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Increased survivorship of testosterone-treated female house mice, *Mus musculus*, in high-density field conditions

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Abstract. Differences in hormone levels influence sexual differences in aggression, survival, home-range size and dispersal in rodents. The role of testosterone in establishing some of these differences in wild house mice was examined. Females treated with either 0·5 mg of testosterone enanthate (TE-treated) or oil (control), and an equal number of untreated males, were released onto three 'highway islands' and periodically recaptured over the following 7 weeks. The islands are segments of highway cloverleafs enclosed by entrance and exit lanes that reduce emigration. Competition was encouraged by releasing large numbers of animals on the islands and by establishing feeding stations to enhance interaction opportunities. Significantly more TE-treated than control females were recaptured, because of differences in survival and not differences in capturability. Survival rates of TE-treated females exceeded those of control females early in the experiment but the reverse occurred near the end of the experiment. The two types of females did not differ in the number of captures at, or proximity of home ranges to, feeding stations. TE-treated females had smaller home ranges than control females, probably because the latter were frequently displaced by the former. Of the females recaptured at the end of the experiment, the TE-treated animals had fewer uterine scars, lighter uteri, heavier preputial glands and more tail scars than control females. TE-treated females resembled males by exhibiting increased intra-sexual aggression and having lower survival rates during the latter phase of the experiment. The benefits of, and hormonal basis for, aggressive behaviour appear similar in male and female house mice. Testosterone may mediate the competitive abilities of female house mice that have been shown in previous studies to be related to dominance and fitness.

Male fitness in most mammals is a function of the number of females fertilized, and thus competition for mates has favoured selection for larger body mass and a more aggressive nature in males than females. Competition for mates in polygynous rodent species is also thought to be responsible for the larger home-range sizes (e.g. DeLong 1967; Ims 1988; Salvioni 1988) and greater age-specific mortality (Rowe et al. 1963; DeLong 1967; Michener 1989) often seen in males compared with females. While sexual differences in body mass, aggression, home-range size, survivorship and dispersal are ultimately controlled by sexual selection (Darwin 1874), they are proximally regulated by differences in circulating levels of steroidal hormones, particularly testosterone, during perinatal and adult life (Phoenix et al. 1959; Bronson & Desjardins 1968; Feder 1981; Holekamp et al. 1984). Moderate increases in neonatal exposure to testosterone can produce behavioural changes that are considered adaptive in some circumstances (vom Saal 1984; Ims 1987, 1989). However, the use of hormone therapy to assess the endocrinological basis of sexually dimorphic behaviour in a field situation has rarely been attempted. Holekamp et al. (1984) induced an increase in disperal tendency in normally philopatric female ground squirrels, *Spermophilus beldingi*, by treating them with testosterone shortly after birth. The ability of testosterone treatment to alter other sexually dimorphic behaviour in the field, and in other species, has not been tested.

Testosterone from exogenous and endogenous sources can affect reproductive, aggressive and dispersal behaviour in female rodents. Adult female rodents produce testosterone from their adrenals (Moberg 1987), ovaries (Kumari et al. 1978; Lu et al. 1979) and placentae (Soares & Talamantes 1982), but plasma levels of testosterone in females are only about 10% of those of males (e.g. Lloyd 1980; Bartke et al. 1973; vom Saal & Bronson 1980).

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However, females exhibit increased aggressive behaviour after treatment with additional testosterone (Suchowsky et al. 1971; Svare et al. 1974), and the neural system modulating hormone-dependent aggression, at least in female rats, has about the same sensitivity to serum testosterone as that in males (van de Poll et al. 1981; Albert et al. 1989). The ability of testosterone to masculinize female behaviour is not limited to the perinatal period, but is a function of a female's age and the dose and duration of testosterone exposure (Whitsett et al. 1972; Svare et al. 1974; Arnold & Breedlove 1985). Perinatal exposure to androgen is not a prerequisite for the production of androgen-induced intra-specific fighting ability later in life. In the laboratory, young adult, ovariectomized females treated with testosterone are more aggressive than untreated females (Mugford & Nowell 1971, 1972; Svare et al. 1974; Gray et al. 1978), and intact, adult females treated with testosterone show significant increases in aggression (Suchowsky et al. 1971). Thus, treating young or adult female mice with testosterone can induce male-like physiological and behavioural changes. Although selection has favoured lower testosterone levels in females than males, the consequences of increasing testosterone in free-living female mice have not been studied.

In the present study we compare the competitive ability and survivorship of young adult, testosterone-treated females, oil-treated control females and untreated males released at high densities in a field experiment. If testosterone can induce more male-typical behaviour in female mice, then testosterone-treated females may have increased intraspecific competitive ability, and therefore greater survivorship than control females. Secondarily, we were interested in whether testosterone would increase female home-range size and would decrease long-term survival over the course of the experiment, phenomena often exhibited in males and attributed to high testosterone levels.

METHODS

Subject Animals and Laboratory Procedures

The subjects were descendants of wild house mice caught near Alberta, Canada, in 1982 (Perrigo & Bronson 1985). All pups destined for release into the field were delivered by Caesarean section approximately 12 h before natural parturition was expected and fostered to a female of the ICR strain. Only those males and females that had one male neighbour in utero (designated 1M animals) were used in the field experiment to control fetal exposure to variations in plasma testosterone and oestrogen known to influence reproductive behaviour (vom Saal 1989). Between 30 and 35 days of age, we treated females with either a subcutaneous injection of 500 μg of testosterone enanthate (TE) in 0·05 ml of peanut oil or with only 0·05 ml of the oil vehicle. The males were untreated. Testosterone enanthate is a long-acting ester that is capable of maintaining ejaculation in castrated rats up to 9 weeks after a single injection and inducing masculine behaviour in female mice (Beach & Sprague 1971; Stevens & Goldstein 1983). Contrary to most experiments that have tested the effect of testosterone on aggressive or sexual behaviour, we did not ovariectomize the females used in laboratory or field experiments. We acknowledged that testosterone can inhabit ovulation (Varon & Christian 1963), but remained interested in the reproductive success of testosterone-treated females. All animals were toe-clipped for individual identification.

