The biology of *Rattus rattoides* with particular reference to the role of olfaction in reproduction - a laboratory study

By

Xu Zhongjian, M. Sc.

(Fujian Teachers' University, Fuzhou, P. R. China)

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This work is dedicated
with love to

my wife, Jin Yaping and son, Xu Xiangyu.

Contents

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Statement

I hereby state that the thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Signature: 

Xu Zhongjian

Date: 6/may-94.

Memorandum

This PhD project was commenced under an agreement between the University of Tasmania, Australia and Fujian Teachers' University, P. R. China that the two universities agreed to co-provide professional supervision to PhD candidates; that the PhD candidate should come to Australia to initiate the PhD programme (for a year) and should go back to China to do research (for two years), and return to Australia again in the fourth year to complete the dissertation.

After being conferred with an M. Sc. in Developmental Biology, Fujian Teachers' University, I was chosen by the University as a Ph D candidate under the agreement. I came to the University of Tasmania in April 1988 to start my Ph D programme in Olfactory Biology, under the supervision of Professor D. Michael Stoddart, an olfactory biologist. After a year's intensive literature research the project plan was designed in early 1989 and approved by Professor Stoddart. I went back in April, 1989 to Fujian Teachers' University in China to carry out the empirical work with the help of Professor Ding Hanbo and Associate Professor Zhang Jian, both developmental biologists.

Fujian Teachers' University granted a special fund to build a four-room laboratory for the project. While the construction was still under way in June, 1989, I started to trap rats in the field and brought them back to the half-finished laboratory. The laboratory was finally finished in September, 1989.

My initial project was to investigate the responses of the agricultural pest, *Rattus rattoides* in behaviour, growth, reproduction to the olfactory cues of its predators for the purpose of finding possible means to repel the rat from crops in certain stages so that to minimize the damage of the crops by the rat. The annual

gland secretion of weasel, *Mustela sibirica,* the urine from *Viverra indica, Viverra zibethia,* fox *(Vulpes vulpes)* and domestic cat *(Felis libyca domestica),* the mixture of urine and faeces of tiger *(Panthera tigris amoyensis)* and the faeces of python *(Python moburus)* were tested from September, 1989 to June, 1990. The primary results showed that the exudates of the above predators were not effective in causing behavioural depression in the species. The results were thoroughly discussed, when Professor Stoddart visited me in China from 4th - 11th of June, 1990, and a decision was made not to continue in this direction. Consequently the project shifted into the investigation of the olfaction in reproductive events such as oestrous cycle, the onset of puberty, the growth and the sexual development of the rat. All the experiments in the newly designed project were completed by June, 1991, after a year's intensive work.

During my two years the experiments in China, Professor Stoddart did his best to provide advice, to purchase RIA kits and send them to China for hormone test and even went to China twice to see the facilities at the first visit before any experiment was initiated and second time in a year later to examine the results by then and help make the wise decision of changing the direction of the research. My Chinese supervisors, Professor Ding Hanbo and Associate Professor Zhang Jian, also tried to give me as much help and encouragement as they could. I would, however, have enjoyed more regular and adequate supervision from Professor Stoddart if there had been no geographic barrier.

Abstract

Rattus rattoides is a pest widely spreading over south-eastern China. In Fujian Province it destroys one fifth of the crops (rice, wheat, sweat potato, peanut, sugar cane, banana and other vegetables) in the field every year. A good range of pesticides have been applied to minimize the pest's damage, yet its biology is hardly studied. This preliminary study is to investigate some aspects of reproductive biology and the roles of olfactory cues in the reproduction of this rodent.

All the experiments were carried out in Fujian, P. R. China during June 1990 to June 1991. The results of the investigations suggest that this species has the potential to be tamed as an experimental laboratory species and olfactory cues from an adult male or female play important roles in the growth and sexual development of young of the two sexes.

1. The average and the maximum body weights were examined. The growth rate of male and female rats before and after weaning were observed. No obvious difference has been found in body size and weight between sexes in adults of this species. The reproductive ability of the females in laboratory was found similar in different months with two low breeding rates in February and August.

2. The sexual maturation of the males developed in three stages: (a), the elevation of testosterone titre in blood; (b), the growth of the body and testes reached a plateau and mature sperm had been transported into the convoluted ducts; (c), the cessation of the growth of epididymes and the success of reproduction.

3. The timing of the onset of puberty (vaginal opening) of females was found to be variable when measured in different seasons, but the body weights at the time of attaining puberty were similar. The onset of puberty of the females was

accelerated by cohabiting with adult males or by the olfactory cues from adult males, and was delayed in the presence of olfactory cues from adult females.

4. Mean lengths of oestrous cycles of the adult females in different seasons were not significantly different from each other. Olfactory cues from adult males or housing under group conditions did not alter the lengths of the females' oestrous cycles.

5. Group-living significantly retarded the growth and sexual development of young males. Olfactory cues from adult males greatly promoted the growth and development of testes in young conspecifics.

