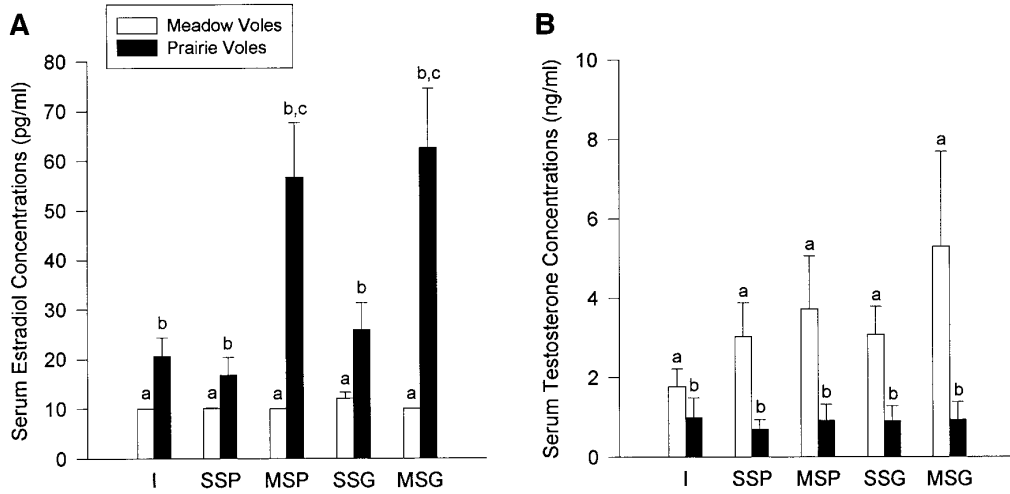


FIG. 4. Mean (\pm SEM) serum estradiol concentrations (pg/ml) in meadow and prairie vole females (**A**) and mean (\pm SEM) serum testosterone concentrations (ng/ml) in meadow and prairie vole males (**B**) that were housed individually (I), in same sex pairs (SSP), mixed-sex pairs (MSP), same-sex groups of four (SSG), or mixed-sex groups of four (MSG) for 28 consecutive days. Bars that share symbols in common are statistically equivalent.

sex groups, or mixed-sex groups ($P > 0.05$). Female meadow voles had higher corticosterone concentrations than conspecific males among animals housed individually ($t = -4.24$, $df = 10$, $P < 0.001$), in same-sex pairs ($t = -4.70$, $df = 13$, $P < 0.001$), in mixed-sex pairs ($t = -5.82$, $df = 11$, $P < 0.001$), in same-sex groups ($t =$

-6.19 , $df = 22$, $P < 0.0001$), and in mixed-sex groups ($t = -3.24$, $df = 14$, $P < 0.01$) (Fig. 5).

Body Mass and Reproductive Organ Mass

Male meadow voles (56.4 ± 1.21 g) weighed more than females (36.6 ± 1.23 g) ($t = 11.5$, $df = 75$, $P <$