Laboratory Dominance Trials and Plasma Testosterone Concentrations

Before we began the field experiments, the behavioural dominance of TE-treated over control females was verified in laboratory trials using an independent sample of mice. Dominance trials were conducted 1 and 2 weeks after injection. Fifteen females from each treatment were tested each week. Each was given a distinctive hairclip that identified her treatment, though the observer was unaware of the identity of the mice. A vaginal lavage was conducted on all females prior to testing to assure that TE-treated females had no cornified cells and that control females were in dioestrus. Females paired for trials were of roughly equivalent mass (mean ± SE; 25·7 ± 2·0 g and 25·5 ± 1·6 g for control and TE-treated females, respectively); where there was a disparity, it was never more than 1 g and the control female was always the heaviest of the pair. One of each type of female was placed into a neutral cage measuring 13 × 18 × 28 cm, between 1300 and 1700 hours and the pair was observed for 15 min or until persistent attacks appeared to threaten a mouse's well-being. Perineal investigations, aggressive grooming and attacks were recorded (Grant & MacKintosh 1963). A female was designated as the 'winner' if she initiated at least four attacks to an

opponent who was not responding with aggressive defence. When attacks were not common or when both females delivered more than four attacks, the trial had 'no winner'.

Four females from each treatment group, at each week post-injection, were killed 48 h after dominance trials and plasma was collected for testosterone assay. The androgen-sensitive preputial glands were also removed while in a semi-frozen state and weighed. Testosterone levels were also determined from a separate group of females, each of which had been treated with TE 7 weeks earlier but none of these females was tested in dominance trials. Testosterone was measured via radioimmunoassay according to the procedures outlined in vom Saal et al. (1990). While the assay measured predominantly testosterone, cross-reactivity with 5 alpha-dihydrotestosterone (DHT) was about 7%.

Field Procedures

Our study sites were the 'highway islands' that are isolated by merging lanes at a highway interchange (Massey & Vandenbergh 1980; Massey 1982; Coppola & Vandenbergh 1987). Highway islands were used because many include habitats preferred by wild house mice and because mice released onto the islands rarely emigrate from them (Massey 1982; Coppola 1986).

The particular interchange we used was developed and seeded in 1981, and the vegetation is typical of an old-field community, 5–10 years after abandonment (Keever 1950). Dog-fennel, *Eupatoriam canadense*, goldenrod, *Solidago* sp., blackberry, *Rubus* sp., broomsedge, *Andropodon* sp. and isolated loblolly pines, *Pinus taeda*, were the dominant native species. However, several species of Korean lespedeza, *Lespedeza* spp., and fescue, *Festuca* spp., which had been seeded during construction of the area were also widespread. Of the four islands available, we chose the southwest (0·64 ha of unmowed area) and northwest (1·02 ha of unmowed area) as sites for mouse releases because of the thick herbaceous cover and because of the low density of pines, which reduce the habitat suitability for house mice.

Competition among females was incited by (1) introducing a number of animals on each island that exceeded the highest density ever recorded on similar sites (Massey & Vandenbergh 1980; Coppola & Vandenbergh 1987), and (2) by establishing a number of feeding stations on each island; three on the southwest and five on the larger, northwest island. We assumed that competitively dominant females and males would defend areas around the permanent supply of food (Ims 1987). We provided cracked corn and sunflower seed every 10 days under the cover of a wooden board, measuring 1 × 1 m, supported by bricks. Feeding stations may have increased the carrying capacity of the island but the number of mice we released exceeded the density of mice ever reported on similarly provisioned islands.

Three to four weeks before the mice were to be released on the islands, traps were set to capture and relocate all native small mammals on the islands. The same trap distribution was used to remove these animals as was later used to recapture experimental house mice. A Sherman live-trap measuring 7 × 7 × 25 cm was placed at 30-m intervals along traplines located 30 m apart and two traps were also placed at each feeding station. This distribution resulted in 46 and 77 traps on the southwest and northwest islands, respectively. During the pre-release removal period, the traps were checked twice a day at least 3 days a week. Cotton rats, *Sigmodon hispidis*, and house mice were the most common residents with far fewer numbers of white-footed mice, *Peromyscus leucopus*, short-tailed shrews, *Blarina carolinensis*, and cottontail rabbits, *Sylvilagus floridanus*.

An experimental replicate consisted of releasing equal numbers of TE-treated and control females, and a number of males equal to the total number of females, on one island. Three replicates were run at two different times of the year. On 11 May 1989 15 TE-treated and 15 control females plus 30 males were released on each island. In addition, 20 of each type of female and 40 males were released on the southwest island on 10 October 1989. The availability of mice, rather than the size of the island, dictated the number of individuals released but in each case the density of mice released was in excess of that ever reported on similar islands. Mice were released at the centre of the island in the late afternoon, 2 days after the females were treated.

Traps were set to recapture mice at four occasions over a 7-week period following release. Each of the four recapture episodes included three consecutive nights of trapping. Traps were set in the late afternoon, checked at dawn, closed for the rest of the day, and reopened again in the late afternoon. The first morning of each of the recapture episodes occurred 7, 19, 32 and 47 days after the day of release resulting

Table I. Laboratory dominance trials comparing the mean (±SE) number of perineal investigation and aggressive grooming behaviour patterns per trial (15 trials each week) and the number of wins for TE-treated and control females

	Number of weeks post-injection			
	One		Two	
	Control	TE	Control	TE
Perineal investigation (per trial)	0·8±0·3	2·4±0·4*	0·6±0·3	1·9±0·5*
Aggressive grooming (per trial)	0·1±0·1	0·4_0·1*	0·2±0·1	0·8±0·4
Number of wins/trials	0/15	5/15†	0/15	4/15

*Significantly different within-week comparison (*t*-test, $P<0·05$).
†Significantly different within-week comparison (chi-squared test, $P<0·05$).

in inter-trapping intervals of 7, 10, 12 and 14 days, respectively.

Analyses of Survivorship, Dominance, Space Use and Reproduction

Using chi-squared contingency tables, we initially analysed survivorship by comparing the number of TE-treated females, control females and male mice that were captured at least once with the number that were never captured. Animals that were never captured were assumed to have either died or dispersed from the island and thus did not 'survive'. However, differences in capture rates may merely reflect differences in behaviour toward traps or activity levels, so we also estimated survival rates independent from capture rates using the Jolly–Seber model (Jolly 1965; Seber 1965) as included in the software program RELEASE (Burnham et al. 1987). All test statistics in RELEASE are distributed as chi-squared. We applied the model separately to the TE-treated females, control females and males to obtain independent estimates of survival probability (φ_i) for each of the four intervals between trapping occasions and capture probability (p_i) for each of the four capture occasions. Islands were compared within treatment before data were pooled.