Chapter 1. Literature review and the objectives

1.1. Introduction

There are about 3,940 species of mammals in the animal kingdom (Stoddart, 1979). Rodents, comprising almost half of the total, are found throughout the world from the highest latitudes to the tropics, where they exploit habitats as diverse as deserts, wetlands, grasslands, rainforests, and high mountain valleys (Bronson, 1989). Dietarily, while focusing mostly on plant parts, most rodents are somewhat omnivorous (Bronson, 1989). The result of this broad diversification is almost 1,700 species classified into over thirty families (Bronson, 1989). Of the total 1,690 species of rodents, 49.7% (840 species) belong to the family of Muridae (Walker, 1975), the best-known rodents so far (Bronson, 1989). Within this family, there are 570 species of *Rattus* and 15 species of *Mus* (Brown, 1985).

A huge amount of research work has been conducted on a limited range of species, particularly on laboratory strains of *Rattus norvegicus* and *Mus musculus* and *Mus domesticus* (Brown, 1985). By contrast, the biology of the majority of the rodents is hardly known.

It was not until late 1970s when olfaction in vertebrates, as an independent sub-discipline, emerged from among other disciplines involving sensory organs such as visual and acoustic sciences. The time of its birth was the First Symposium on Vertebrate Chemical Signals held in 1976 at Saratoga Springs, New York and the collection of the proceedings to the meeting, *Chemical Signals in Vertebrates* edited by Müller-Schwarze and Mozell (1977). The first conference exclusively discussing olfaction in mammals was held under the auspices of the Zoological Society of London in 1979 (Stoddart, 1980). Six symposia on vertebrate olfaction

have so far been held in different cities in the United States and the United Kingdom. Chemical Signals in Vertebrates I-VI containing the proceedings of the symposia have been published. Apart from that, several books on vertebrate olfaction are also available, *Mammalian olfaction, reproductive processes, and behavior* by Doty (1976); *The ecology of vertebrate olfaction* by Stoddart (1980); *Pheromones and reproduction in mammals* edited by Vandenbergh (1983); *Mammalian semiochemistry: the investigation of chemical signals between mammals* by Albone (1984); *Social odours in mammals* edited by Brown and Macdonald (1985).

Rodents are generally nocturnal animals. Apart from their sharp acoustic sense they usually have poor sight but a well-developed olfactory system. Because both the acoustic and olfactory senses are vital to them in their life, their sense of olfaction and the ability to spread their distinctive odours are more useful not only for the detecting of prey or predators but also for the utmost strategy, reproduction.

During reproductive seasons rodents depend heavily on the odorants spread in their environment by their conspecifics to locate mates (Johnston, 1983), to determine the receptivity of the opposite sex (McClintock, 1983), to adjust the behaviours of both of the counterparts until eventually copulation takes place (Johnston, 1983). Without olfactory cues none of the processes could occur among the rodents. Since olfaction plays a most important role in the reproduction of small mammals it has been intensively studied during last three decades since the emergence of the discipline. Many aspects of reproduction, such as the roles of olfaction in sexual recognition and courtship scent marking (Johnston, 1983), in the synchrony of oestrous cycles (Whitten, 1956; McClintock, 1978; 1983), in the acceleration or delay of puberty (Vandenbergh, 1967; Drickamer, 1974a; 1974b;

1986), in pseudopregnancy (Lee-and Boot, 1955; 1956) and in pregnancy blockade (Bruce, 1959), have been thoroughly studied and well documented.

Recently, research into the olfactory biology of mammals has extended to the genetic and chemical aspects of individual odours with fundamental functions in sexual recognition and mating preferences (Andrews and Boyse, 1978; Brown, 1979; Halpin, 1986; Yamazaki *et al.*,1976; Yamazaki *et al.*,1978; Yamazaki *et al.*, 1988), in the induction of puberty in juvenile females (Lendrem, 1985), and in pregnancy blockade (Yamazaki *et al.*, 1983). Research in this field involves a combination of techniques as well as knowledge of molecular genetics and olfactory biology. The marriage of traditional ecological and behavioural studies and modern molecular-genetics will doubtless bring tremendous benefit to the development of olfactory biology.

1.2 Rodent olfactory biology - An overview

Rodents, as mentioned above, depend heavily on olfactory communication in feeding, foraging, and reproduction. To achieve these they use urine, faeces, specialized scent glands, and vaginal secretions as the vectors of messages. They do this by means of urine-marking, urine-spraying, anogenital dragging, sand bathing, and scent-gland rubbing (Brown, 1985). The advantages of sending messages through scents, compared with other ways of communication, such as visual and acoustic, are that they remain effective even after the sender is absent (Bronson, 1976; Johnson, 1973; Wilson, 1975) and the message can be carried quietly to distance in darkness (Wilson, 1975; Bronson, 1976). It is therefore an efficient and economic way of exchanging important information.

A large amount of investigation has by now been conducted in uncovering the

roles of olfactory cues in rodent reproduction - a phase in the population development which is both long and sees individuals at their most vulnerable (Vandenbergh, 1983).