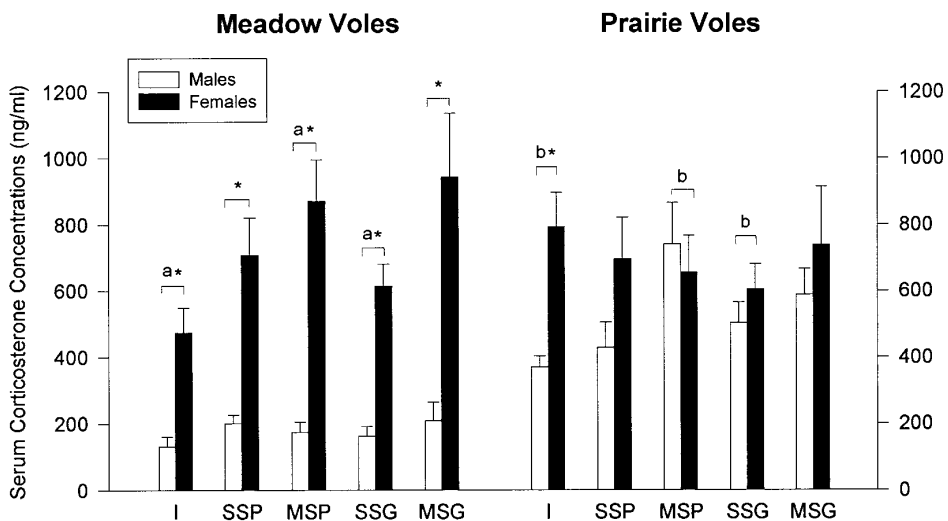


FIG. 5. Mean (\pm SEM) serum corticosterone concentrations (ng/ml) in male and female meadow and prairie voles that were housed individually (I), in same-sex pairs (SSP), mixed-sex pairs (MSP), same-sex groups of four (SSG), or mixed-sex groups of four (MSG) for 28 consecutive days. For a single housing condition, bars with different letters (a or b) indicate significant differences between species. For a single housing condition, an asterisk indicates significant sex differences within a species.

0.0001), while mean body mass did not differ between male ($42.9 \pm 1.25\text{g}$) and female ($39.8 \pm 1.23\text{g}$) prairie voles ($P > 0.05$). Heavier meadow vole males exhibited higher immune responses ($r^2 = 0.66$, $P < 0.05$). No such relationship between body size and immune function was observed among female meadow voles or among male or female prairie voles ($P > 0.05$ in each case).

Male meadow voles had significantly heavier testes and seminal vesicles than male prairie voles in each of the housing conditions ($F(1, 67) = 225.36$, $P < 0.0001$ for testes and $F(1, 66) = 23.81$, $P < 0.0001$ for seminal vesicles; data not shown). Uterine horn mass data among female meadow and prairie voles were not normally distributed, therefore statistical analyses were conducted on log transformed data. Female meadow voles and prairie voles that were either paired or group-housed with conspecific males had significantly greater uterine horn mass than conspecific females housed individually or with same-sex conspecifics ($F(4, 33) = 3.46$, $P < 0.05$ for meadow voles and $F(4, 32) = 5.58$, $P < 0.01$ for prairie voles; data not shown).

DISCUSSION

Although hormonal mediation of sex differences in immune function is well established in laboratory studies (Olsen and Kovacs, 1996; Schuur and Verheul, 1990), the adaptive functional implications of these sex differences are rarely considered (Folstad and Karter, 1992). The present study directly assessed endocrine status and immune function in closely related species with different mating systems. Regardless of the mating system characterized, individually housed males and females exhibited similar immune responses among all arvicoline rodents examined in Experiment 1. Unlike previous studies of rats, mice, and humans (Olsen and Kovacs, 1996; Schuur and Verheul, 1990), differences in circulating testosterone and estradiol concentrations in males and females, respectively, were not related to splenocyte proliferation as a measure of immunocompetence. Additionally, circulating testosterone concentrations were not related to the mating system as originally hypothesized. Polygynous montane voles had higher testosterone concentrations than monogamous prairie voles, but monogamous pine voles also had higher testosterone concentrations than prairie voles. Taken together, these data do not support the hypothesis that sex differences in immune function are more pronounced among polygynous than monogamous

species or that immunocompetence is related to species differences in circulating sex steroids (Zuk, 1990, 1994).

To test the contribution of social isolation to the results obtained in Experiment 1, we housed animals with either same- or opposite-sex conspecifics for 28 days. Exposure to differential housing conditions unmasked both species and sex differences in immunocompetence. Overall, prairie voles exhibited more robust immune responses than meadow voles. Additionally, male meadow voles exhibited higher immune responses than conspecific females in same- and mixed-sex pairs. In contrast, female prairie voles had higher immune responses than conspecific males among same-sex pairs. These data suggest that social factors can have significant effects on immune function; however, the precise mechanisms mediating these species and sex differences remains unspecified.