We assessed dominance by comparing the number of TE-treated females, control females and male mice caught at feeding stations and the mean distance of all captures for each individual from the nearest feeding station. Dominant animals were presumed to establish home ranges near feeding stations. We also calculated the area that an individual used for each mouse that was captured three times or more using the location analysis program MCPAAL (Stüwe & Blohowiak, unpublished). Body weights, measured during recapture, were an index of reproductive activity and general health. Females having a mass of 27 g or more, and having obvious abdominal distension, were assumed to be pregnant because laboratory observations indicate that it is rare for females to weigh more than this unless they are pregnant (Zielinski, personal observation). At the last recapture episode, during the spring of 1989 only, all remaining females were returned to the laboratory and dominance trials were conducted by pairing control and TE-treated females on the basis of mass. Following these trials the females were killed, their preputial glands and uteri were weighed and their uterine implantation scars and tail scars were counted. Our primary measure of reproductive success was obtained by comparing the mean number of uterine scars of TE-treated and control females.

RESULTS

Dominance Trials, Testosterone Levels and Preputial Mass of Laboratory-housed Females

In the laboratory, TE-treated females were winners in all dominance trials in which a winner could be assigned (Table I). The number of winners among treated and control females was significantly different at 1 but not at 2 weeks

Table II. Mean (± SE) plasma testosterone levels, body mass and preputial mass for control and TE-treated females 1-, 2- and 7-weeks post-injection

	1 week		2 weeks		7 weeks	
	Control	TE	Control	TE	Control	TE
Plasma testosterone (ng)	0·29 ± 0·05	5·98 ± 2·20*	0·38 ± 0·06	5·20 ± 2·20*	0·10 ± 0·02	0·25 ± 0·03*
Body mass (g)	13·5 ± 0·6	14·3 ± 0·8	16·7 ± 0·5	16·6 ± 0·6	20·0 ± 1·0	17·5 ± 0·7*
Preputial mass/ body mass (mg/g)	0·03 ± 0·00	0·32 ± 0·06*	0·04 ± 0·00	0·86 ± 0·13*	†	†

*Significantly different within-week comparison (*t*-test, $P < 0·05$).
†Preputial mass data were not collected for the 7-week group.

post-injection ($\chi^2 = 3·84$, $df = 1$, $P < 0·05$ and $\chi^2 = 2·59$, $df = 1$, $P > 0·05$, respectively). TE-treated females initiated significantly more perineal investigations than control females at both weeks (1 week: $t = 3·34$, $df = 34$, $P < 0·005$; 2 weeks: $t = 2·21$, $df = 26$, $P < 0·025$; Table I). Aggressive, or rough, grooming occurred more frequently in TE-treated females at 1 ($t = 2·03$, $df = 34$, $P < 0·025$) but not 2 weeks ($t = 1·49$, $df = 26$, $P < 0·10$) post-injection (Table I). Attacks were usually preceded by genital sniffing, rough-grooming and tail rattling, all of which have been associated with aggression in previous studies (e.g. Svare et al. 1974). Control females were consistently passive and in only one of 30 trials did a control female initiate an attack on a TE-treated female.

Plasma testosterone levels were significantly higher in TE-treated than control females at 1, 2 and 7 weeks after injection (Table II). However, the difference at 7 weeks is probably meaningless because the level in TE-treated females at this time is no different than the control level at 1 and 2 weeks. The 1- and 2-week post-injection levels were within the mean physiological levels reported for males (reviewed in Carlier et al. 1990).

Body mass of females did not differ on the basis of treatment 1 and 2 weeks after injection, but the TE-treated females were significantly lighter than the control females by 7 weeks post-injection ($t = 2·13$, $df = 16$, $P < 0·25$; Table II). The mass of preputial glands, standardized by body mass, were significantly greater in the TE-treated than control females ($t = 4·7$, $df = 6$, $P < 0·005$ and $t = 5·8$, $df = 6$, $P < 0·005$, at 1 and 2 weeks, respectively). Thus, TE-treated females were more frequently dominant, had higher testosterone levels and had larger preputial glands than the control females.

Table III. Number of TE-treated and control females and males either caught at least once or never caught after release

	TE	Control	Male
NW: Spring 1989			
Caught	11	6	20
Not	4	9	10
Total number released	15	15	30
SW: Spring 1989			
Caught	12	7	13
Not	3	8	17
Total number released	15	15	30
SW: Autumn 1989			
Caught	9	6	18
Not	11	14	22
Total number released	20	20	40
All islands			
Caught	32	19	51
Not	18	31	49
Total number released	50	50	100

Survivorship of Field-released Animals

There were no significant differences in survival or capture rates among islands within treatment group, thus data among islands were pooled to assess the overall effect of treatment on survivorship. Analysed simply on the basis of whether a particular animal was either caught or not caught after release, the TE-treated females had significantly greater survivorship in the wild than the control females ($\chi^2 = 5·02$, $df = 1$, $P < 0·025$, Table III). The pattern was similar for all three replicates. However, this broad measure of survivorship does not distinguish between the probability of survival and probability of capture and does not address the change in relative survivorship over time. Program

Table IV. Mean (SE) survival (φ_x) and capture (p_x) rate estimates (x = the order of between-capture interval and capture occasion, respectively)

	Females		
	Control	TE-treated	Males
Survival rates			
φ_1	0·44 (0·08)[a]	0·66 (0·07)[b]	0·56 (0·06)[ab]
φ_2	0·60 (0·12)[a]	0·83 (0·07)†	0·53 (0·07)[a]
φ_3	0·92 (0·09)	0·98 (0·07)	0·83 (0·09)
$\varphi_4 p_4$*	0·90 (0·09)[a]	0·48 (0·09)[b]	0·66 (0·10)[ab]
Capture rates			
p_1	0·77 (0·11)	0·85 (0·07)	0·79 (0·07)
p_2	0·83 (0·10)	0·69 (0·09)	0·79 (0·08)
p_3	0·82 (0·11)	0·92 (0·07)	0·87 (0·08)

Different letters, within a row, indicate statistical differences at $P<0·05$. The absence of letters indicates lack of statistically significant results.