Van der Lee and Boot (1955) discovered that if female mice were caged in groups the suppressive effect among the conspecifics increases the cyclic length of their oestrus. This was subsequently termed Lee-Boot effect. The suppressive effect can be abolished by ovariectomy and restored by oestradiol implants (Clee *et al.*, 1975), indicating that the cues which prolong the cyclic length are ovary-dependent. The suppressive effect in female mice can also be abolished and normal cycling restored with the presence of a male conspecific mouse (Whitten, 1956), the well known Whitten effect. A recently inseminated female mouse will return to oestrus and be remated if she encounters a strange male or its urinary odour (Bruce, 1959; Bruce and Parrott, 1960), the so-called Bruce effect. The urinary odour from adult male mice, particularly socially dominant mice, can accelerate the attainment of puberty in female mice (Castro, 1967; Vandenbergh, 1967), the Vandenbergh effect (Stoddart, 1980). Exposing female mice to male chemosignals prior to mating reduces the latency and increases the duration of contact with males. It also increases the litter size (Fan and Vandenbergh, 1991). Increasing densities can delay the onset of puberty in natural, semi-natural and laboratory populations of house mice (Coppola, 1986; Coppola and Vandenbergh, 1985; Lidicker, 1976; Massey and Vandenbergh, 1980). Female mice housed in groups reach sexual maturity later than those caged alone (Castro, 1967; Vandenbergh *et al.*, 1972; Drickamer, 1974b). The puberty can also be delayed by exposing immature female mice to urine from group-caged adult females (Cowley and Wise, 1972; Drickamer, 1982). Population growth as well as female puberty attainment in semi-natural

enclosures can be slowed with treatment of urine from grouped adult females and accelerated with treatment of urine from adult males relative to control enclosures treated with water (Drickamer, 1991).

The fast growth of olfactory biology in last three decades provides detailed information which helps understanding population dynamics of rapidly breeding rodents and hence promoting investigation into pest management options. It may also suggest techniques for increasing the yield from stock species (Fan and Vandenbergh, 1991).

1.2-1. Prey and predator

1.2-1-1. The responses of the prey to the exudates of the predator

Animals tend to avoid predators or areas odorized by predators (Dickman and Doncaster, 1984; Stoddart, 1976; 1982a; 1982b; Sullivan and Crump, 1984) . Olfactory cues from predators can cause stress responses in the prey. Laboratory rats "freeze" when they are exposed to the body odour of a cat (Griffith, 1920). The evidence of the primary role of olfaction was provided by Griffith (1920), who confirmed that rats escape from a cat's presence and do not rely upon visual stimulation for when the cat was in a glass jar the rats paid no attention to the cat and its movements. When the rats were put in an enclosed space which had previously contained a cat, however, they responded to the odour by "freezing". The "fear" responses of a rat to a confined cat were reported by Mollenauer *et al.* (1974), further supporting the discovery of Griffith (1920). The responses included almost total suppression of drinking and long periods of freezing, broken by brief bursts of high-speed activity, whereas olfactory bulbectomized rats showed neither freezing nor suppression of drinking.

The presence of weasel, *Mustela nivalis* (Stoddart,1976; 1980) or stoat, *M. erminea* (Gorman, 1984) anal gland secretion or tiger urine, *Felis tigris* (Stoddart, 1980; 1982) can substantially reduce the number of captures, hence obscuring the estimation of population size of a prey species. The odour of a familiar predator (weasel) appears to be comparatively more effective than the odour of an unfamiliar predator species, such as a tiger (Stoddart, 1980). These results cannot be induced by a novel non-predator odour; eg. the odour of rabbits (*Oryctolagus cuniculus*) causes no change in the number of captures (Stoddart, 1980). An earlier experiment also supported the view that the prey's response was to the odour given specifically by a predator but not to any novel odour. Courtrey *et al.* (1968) reported that the odour of cats in a 7-foot runway decreased the running time of test rats, but another non-predator odour, "Ban" deodorant, did not.

Voles tend to avoid the odours of stoat (Gorman, 1984) or shrews in the wild (Fulk, 1972). They also avoided shrew odour as well as a shrew *per se* in a laboratory test (Fulk, 1972). In the same experiment, Fulk found that the utilization of the nest boxes with shrew droppings developed in three phases: avoidance, exploration and normal use. The habituation of prey to predator odour is demonstrated here, though shrew is only an occasional predator of voles. The tendency of sensory adaptation was also reported by Gorman (1984) in voles.

In a series of studies, Sullivan *et al*(1985a; 1985b) have tested the effect of the odours of faeces or urine from natural predators on the feeding behaviours of some herbivores. The consumption by snowshoe hare (*Lepus americanus*) of willow browse and coniferous seedlings was significantly reduced by lynx and bobcat faeces and weasel anal gland secretions, and also by urine from lynx (*Lynx canadensis*), bobcat (*L. rufus*), wolf (*Canis. Lupus*), coyote (*C. latrans*), fox

(*Vulpes vulpes*) and wolverine (*Gulo gulo*). Faeces of coyotes and wolf and urines of coyotes, wolf, fox, wolverine, lynx and bobcat again provided effective suppression of black-tailed deer (*Odocoileus hemionus columbianus*) feeding damage. Of them all, coyote urine provided the most consistent suppression of deer browsing on salal. Wolverine urine was excellent in protecting lodgepole pine seedlings in field bioassays during spring and autumn, even though the odour samples were not protected from adverse weather conditions (Sullivan, 1986).