Differences in sex steroid concentrations were not correlated with immunocompetence among either prairie or meadow voles. Circulating estradiol concentrations were higher among female prairie voles than female meadow voles (both of which were presumably in estrus) and circulating testosterone concentrations were higher among male meadow voles than male prairie voles. Additionally, male meadow voles had heavier reproductive organ mass compared to male prairie voles. Reproductive organ mass (e.g., testes and seminal vesicles) is considered a good indicator of long-term circulating testosterone concentrations because it is less affected by the transient nature of testosterone secretion, at any given time (Bronson, 1989). Taken together, relatively high estradiol concentrations among female prairie voles and low testosterone concentrations among male prairie voles may act in concert to mediate the observed species difference in immune function in which prairie voles had higher immune responses than meadow voles.

Corticosterone has been reported both to enhance (Dhabhar and McEwen, 1996; Dhabhar, Miller, McEwen and Spencer, 1995) and to suppress (Bateman *et al.*, 1986) immune function in rats and mice. In prairie voles, basal corticosterone concentrations are approximately 10 times higher than those of laboratory mice, yet prairie voles display higher immune responses than mice (Klein, Taymans, DeVries, and Nelson, 1996). In the present study, prairie voles had higher corticosterone concentrations than meadow voles, and females had higher corticosterone concentrations than conspecific males. Differential housing changed the relationship of corticosterone concentrations between species and sexes (Fig. 5). Although prairie voles had higher

corticosterone concentrations than meadow voles across most housing conditions, prairie voles exhibited higher immune responses. These data suggest that corticosterone in arvicoline rodents may have immunoenhancing properties as previously described for rats and mice (Dhabhar and McEwen, 1996; Dhabhar *et al.*, 1995).

Assessment of estradiol, testosterone, and corticosterone concentrations was not conducted until the end of the 28-day housing period. In an attempt to use a well-established housing regimen that alters immune function in mice (Karp *et al.*, 1993), all animals were undisturbed for the 28-day period. Consequently, estradiol, testosterone, and corticosterone concentrations presented in this study represent only one brief time point during a cascade of hormonal changes that occur over time. Studies in both birds (Wingfield, 1994) and baboons (Alberts, Sapolsky, and Altmann, 1992) have suggested that the relationship between hormones and aggression, as well as between hormones and the immune system, may be apparent only during times of social instability (i.e., prior to the establishment of social status). Future studies will need to elucidate the changes in hormonal concentrations throughout the 28-day housing period (especially during initial exposure) in order to understand fully how differential housing affects immune function.

Following exposure to different social factors, sex differences in proliferative responses were observed among prairie voles as well as among meadow voles. Selection pressures influencing the evolution of sex differences in immune function may be more pronounced among monogamous species than initially hypothesized. Selection pressures are often reduced among monogamous species because regardless of variation in the quality of a male, the probability that a monogamous male will acquire a mate during the breeding season is hypothesized to be high (Read, 1991). Assuming that the species-specific sex differences in immune function observed in Experiment 2 are related to the mating system, these data contradict current hypotheses suggesting that sexual dimorphism in immunocompetence should be greater among polygynous species than among monogamous species (Zuk, 1990).

Sex differences in body size are generally more pronounced among polygynous mammalian species, with males being larger than females (Andersson, 1994). Both polygynous and monogamous males were heavier than conspecific females in Experiment 1; however this was not the case in Experiment 2, in which sex differences in body mass were apparent only among polygynous meadow voles. In Experiment 2 larger size was

related to higher immune responses among male meadow voles. This relationship suggests that within this species males may use size as an advertisement for greater immunocompetence. Future studies will need to establish the reproductive advantage of the relationship between body size and immune function by examining whether female meadow voles prefer to mate with larger males.

In sum, the data from the present study suggest that: (1) social factors can significantly influence species and sex differences in immune responsiveness, (2) sex differences in immune function are not necessarily more pronounced among polygynous species than among monogamous species, and (3) the precise hormonal mechanisms mediating the observed species and sex differences may involve the interplay of many steroidal hormones acting over time. Taken together, these data provide a new perspective into the adaptive significance of the relationship between the endocrine and the immune systems. This study also suggests that social factors have significant effects on immunity and should be considered in studies of sex differences at both proximate and ultimate levels.

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