*Neither the survival rate for the final between-capture interval (φ_4) nor the capture rate for the final capture occasion (p_4) can be estimated independently. They are combined to yield an approximate estimate of survival rate for the final between-capture interval.

†Difference between TE-treated and control females approaching significance ($P<0·079$).

RELEASE allowed us to address both of these problems. In no case was the probability of capture different between the TE-treated and control females or between either type of female and males (Table IV). This suggests that the animals did not differ in behaviour towards, or encounter rate with, traps. Survival rates for all animals decreased immediately after release, increased for the interval between trapping occasion 2 and 3 and between 3 and 4, and then, especially in the TE-treated females and males, decreased immediately before the final trapping occasion (Fig. 1). However, and most importantly, the TE-treated females had a significantly greater probability of surviving the first interval than control females and an almost significantly greater probability for the second interval (Table IV). Competition was expected to be the most severe during the first two intervals. Despite an advantage early in the experiment, by the end of the experiment, the TE-treated females appeared to survive more poorly than control females. The survival rate for males differed from either type of female only for the interval between trapping occasion 1 and 2 when it was significantly lower than that for TE-treated females (Table IV).

The pattern of survivorship over time was analysed by examining survival rate changes for each sex-class type. The survival rates increased for all animals from the interval between the first and second trapping occasion through the interval between the second and third occasion, but either stabilized or decreased in the last interval (Fig. 1). Considering the change in survival rates between the penultimate and ultimate intervals, there was a significant decrease for TE-treated females ($t=4·39$, $df=33$, $P<0·0001$), a non-significant decrease for males ($t=1·27$, $df=37$, $P<0·11$), and no change for control females ($t=0·16$, $df=20$, $P<0·45$).

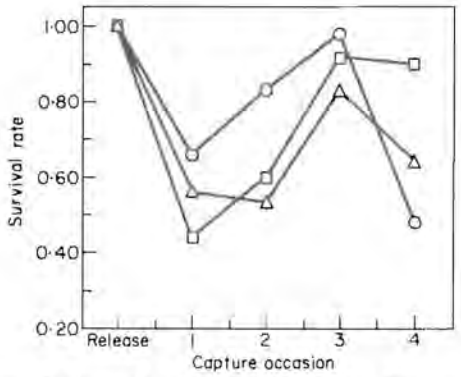

Figure 1. Survival rate (φ) estimates for TE-treated females (○), control females (□), and males (△) for the periods between recapture occasions (all replicates pooled).

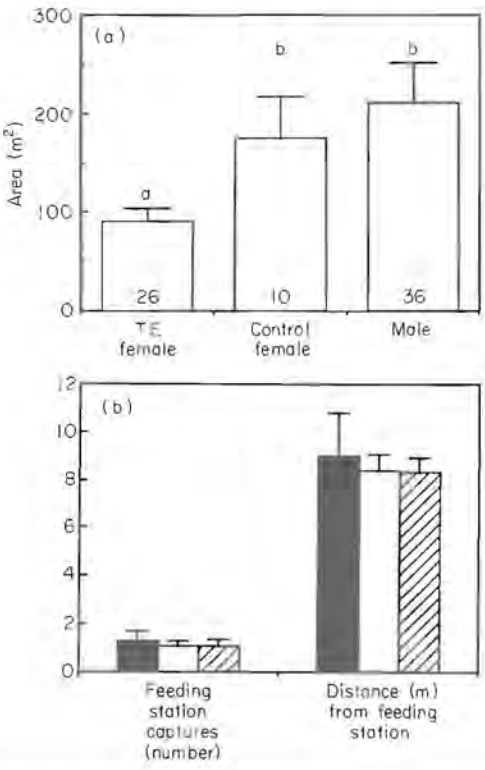

Figure 2. (a) Mean (± SE) area used by TE-treated females, control females and males. Letters above bars distinguish significantly different means ($P<0.05$) and numbers within bars are sample sizes. (b) Mean (+SE) number of feeding station captures and distance of captures from a feeding station for TE-treated (□), control females (■), and males (▨). No significant differences were found among sexes or treatment groups.

Spatial Variables

Although an animal had to be captured a minimum of three times to be included in the analysis of home range, the mean (± SE) number of captures of those included were 6·1 ± 0·5 and 5·6 ± 0·2 for males and females, respectively. TE-treated females had significantly smaller home-ranges than control females ($t=2.51$, $df=34$, $P<0.01$) and males ($t=2.53$, $df=60$, $P<0.01$; Fig. 2a). However, neither the mean number of captures at feeding stations nor the mean distances of captures from a feeding station differed among TE-treated females, control females and males (Fig. 2b).

Reproduction and Body Mass

The number of TE-treated females that had a body mass of 27 g or more at any time during the study (6 of 32 recaptured individuals) was significantly less than the number of control females (9 of 19; $\chi^2=4.7$, $P<0.05$). In addition, of the females that were recaptured at the last session and killed, TE-treated females had fewer uterine scars ($F=79.29$, $df=5$, $P<0.001$) and lighter uteri ($F=24.1$, $df=5$, $P<0.0005$) than control females (Fig. 3).

Testosterone treatment also affected the mass of non-pregnant animals (those weighing less than 27 g by our definition) throughout the course of the experiment. While the mean body mass of the females were indistinguishable at release, TE-treated females weighed less than control females at each of the subsequent recapture episodes (Fig. 4). This agrees with the laboratory finding that at 7 weeks post-injection TE-treated females weighed significantly less than controls.

Post-experiment Dominance Trials, Preputial Mass and Tail Scars

At the last recapture episode for the two spring 1989 replicates, seven TE-treated and seven control females were returned to the laboratory. They were paired on the basis of mass and tested in dominance trials identical to those described above for laboratory females. The results were similar to those of laboratory-housed females tested 1 and 2 weeks after injection. Testosterone-treated females were winners in four of the seven pairs, while in the other three pairs a winner could not be unequivocally determined. Thus, in no case did a control female win a contest.

Each female recaptured at the end of the third replicate (autumn 1989, southwest island) was killed when in dioestrus, weighed and her preputial glands were weighed. The TE-treated females had preputial glands that weighed significantly more than the control females (means ± SE: TE = 1·19 ± 0·07 mg/g, control = 0·68 ± 0·08 mg/g; $t=3.45$, $P<0.01$), even 8 weeks after the injection (Fig. 3). Only TE-treated females had scars from tail wounds (Fig. 3). Scars were numerous, with individual females having 1–12 distinct wounds. These were probably not self-inflicted because none of the TE-treated females that were laboratory-housed had tail scars when examined 7 weeks after treatment.