It was proven that the feeding suppressions were not caused by the novelty of odours but by special odorants from predators, for the feeding behaviour was not suppressed by dog urine (Sullivan *et al.*, 1985a), deer urine and hare blood (Sullivan, 1986), human urine or ammonia odour (Sullivan *et al.*,1985b).

1.2.1-2. Synthetic analogues of the functioning compounds in the exudates from predators and field bioassay

Logically the next step, after knowing that predator exudates can depress a prey's feeding behaviour, is to extract, to identify, and to synthesize the effective components of those exudates for application purpose.

Fox droppings have been reported to be effective in the suppression of the activity of laboratory rats (Vernet-Maury, 1980; Vernet-Maury *et al.*,1984). Nine compounds in fox faeces have been shown to be able to induce stress in rats (Vernet-Maury *et al.*,1984): a dihydrothiazole, two cyclic polysulfides, five mercaptoketone, and a mercaptan. From the results of bioassay the synthetic analogues of the identified compounds, 2,5-dihydro-2,4,5-trimethyl-thiazole, 3,3-dimethyl-1,2-dithiolane and the mix of *cis*-and *trans*-8-mercaptomenthones turned out to be very effective in repelling rats in the laboratory.

One of the first practical demonstrations of the use of predator odour in crop protection was reported by Sullivan and Crump (1984), in which, synthetic components of stoat anal gland secretion, 3-propyl-1,2-dithiolane and 2,2-dimethyl-thielane from mink (*M. vison*) were very effective in depressing feeding behaviour of snowshoe hares. The principal compound of fox urine, 3-methyl-3-butenyl methyl sulfide, had similar effect (Sullivan and Crump, 1986). Gophers (*Thomomys talpoides*) clearly avoided 2,5-dihydro-2,4,5-trimethylthiazoline, a component of fox faeces but did not avoid 3-methyl-3-butenyl methyl sulfide from urine in laboratory bioassays. The effect of 3,3-dimethyl-1,2-dithiolane, however, was not that strong in the rat (Sullivan *et al.*, 1986b, Vernet-Maury *et al.*, 1984). Stoat anal gland excretion, 2-propylthietane and 3-propyl-1,2-dithiolane, significantly reduced vole captures when they were mixed (1:1), while a compound of fox faeces, 2,5-dihydro-2,4,5-trimethylthiazoline did also reduce vole captures significantly. Deer mice, however, did not show any negative response to any predator odours tested (Sullivan *et al.*, 1988a). In a five-month field bioassay over the winter, both the mixture of 2-propylthietane and 3-propyl-1,2-dithiolane from stoat and 2,5-dihydro-2,4,5-trimethylthiazoline significantly reduced the attack by two species of vole (*M. montanus* and *M. pennsylvanicus*) in respective orchard blocks. The feeding damage, in terms of the amount of bark and vascular tissues removed from trees, was reduced by 60-97% (Sullivan *et al.*, 1988a).

There are obviously some differences in responses to various compounds of predator exudates among different species but the general goal is that one or more components of a predator's exudates have the effect of protecting vegetation from a certain species of herbivore. It can be expected, upon this basis, that a suitable way of protecting crops from feeding damage by herbivores such as deer and rodents

could be produced by means of synthetic chemicals used as repellents, after the problems of the durability of the repellents in nature and the proper way of delivering them can be solved.

1.2-2. Scent marking and preference bias in reproduction

Scent marking in rodents has multiple functions, such as species recognition, individual identification, social status discrimination, sexual recognition, and reproductive advertisement (Johnston, 1983). Since the major part of the adulthood of most rodents is spent in reproductive activities, e.g. mating, pregnancy and lactation, most scent-marking behaviours are for the advertising of receptiveness and sexual arousal.

The vehicles for the reproductive message of females vary with species. It is believed, however, that the most likely sources of reproductive advertisements are urine, which contains the metabolic products of many hormones and the secretions of the reproductive organs. Other sources can be the secretions of reproductive organs like the vagina or specialized glands, such as preputial glands and flank glands (Johnston, 1983).

Scent-marking frequency is often highest during the reproductive season (Ewer, 1973; Johnston, 1983). Obviously the purpose of this behaviour is to advertise reproductive status and to attract the opposite sex. In the rat, both female and male preputial gland secretions are attractive to the opposite sex (Stanley and Powell, 1941). The saline homogenate of the rat preputial gland contains a substance which is attractive to the opposite sex, but the other tissues, such as submaxillary-sublingual glands and foot pads, do not produce any behavioural change at the same homogenate concentration (Orsulak and Gawienowski, 1972;

Gawienowski *et al.*, 1975). Sexually experienced males prefer the odour of receptive females to that of non-receptive ones, whereas sexually naive males show no reliable preference (Carr *et al.*, 1965; 1970; Hayashi and Kimura, 1974). Females, regardless of their sexual experience, prefer the odour of intact males to that of castrated ones (Carr *et al.*, 1965). Similarly, female rats prefer the odour of the preputial extract of intact male to that from castrated rats but do not respond to the odour of female preputial extract (Gawienowski *et al.*, 1975).

In the mouse, the secretions of preputial glands of males exerted an attractant effect on the female (Bronson and Caroom, 1971). Female mice become more active when in the presence of odour drawn from a cage of males (Ropartz, 1968, reviewed in Bronson, 1971). Female mice also prefer urine collected from intact males to that obtained from castrates (Scott and Pfaff, 1970). It was demonstrated that, regardless of their reproductive status, intact naive female mice showed a preference for normal males over castrated ones (Hayashi and Kimura, 1973), and postnatal exposure of male odour will help the development of the female's preference for males in adult life (Hayashi and Kimura, 1978).

In lemmings, both sexually experienced and naive male brown lemmings (*Lemmus sibiricus*) and collared lemmings (*Dicrostonyx groenlandicus*) prefer the odour of conspecific females to the odour of males. Sexually experienced males also show significant preference for oestrous over non-oestrous conspecific females (Huck and Banks, 1984). Furthermore, sexually experienced males prefer the odour of an unmated receptive female to that of a female that has recently copulated with another male (Huck, *et al.*, 1984). A male lemming can discriminate between the odour of bedding from a novel oestrous female and the odour of bedding from the female with which he has just copulated even when the bedding is collected before the mating occurs (Huck, *et al.*, 1984).

Sexually experienced male mice prefer the odour of a female in oestrus, and the vaginal smear or vaginal tissue from oestrous females, whereas sexually naive males showed no preference (Hayashi and Kimura, 1974). Male hamsters (*Mesocricetus auratus*) are more attracted to intact females than to vaginectomized females when contact was prevented (but not when contact was allowed), indicating that the presence of vaginal secretion is necessary to attract males from distance (Kwan and Johnston, 1980).

Above all, during reproduction, male scent marking is to lure the female into endocrinal and behavioural receptivity for copulation, whereas female scent marking is to advertise of her receptiveness, to attract males to investigate her readiness and to copulate. No matter what species they are and what sources of scent they use to mark around the purpose is common and simple -- to meet with the opposite sex and to mate.

1.2-3. Pregnancy blockade

Bruce (1959) was the first to report that an encounter by a newly mated female with a "strange" (*i.e.* not the stud) male resulted in a pregnancy failure and a new mating within 3-6 days (Bruce effect).

A large body of investigation into the phenomenon of pregnancy blockade has subsequently followed. Male mice of a different genetic strain are more effective than the males from the same strain of the pregnant females in inducing the Bruce effect (Bruce, 1959). Inadequate physical conditions of housing also result in the pregnancy failure (Bruce, 1963). Physical contact of the strange male with the pregnant female is not necessary to produce the blockade (Bruce, 1960), and furthermore, anosmic females do not show the Bruce effect (Bruce and Parrott,

1960), implying that it is olfactory cues that actually induce the phenomenon. Newly mated female mice placed in proximity to alien males (Bruce, 1960) or into boxes soiled by alien males for up to 3 days can also produce the blockade, suggesting that the odorous substances involved are highly evanescent (Parkes and Bruce, 1962). The pregnancy block was also observed when females were exposed to fresh male urine (Dominic, 1971). The pregnancy-block pheromone is supposed to associate with androgens either directly, or indirectly through some androgen-dependent gland (Dominic, 1965), and it is a peptide or a substance bound with the peptide fraction (Marchlewska-Koj, 1977; 1981). The site of the pheromone production, however, is unknown.

The Bruce effect is found not only in laboratory house mice but also in wild house mice (Chipman *et al.*, 1966) and other wild rodents, such as field voles, *Microtus agrestis,* (Clulow and Clarke, 1968; Milligan, 1976a; 1976b), prairie voles, *Microtus ochrogaster,* (Stehn and Richmand, 1975), and meadow voles, *Microtus pennsylvanicus,* (Clulow and Langford, 1971; Clulow and Mallory, 1974); deer mice, *Peromyscus maniculatus,*(Birdsall and Nash, 1973; Terman, 1969); and collared lemmings, *Dicrostonyx groenlandicus,* (Mallory and Brooks, 1980).

All those researches were carried out in the laboratory. The expression of the pregnancy block phenomenon under natural conditions and its possible adaptive significance are poorly understood (Marchlewska-Koj, 1983).

Several explanations for the evolution of the Bruce effect have been postulated. Chipman *et al.* (1966) hypothesized that females living in high density populations would probably encounter strange males for brief periods, and the Bruce effect could reduce reproduction and suppress population growth. Rogers

and Beauchamp (1976) speculated that pregnancy blocking would minimize gene flow between demes; by terminating the pregnancy of a female immigrant, resident males could prevent the incorporation of genes from an unrelated litter into their reproductive unit. By terminating the pregnancy of a recently inseminated female a male can prevent postparturitional parental investment in genetically unrelated pups and guarantee that her offspring will carry his genetic material (Barash, 1977; Dewsbury, 1978; Wilson, 1975). Although these hypotheses are apparently acceptable, a question has been raised (Wilson, 1975; Bronson; 1979; Bronson and Coquelin; 1980) as to how pregnancy blockade could have evolved since the reproductive success of females can hardly be enhanced by the loss of their offspring. What the Bruce effect really means in terms of modifying reproduction of natural populations is still unknown and, therefore, intensive studies in natural populations as well as in the laboratory strains are necessary for a final conclusion.