DISCUSSION

Female persistence on the islands was clearly affected by TE-treatment, especially in the early,

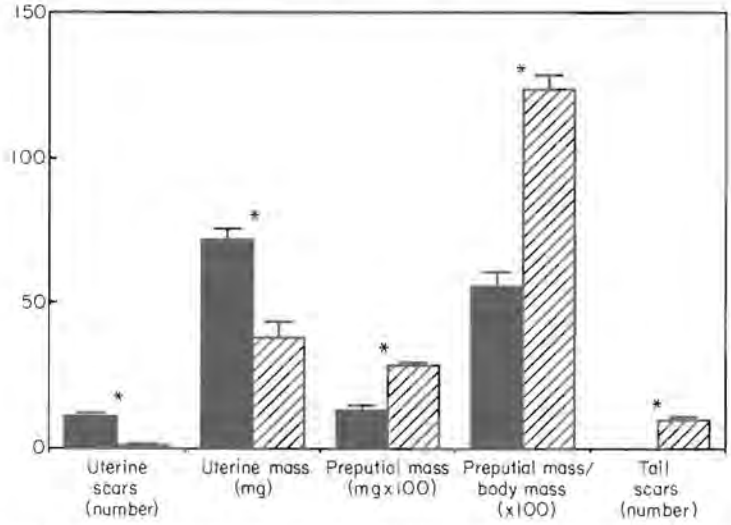

Figure 3. Comparisons of mean (±SE) uterine scars, uterine mass, preputial mass and tail scars between TE-treated (▨) and control females (■) that were recaptured at the end of the experiment. * Indicates significant treatment differences (see text).

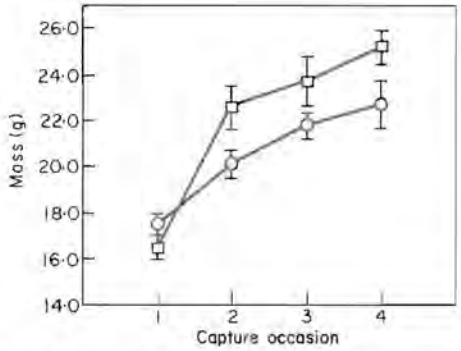

Figure 4. Mean (±SE) body mass for TE-treated (○) and control (□) females as a function of recapture episode. Episode 4 was about 7 weeks post-release.

high-density, phase of the experiment. Our laboratory data suggest that the poor survival rate of control females was probably caused either by their dispersal or death as a result of interactions with TE-treated females. Because intra-sexual aggression usually exceeds inter-sexual aggression in most mammals, including rodents (Floody 1983), we suspect that TE-treated females were primarily aggressive toward females and that they were effective in preventing the establishment of home ranges by some control females. Because the TE-treated and control females did not have different capture rates, we reject the theory that control females were caught less frequently because they were pregnant or lactating more often than treated females. In addition, although pregnancy may reduce subsequent survival, we doubt that higher pregnancy rates in control than TE-treated females contributed to differences in survival because the survival differences were greatest before first parturition dates.

Our conclusions are surprising given the long-standing view that female house mice are passive and that testosterone is ineffective in promoting aggression in adult female mice (Levy 1954; Tollman & King 1956; Bronson & Desjardins 1968). The previous conclusion that female mice are not aggressive is probably because: (1) female mice fight infrequently in the type of situation typically used to test aggression in males, that is, during pairwise encounters with adult conspecifics in neutral arenas (e.g. Fredericson 1952), and (2) most tests are conducted with albino strains of mice, both sexes of which are less aggressive than wild mice (Scott 1966). However, female–female aggression frequently occurs in competition for food (Brain & Evans 1975; Albert et al. 1989) and when females are lactating (Svare 1989).

While early findings suggested that perinatal exposure to testosterone was necessary for females to exhibit aggression in response to androgens in adulthood (Bronson & Desjardins 1968), more

recent data suggests that treatment in adulthood can be as effective (Svare et al. 1974; vom Saal et al. 1976; Gray et al. 1978). It was surprising, however, to find that one injection of TE was sufficient to affect preputial gland mass for at least 7 weeks and behaviour and plasma testosterone for at least 2 weeks. A single, 0·5 mg dose of testosterone propionate (TP) has been shown to increase aggression in female white-footed mice (Gleason et al. 1979), but in house mice single injections of TP are ineffective (Levy 1954; Tollman & King 1956; White et al. 1969). Testosterone propionate must usually be administered daily, for several weeks, before ovariectomized, albino, female house mice behave aggressively (Edwards 1970; Svare et al. 1974). Our treatment was probably effective because we used wild females, which are naturally more aggressive, and because females were treated with a long-acting testosterone ester.

Mice were treated only once and then released, so we were most likely observing the 'activational', or proximate, effects of testosterone on aggression. However, given the long-acting nature of TE, early exposure (shortly after the treatment) may have sensitized brain regions to the effects of testosterone, or its metabolites, that were still circulating weeks later; producing an 'organizational' effect. Others have suggested this possibility as a result of chronic TP treatment, even in adulthood (65 days of age, Svare et al. 1974), and TP can have 'organizational' effects on masculine behaviour even when first administered to 30-day-old females that are subsequently tested after a second exposure at 45 days of age (Edwards 1970). We did not determine whether the aggression-enhancing effects were caused by testosterone, one of its metabolites or its aromatization to oestrogen (Naftolin et al. 1971). Injected oestradiol is less effective than testosterone in inducing aggressive behaviour (Edwards & Herndon 1970; Simon & Gandelman 1978; Gleason et al. 1979), but this may be due to its inactivation by α-fetoprotein (Raynaud et al. 1971).