1.3 Oestrous cycles

An oestrous cycle includes three stages. The first stage is that of follicular development, and is characterised by a vaginal smear rich in cornified cells caused by estrogen (Nequin *et al.,* 1979). After that, ovulation occurs, with a transitional vaginal smear of cornified epithelium cells and leukocytes (Lamond, 1959; Schwartz, 1973). The last stage of the oestrous cycle is a long phase of corpus luteum with a vaginal smear dominated by leukocytes caused by high progesterone concentrations (Parkes, 1929). If the female is not inseminated by a male, the cycle returns to the beginning of follicular development. Normally, it takes 4-6 days to complete a cycle in laboratory mice (Allen, 1922; Parkes, 1928). The duration of each of the stages can be shortened or lengthened by social or environmental cues, thus causing the enhancement or suppression of an oestrous cycle (McClintock,1983; Vandenbergh,1986). The cues can be chemosignals emitted by conspecifics as well as contact between conspecifics and physical factors such as temperature, food, photoperiod, among others (Emlen and Demong, 1975; Kiester and Slatkin, 1974).

Among these various cue types, that of chemosignals is the goal of olfactory biology and has been intensively studied during last few decades. The oestrous cycle has been shown to be enhanced by chemosignals from males (Whitten, 1956), suppressed both by caging females in dense groups and by isolating them from the odours of males (van der Lee and Boot, 1956; Muhlbock, 1958) and by the treatment with the urine from pregnant or lactating females (Hoover and Drickamer, 1979).

1.3-1 Oestrus enhancement (i .e. shortening of the cycle)

The Whitten Effect was named after the discovery by W. K. Whitten (1956, 1958) that the oestrous cycles of the mouse will be enhanced in the presence of a male mouse or his urine.

It has been demonstrated that the effective factor is contained in male's urine (Bronson and Whitten, 1968; Marsden and Bronson, 1964; Whitten, 1966). The induction of oestrus is androgen-dependent (Bronson and Whitten, 1968). The authors found that the shortening effect of oestrous cycling was abolished when the male urine donors were castrated or the female urine odour recipients were androgenized. Chipman and Albrecht (1974) postulated that preputial secretion is at least partly responsible for the enhancement of oestrous cycles; they found that the urine from preputialectomised males was significantly less effective than urine from intact males although it was slightly more effective than urine from castrated males. Bladder urine had the same effectiveness in inducing oestrus as externally collected urine (Bronson and Whitten,1968). This oestrous-inducing cue was assumed to be species-specific because urine from conspecific males accelerated the attainment of oestrus but the urine from different species did not (Marsden and Bronson, 1965).

Whitten *et al.* (1968) further reported that the affective agency in male urine was volatile and it acted through olfactory receptors of the recipient. They ascertained this by putting a group of 15 males in a wire cage in the middle of a 6-meter-canal, one group of females at each side of the canal 2-meters apart from the males' cage, and another group of females directly under the male's cage, thus exposing them to the males' urine. The fourth group then was put in a place remote from the males. All females were also housed in wire cages. By an air flow of 6m/min, the males' odour was carried downwind to the females. Within 4 days of

testing, 33 out of 47 females downwind and 37 out of 44 under the male's cage were in oestrus, whereas only 17 out of 49 females upwind showed oestrus. The proportion of females in the downwind group exhibiting oestrus was significantly greater than that of the group upwind, but was not significantly less than that in the group directly under the males' cage. The proportion of females in oestrus in the upwind group was about the same as that in the group in the remote place. These results were confirmed by Gangrade and Dominic (1984), who induced the Whitten effect in grouped female mice by exposing them to grouped males, and also to grouped males housed within a perforated cage which prevented physical contact of the females with the males.

After comprehensive studies of the volatile components of mice urine, Schwende *et al.* (1986) were able to identify two structurally unique substances [2-(*sec*-butyl)thiazoline and dehydro-*exo*-brevicomin] in association with the intact male. Jemiolo *et al.* (1986) then verified that the synthetic analogues of these two constituents of the urine from male mice are just as effective as normal male urine in bringing about the Whitten effect.