The high survival rate of TE-treated females did not continue throughout the duration of the experiment. During the interval between the third and fourth trapping occasions, the survivorship of TE-treated females dropped significantly and was also significantly lower than that of control females. Survival and capture probabilities cannot be estimated independently for the interval between the third and fourth trapping occasions (Burnham et al. 1987), but previous capture rates were similar for TE-treated and control females so the difference was probably due to a difference in survival. We suggest several factors that could account for the decrease in survival of TE-treated females near the end of the experiment. First, the anabolic action of testosterone draws heavily on lipid stores (Wade & Gray 1979) and the action of injected testosterone may have eventually decreased survival by eliminating fat reserves. The decreased body mass in TE-treated females toward the end of the experiment supports this possibility. Although female mice treated with TE and maintained on ad libitum food gain mass compared with controls (Stevens & Goldstein 1983), this seems less probable in free-ranging females that may be food-limited. Second, TE-treated females may have been perceived as sufficiently masculine by males so that by the end of the experiment treated females were negatively affected by male harassment. Testosterone-treated females produce an aggression-promoting pheromone that incites attacks by males (Mugford & Nowell 1972; Lee & Griffo 1973). The high incidence of tail scars in TE-treated females may have resulted from attacks by males because male mice and rats tend to direct bites toward the rear while females attack the face of intruders (DeBold & Miczek 1984; vom Saal, personal communication). However, it is equally likely that the scars could have resulted from interactions between TE-treated females because group-housed, testosterone-treated females are aggressive towards one another and towards intruders (Bronson & Desjardins 1971; Gray et al. 1978). Finally, testosterone may have caused behavioural changes in the treated females that increased their risk to predators as has been shown for testosterone-treated male lizards and birds (Marler & Moore 1989; Trobec & Oring 1972). Perhaps the substantial, but not significant, decline in male survival observed during the last interval occurred for the same reason. Males and testosterone-treated females may engage in more risky behaviour than control females.

The TE-treated females neither dominated feeding stations nor had larger home ranges than control females, both of which might have been expected if testosterone produced masculine behaviour in treated females. Because the use of feeding stations was relatively low by all animals, we suspect that natural food was abundant enough to preclude the establishment of territories at or near stations. Territoriality is less common when

food is abundant (Carpenter 1987). Recent evidence indicating sexual differences in hippocampal size in polygynous vole species has been used to explain sexual difference in space use and home range size (Jacobs et al. 1990). If the sexual differences in spatial abilities in house mice also result from differences in relative hippocampus size then it would be unlikely that treating females with testosterone, especially as adults, would influence space use patterns. Why then, might TE-treated females have used significantly smaller areas than control females? We believe that in the high-density, early phase of the experiment interactions among females were common and control females were frequently displaced by the more dominant TE-treated females. Thus, the spatially disparate recaptures of control females reflected their continued harassment and evictions by resident TE-treated females, making them appear to have larger home-ranges. These results are similar to other highway island studies of house mice where resident females had 67% smaller home ranges than female 'interlopers' that were added to the population (Coppola 1986). It is possible, however, that the smaller home ranges could be a pharmacological effect because females treated peripubertally with TE have decreased open-field behaviour compared to controls (Stevens & Goldstein 1983).

We deliberately used non-ovariectomized females to examine the effect of testosterone treatment on reproduction in the field, although we expected very little reproductive activity. Surprisingly, 27·0% of the treated females that were recaptured at the end of the experiment had at least one uterine scar indicating that the effects of TE-exposure in young adulthood on subsequent reproduction were not permanent. This is in agreement with the results of Barkley & Goldman (1977) who found that 70 days after removing a testosterone implant females showed normal vaginal and ovarian cyclicity following an ovarian graft. Similarly, selection for female aggression can occur without affecting fertility or body weight (Ebert & Hyde 1976). Thus, it appears that females can exhibit above average levels of aggression, and possibly increased plasma testosterone, without completely sacrificing reproductive performance.

Observations of wild house mice indicate that females will actively defend territories (Reimer & Petras 1967; Lidicker 1976) and that dominant females have the greatest fitness (Lloyd & Christian 1969; Franks & Lennington 1986). Aggression can also favour increased reproductive success in female yellow-bellied marmots, *Marmota flaviventris* (Svendson 1974) and voles, *Microtus* sp. (Taitt & Krebs 1985). However, androgen levels have not been correlated with female dominance in any species. Our results, and the laboratory findings of others, suggest that testosterone can mediate the competitive and territorial behaviour of female mice. Testosterone is more effective in inducing aggressive behaviour in females than is oestradiol (Edwards & Herndon 1970; Simon & Gandelman 1978; Gleason et al. 1979) even though plasma levels of testosterone in females are only a fraction of that found in males. Females exhibit increased aggressive behaviour after treatment with supraphysiological doses of testosterone (Suchowsky et al. 1971; Svare et al. 1974) and the neural system modulating hormone-dependent aggression in females, at least in rats, has about the same sensitivity to serum testosterone as that of males (van de Poll et al. 1981; Albert et al. 1989). Furthermore, the aggressive response of female rats to testosterone treatment is greatest if they are forced to compete for resources (Albert et al. 1989), indicating that the effect of the hormone is dependent on circumstances where competitive ability would be favoured. Thus, the benefits of, and the hormonal basis for aggressive behaviour appear similar in males and females. The island situation used in the present study probably created a competitive setting where female aggression would be favoured and testosterone simply enhanced the competitive abilities of treated females. Additional studies where females are treated with testosterone and oestradiol at the lower and upper end of their physiological plasma levels will help refine our understanding of the consequences of hormonal variation on reproduction and aggression in female mice.

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REFERENCES

Albert, D. J., Jonik, R. H., Walsh, M. L. & Petrovic, D. M. 1989. Testosterone supports hormone-dependent aggression in female rats. *Physiol. Behav.*, **46**, 185–189.

Arnold, P. A. & Breedlove, S. M. 1985. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. *Horm. Behav.*, **19**, 469–498.

Barkley, M. S. & Goldman, B. D. 1977. A quantitative study of serum testoterone, sex accessory organ growth, and the development of inter-male aggression in the mouse. *Horm. Behav.*, **8**, 208–218.

Bartke, A., Steele, R. E., Musto, N. & Caldwell, B. V. 1973. Fluctuations in plasma T levels in adult male rats and mice. *Endocrinology*, **92**, 1223–1228.

Beach, G. A. & Sprague, R. H. 1971. Effects of different forms of testosterone on mating in castrated rats. *Horm. Behav.*, **2**, 71–72.

Brain, P. F. & Evans, C. M. 1975. Attempts to influence fighting and threat behaviors in adult isolated female CFW mice in standard apparent aggression tests using injected and subcutaneously implanted androgens. *Physiol. Behav.*, **14**, 551–556.

Bronson, F. H. & Desjardins, L. 1968. Aggression in adult mice: modification by neonatal injections of gonadal hormones. *Science*, **161**, 705–706.

Bronson, F. H. & Desjardins, L. 1971. Steroid hormones and aggressive behavior in mammals. In: *The Physiology of Aggression and Defeat* (Ed. by B. E. Eleftheriou & J. P. Scott), pp. 43–63. New York: Plenum Press.

Burnham, K. P., Anderson, D. R., White, G. C., Brownie, C. & Pollock, K. H. 1987. *Design and Analysis Methods for Fish Survival Experiments Based on Release-recapture*. Bethesda, Maryland: American Fisheries Society.

Carlier, M., Roubertoux, M., Kottles, M. L. & Degrelle, H. 1990. Y-chromosome and aggression in strains of lab mice. *Behav. Gen.*, **20**, 137–156.

Carpenter, F. L. 1987. Food abundance and territoriality: to defend or not to defend? *Am. Zool.*, **27**, 387–399.

Coppola, D. M. 1986. Reproductive status and the assimilation of introduced females into a wild population of house mice. *Acta theriol.*, **31**, 221–237.

Coppola, D. M. & Vandenbergh, J. G. 1987. Induction of a puberty-regulating chemosignal in wild mouse populations. *J. Mammal.*, **68**, 86–91.

Darwin, C. 1874. *The Descent of Man and Selection in Relation to Sex*. 2nd edn. New York: A. L. Burt.

DeBold, J. F. & Miczek, K. A. 1984. Aggression persists after ovariectomy in female rats. *Horm. Behav.*, **18**, 177–190.

DeLong, K. T. 1967. Population ecology of feral house mice. *Ecology*, **48**, 611–634.

Ebert, P. D. & Hyde, J. S. 1976. Selection for agonistic behavior in wild female *Mus musculus*. *Behav. Gen.*, **6**, 291–304.

Edwards, D. A. 1970. Post-neonatal androgenization and adult aggressive behavior in female mice. *Physiol. Behav.*, **5**, 465–467.

Edwards, D. A. & Herndon, J. 1970. Neonatal estrogen stimulation and aggressive behaviour in female mice. *Physiol. Behav.*, **5**, 993–995.

Feder, H. H. 1981. Perinatal hormones and their role in the development of sexually dimorphic behaviors. In: *Neuroendocrinology of Reproduction* (Ed. by N. T. Adler), pp. 127–157. New York: Plenum Press.

Floody, O. R. 1983. Hormones and aggression in female mammals. In: *Hormones and Aggressive Behavior* (Ed. by B. B. Svare), pp. 39–89. New York: Plenum Press.

Franks, P. & Lennington, S. 1986. Dominance and reproductive behavior of wild house mice in a seminatural environment correlated with T-locus genotype. *Behav. Ecol. Sociobiol.*, **18**, 395–404.

Frederickson, E. 1952. Aggressiveness in female mice. *J. comp. physiol. Psychol.*, **45**, 254–257.

Gleason, P. E., Michael, S. D. & Christon, J. J. 1979. Effects of gonadal steroids on agonistic behavior of female *P.I. Horm. Behav.*, **12**, 30–39.

Grant, E. C. & MacKintosh, J. H. 1963. Comparison of social postures of some common laboratory rodents. *Behaviour*, **21**, 246–259.

Gray, L. E., Jr, Whitsett, J. M. & Zieseuis, J. S. 1978. Hormonal regulation of aggression toward juveniles in female house mice. *Horm. Behav.*, **11**, 310–322.

Holekamp, K. E., Smale, L., Simpson, H. B. & Holekamp, N. A. 1984. Hormonal influences on natal dispersal in free-living Belding's ground squirrels (*Spermophilus beldingi*). *Horm. Behav.*, **18**, 465–483.

Ims, R. A. 1987. Determinant of competitive success in *Clethrionomys rofocanus*. *Ecology*, **68**, 1812–1818.

Ims, R. A. 1988. Spatial clumping of sexually receptive females induces space sharing among male voles. *Nature, Lond.*, **335**, 541–543.

Ims, R. A. 1989. Kinship and origin effects on dispersal and space sharing in *Clethrionomys rufocanus*. *Ecology*, **70**, 607–616.

Jacobs, L. G., Gaudlin, S. J. C., Sherry, D. F. & Hoffman, G. E. 1990. Evolution of spatial cognition: sex specific patterns of spatial behavior predict hippocampal size. *Proc. natn. Acad. Sci.*, **87**, 6349–6352.

Jolly, G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika*, **52**, 225–247.

Keever, C. 1950. Causes of succession on old-fields of the Piedmont, North Carolina. *Ecol. Monogr.*, **20**, 229–250.

Kumari, C. L., Datta, J. K., Das, R. P. & Roy, S. 1978. Evidence for a role of androgens in the growth and maturation of ovarian follicles in rats. *Horm. Res.*, **9**, 112–120.

Lee, C. T. & Griffo, W. 1973. Early androgenization and aggression phenomena in inbred mice. *Horm. Behav.*, **4**, 181–189.

Levy, J. V. 1954. The effects of testosterone propionate on fighting behaviour in C57 BL/10 young female mice. *Proc. W. Virginia Acad. Sci.*, **26**, 14.

Lidicker, W. Z. 1976. Social behaviour and density regulation in house mice living in large enclosures. *J. Anim. Ecol.*, **45**, 677–697.

Lloyd, J. A. 1980. Interactions of social structure and reproduction in populations of mice. In: *Biosocial*

Mechanisms of Population Regulation (Ed. by M. N. Cohen, R. S. Malpass & H. G. Klein), pp. 3–21. New Haven, Connecticutt: Yale University Press.

Lloyd, J. A. & Christian, J. J. 1969. Reproductive activity of individual females in three experimental freely growing populations of house mice (*Mus musculus*). *J. Mammal.*, **50**, 49–59.

Lu, J., Hopper, B., Vargo, T. & Yon, S. 1979. Chronological changes in sex steroid gonadotropin and prolactin secretion in aging female rats displaying different reproductive states. *Biol. Reprod.*, **21**, 193–203.

Marler, C. A. & Moore, M. C. 1989. Time and energy costs of aggression in testosterone-implanted free-living male mountain spiny lizards (*Sceloporus jarrovi*). *Physiol. Zool.*, **62**, 1334–1350.

Massey, A. 1982. Ecology of house mouse populations confined by highways. *J. Elisha Mitchell Sci. Soc.*, **98**, 135–143.