1.3-2 Oestrus suppression

The phenomenon of oestrus suppression has been intensively studied in mice. As early as the late 1930s (Merton, 1938) and early 1940s (Andervont, 1944), it was reported that house mice (***Mus musculus***) living in groups displayed prolonged oestrous cycles. Bloch (1955) described constant oestrus in albino mice; van der Lee and Boot (1955; 1956) observed the frequent occurrence of prolonged dioestrous intervals in mice housed four to a cage and presented evidence to show that these delays resulted from pseudopregnancy. Similar prolonged cycles have

been recorded by Lamond (1958). Cessation of oestrous cycles has been observed by Whitten (1957; 1959) in mice housed in large groups (30/cage). He recorded that when female mice were caged in groups of thirty, regular oestrous cycles did not occur in the majority of individuals. The vaginal smears of these mice were mucified and some remained so for 40 days. Cycles promptly returned when the mice were caged individually. It is very clear, here, that grouping mice at high density will suppress oestrus in adult females. Furthermore, Whitten discovered that regular oestrous cycles did not occur in blind mice when they were grouped or in mice when they were separated by partitions in the cage. A large cage was subdivided by either solid or perforated partitions into thirty small compartments. Inhibition occurred with both forms of partition, though it was less than in the undivided cage. This indicates that visual or tactile stimuli are not necessary for the suppression to occur and it is airborne cues which brings about the effect. Champlin (1971) also reported that oestrous cycle frequencies of two test laboratory strains decreased with increasing cage density (1-8/cage). The suppression took the form of a relative lengthening of the dioestrus phase of the cycle. On finding that the suppression could be initiated by housing single females in cages recently soiled by groups of females, Champlin postulated that a pheromone might be the stimulus for the suppression. Hoover and Drickamer (1979) treated singly caged female mice with urine from pregnant or lactating females by means of perforated capsules placed in the cage of the test mouse. The test mice showed longer periods of oestrus than controls, which were treated with either water or urine from singly caged females. This result also indicates that the factor responsible for the longer periods of oestrus is an airborne one.

Despite this clear indication there is no report of the chemical structure of the volatile substance(s) which induce the suppression of oestrous cycles, although

volatiles which delay puberty have been identified and the synthetic analogs have been checked and verified for biological activity (Jemiolo,1986; Novotny *et al.*, 1986).

It was noted that the mutual suppression of ovarian cyclical activity was largely dependent upon the ovaries of the suppressing mice and that the suppressive effect lost in ovariectomized mice could be restored by the injection of oestradiol (Clee *et al.*, 1975; Paudey 1985). Paudey (1985) reported that when females were exposed to spayed and oestrogenised females, they suffered an oestrus block. He thus assumed that the oestrus-suppressing pheromone was dependent on estrogen.

The phenomenon of oestrus suppression has also been reported in deer mice, *Peromyscus maniculatus* Bairdii (Lombardo and Terman, 1981), Indian field mice, *Mus booduga* Gray (Dominic and Pandey,1979), wild hopping mice, *Notomys alexcis* (Breed, 1976), prairie vole, *Microtus ochrogaster* (Getz *et al.*, 1983), California vole, *Microtus californicus* (Batzli *et al.*, 1977), field vole, *Microtus agrestis* (Milligan, 1974). In rats *Rattus norvegicus* (Aron, 1973; McClintock, 1981; Roser and Chateau, 1974) and *Sigmodon hispidus* (Evans and McClure, 1986) and guinea pig, *Cavia porcellus* (Jesel and Aron, 1974). However, the effect of group living is opposite to that of mice; group living shortened, instead of lengthened, the estrous cycles.

1.3-3 Oestrus synchrony and mechanism

The ovarian cycle of mammals consists of, as mentioned above, three phases which repeat spontaneously: follicular development, ovulation, and formation of the corpus luteum. The time for follicular development can either be lengthened or shortened; once the follicles are mature, the time of ovulation itself can be delayed

or advanced; finally, the life span of the corpus luteum can also be lengthened or shortened. Consequently, the oestrous cycle can be enhanced or suppressed by modulating each of the three phases (McClintock, 1983).

Synchronization of oestrus results from mutual entrainment of a set of opposite pheromones; one phase delays the cycle and the other phase advances it (McClintock, 1983). Synchronization occurs more rapidly when these two opposing signals follow quickly one after the other (Winfree, 1980). As a matter of fact, synchronization of oestrus is the result of phase advance (enhancing) or phase delay (suppression) of the cycles.

Although it is not necessary for every enhancement or suppression to result in synchrony, it has to be considered as the consequence of these events whenever synchrony is discussed.

The mechanism of synchronization of the oestrous cycles in the mouse cannot be explained in the same way as it can in the rat (McClintock, 1983). In mice, females appear to produce signals that only suppress the estrous cycle, whereas in rats, females produce two opposite signals, one advances and the other delays the phases. The mechanism of advance or delay of oestrus, nevertheless, is still not understood, though some workers have addressed it.

Odour from females in follicular phase (from dioestrus to proestrus) enhanced the cycle of other females in group, while odour from females in ovulatory phase (from proestrus to metoestrus) had the opposite effect, or suppressed the cycle. Odours from the luteal phase (from metoestrus to dioestrus) did not alter the timing of the oestrous cycle significantly (McClintock, 1983). This is true, however, only in rats.