Massey, A. & Vandenbergh, J. G. 1980. Puberty delay by a urinary cue from female mice in feral populations. *Science*, **209**, 821–822.

Michener, G. 1989. Sexual differences in interyear survival and lifespan of Richardson's ground squirrels. *Can. J. Zool.*, **67**, 1827–1831.

Moberg, G. P. 1987. Influence of the adrenal axis upon the gonads. In: *Oxford Reviews of Reproductive Biology, Vol. 9* (Ed. by J. R. Clarke), pp. 456–496. Oxford: Clarendon Press.

Mugford, R. A. & Nowell, N. W. 1971. The relationship between endocrine status of female opponents and aggressive behaviour of male mice. *Anim. Behav.*, **19**, 153–155.

Mugford, R. A. & Nowell, N. W. 1972. The dose-response to testosterone propionate of prepubital glands, pheromones and aggression in mice. *Horm. Behav.*, **3**, 39–46.

Naftolin, F., Ryan, K. J. & Petro, Z. 1971. Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. *Endocrinology*, **90**, 295–297.

Perrigo, G. & Bronson, F. H. 1985. Sex differences in the energy allocation strategies of house mice. *Behav. Ecol. Sociobiol.*, **17**, 297–302.

Phoenix, C. H., Goy, R. W., Gerall, A. A. & Young, W. C. 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, **65**, 369–382.

van de Poll, N. E., de Jange, F., van Oyon, H. G., von Pelt, J. & de Brun, J. P. C. 1981. Failure to find sex difference in testosterone activated aggression in two strains of rats. *Horm. Behav.*, **15**, 94–105.

Raynaud, J. P., Mercier, B. C. & Baulieu, E. E. 1971. Rat estradiol binding plasma protein (EBP). *Steroids*, **18**, 767–788.

Reimer, J. D. & Petras, M. L. 1967. Breeding structure of the house mouse, *Mus musculus*, in a population cage. *J. Mammal.*, **48**, 88–99.

Rowe, F. P., Taylor, E. J. & Chudley, A. H. J. 1963. The numbers and movements of house-mice (*Mus musculus* L.) in the vicinity of four corn-ricks. *J. Anim. Ecol.*, **32**, 87–97.

vom Saal, F. S. 1984. The intrauterine position phenomenon: effects on physiology, aggressive behavior, and population dynamics in house mice. In: *Biological Perspectives on Aggression. Vol. 169* (Ed. by K. Flannely, R. Blanchard & D. Blanchard), pp. 135–179. New York: Alan Liss.

vom Saal, F. S. 1989. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. *J. Anim. Sci.*, **67**, 1824–1840.

vom Saal, F. S. & Bronson, F. 1980. Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science*, **208**, 597–599.

vom Saal, F. S., Gandelman, R. & Svare, B. 1976. Aggression in male and female mice: evidence for changed neural sensitivity in response to neonatal but not adult androgen exposure. *Physiol. Behav.*, **17**, 53–57.

vom Saal, F. S., Quadagno, D., Even, M., Keisler, L. & Khan, S. 1990. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. *Biol. Reprod.*, **43**, 751–761.

Salvioni, M. 1988. Home range and social behavior of three species of European *Pitymys* (Mammalia, Rodentia). *Behav. Ecol. Sociobiol.*, **22**, 203–210.

Scott, J. P. 1966. Agonistic behaviour of mice and rats: A review. *Am. Zool.*, **6**, 683–701.

Seber, G. A. F. 1965. A note on the multiple-recapture census. *Biometrika*, **52**, 24–259.

Simon, N. G. & Gandelman, R. 1978. The estrogenic arousal of aggressive behavior in female mice. *Horm. Behav.*, **10**, 118–127.

Soares, M. & Talamantes, F. 1982. Gestational effects on placental and serum androgen, progesterone and prolactin-like activity in the mouse. *J. Endocrinol.*, **95**, 29–36.

Stevens, R. & Goldstein, R. 1983. Organisational effects of neonatal and pubertal testosterone on sexually differentiated behaviors in the open-field and head-dip apparatus. *Q. Jl exp. Psychol.*, **35B**, 81–92.

Suchowsky, G. K., Pegrassi, L. & Bonsignori, A. 1971. Steroids and aggressive behavior in isolated male and female mice. *Psychopharmacologia*, **21**, 32–38.

Svare, B. 1989. Recent advances in the study of female aggressive behavior in mice. In: *House Mouse Aggression. A Model for Understanding the Evolution of Social Behaviour* (Ed. by P. F. Brain, D. Mainardi & S. Parmigiani), pp. 135–159. New York: Harwood Academic Publishers.

Svare, B., Davis, P. B. & Gandelman, R. 1974. Fighting behavior in female mice following chronic androgen treatment during adulthood. *Physiol. Behav.*, **12**, 399–403.

Svendson, G. E. 1974. Behavioral and environmental factors in the spatial distribution of a yellow-bellied marmot population. *Ecology*, **55**, 760–771.

Taitt, M. J. & Krebs, C. J. 1985. Population dynamics and cycles. In: *Biology of the New World Microtus* (Ed. by R. Tamarin), pp. 567–620. Shippensburg, Pennsylvania: American Society of Mammalogists.

Tollman, J. & King, J. A. 1956. The effects of testosterone propionate aggression in male and female C57 B1/10 mice. *Br. J. Anim. Behav.*, **6**, 147–155.

Trobec, R. J. & Oring, L. W. 1972. Effects of testosterone propionate implantation on lek behaviour of sharp-tailed grouse. *Am. Midl. Nat.*, **87**, 531–536.

Varon, H. H. & Christian, J. J. 1963. Effects of adrenal androgens on immature female mice. *Endocrinology*, **72**, 210–222.

Wade, G. N. & Gray, J. M. 1979. Gonadal effects on food intake and adiposity: a metabolic hypothesis. *Physiol. Behav.*, **22**, 583–594.

White, M., Mayo, S. & Edwards, D. A. 1969. Fighting in female mice as a function of the size of the opponent. *Psychonom. Sci.*, **6**, 14–15.

Whitsett, J. M., Grenson, G. H., Peters, P. J. & Hamilton, T. H. 1972. Neonatal organization of aggression in mice: correlation of critical period with uptake of hormone. *Horm. Behav.*, **3**, 11–21.