By contrast, female mice produce suppressive chemicals which lengthen the

dioestrous phase by prolonging the lifespan of the corpus luteum (Ryan and Schwartz, 1977). van der Lee and Boot (1955) originally hypothesized that the increase in cycle length was mediated by an increase in prolactin secretion, whereas Whitten (1959) postulated that the lengthening of dioestrous phase was the result of an increase in secretion of follicular-stimulating hormone (FSH). It was demonstrated later that the corpus luteum was maintained by a combination of prolactin and FSH (Choudary and Greenwald, 1969). An increase of prolactin secretion is more likely to be part of the primary ovarian response to group living in mice (Bronson, 1976). When ovariectomized and low-dose estrogen-treated females lived together, their prolactin levels were increased. In addition, treatment with a dopamine agonist (bromocriptine) that lowers prolactin levels blocked the suppression of oestrus (Bronson, 1983).

The phase of the cycle at which the females are grouped is also important in producing the effect of oestrus suppression. Grouping females at oestrus was found to be most effective for the suppression of the following oestrous cycle to occur. In contrast, grouping females just before ovulation, at proestrous stage, did not alter the timing of the next oestrous cycle (Ryan and Schwartz, 1977).

The varieties in response may result from changes in the endocrine state and sensitivity of the recipient, such as during oestrus when fresh corpora lutea are developing, estrogen may have increased olfactory sensitivity in the rat (Pietras and Moulton, 1974). The varied responses could also result from changes in the endocrine state of the females that are producing the signal (McClintock, 1983).

A number of other treatments are known to influence the lengths of the oestrous cycle. Preputialectomy attenuated oestrus suppression (Chipman, 1969). Excision of the vomeronasal organ reduced the number of females with delayed

oestrous cycles (Reynolds and Keverne, 1979). Ablation of the olfactory bulbs prevented spontaneous pseudopregnancy in grouped females (Whitten, 1956; Mody, 1963). Ovariectomy attenuated the abilities to suppress oestrous cycles in other females (Chipman, 1967; Clee, Humphreys, and Russell, 1975). Ovariectomy did not, however, completely abolish the suppressive effect (Chipman, 1967; Kimura, 1971), suggesting that there might be other effective signals present.

1.3-4 The determination of oestrus phases

The determination of oestrous phases is a crucial element in any study which needs to measure the effect of odours from males or females on the reproduction of other females.

Generally speaking, there are two ways for determining oestrous stages (Whitten and Champlin, 1978). One is to take a vaginal smear, a widely accepted procedure for determining the stages of the oestrous cycle in rodents, following its initial use in a study of guinea pig reproduction by Stockard and Papanicolaon in 1917. The other way is to examine the gross changes in the appearance of vagina during oestrus. This technique was first mentioned by Allen (1922) and further developed by Champlin, Dorr, and Gates (1973).

The technique of vaginal smear depends upon the changes of proportions of different type of cells (i.e. leukocyte, nucleated or cornified epithelium cells) in the vaginal wall. The judgement is made according to the criteria of Rugh (1968) and Vandenbergh (1969). The usual procedure is by scraping the dorsal vagina wall with a tooth pick, or smooth spatula (Whitten, 1958), or silver loop, or glass rod (Fan, 1988), or by vaginal lavage with a cotton swab (Emery and Schwarbe, 1936), or by rinsing a pipette in the vagina (Rugh, 1968).

The smear is either stained, air dried and left unstained (Whitten and Champlin, 1978), or wet-mounted (Drickamer, 1986) before microscopic examination.

Gross changes in the appearance of the vagina were observed in the mouse, and described as the judgement of oestrous stages (Allen, 1922). This method has been further developed by Champlin *et al.*, (1973) and Whitten and Champlin (1978). The advantage of this method is that it is a visual method and does not require inserting a device into the vagina which might stimulate copulation and initiate the related neuroendocrine changes. The visual method uses the changes in the size of the vaginal opening, the degree of vaginal swelling, the colour, the moisture of the tissues and the presence or absence of obvious cellular debris within the vagina to determine oestrous stages. This technique, however, is not as popular as the method of vaginal smearing nor has it been used in species other than albino mice, probably because it depends too much on the experimenter's experience.

A graphical method was established by Fox (1985) to estimate the length of oestrous cycles. It is based on the observation of intervals between births and the gestation lengths. By applying the observed values into a regression model the length of the oestrous cycle can be calculated. This method is particularly useful when a researcher only wants to know the lengths of the oestrous cycles of a species and does not want to know specifically in which stage of the cycles the test individuals are.

In this study the technique of vaginal lavage analysis was used, with care being taken to avoid artefacts introduced during the lavage procedure itself. A drop of distilled water was sucked into a pipette and the dorsal vaginal wall was lavaged 2-3 times gently without actually touching the wall with the tip of the pipette. The

water drops with the cells washed down from the vaginal wall were then sucked into the tip again and smeared onto a clean slide marked with the individual's number. The wet-mounted slides were then examined under microscope to determine the stages of oestrous cycles.

1.4 Puberty acceleration and delaying

Vandenbergh tested the influence of the presence of an adult male mouse (1967) and the urine from adult male mice (1969) on the onset of sexual maturation of female mice. The results show that both adult male mouse and the urine from adult male mice accelerate the onset of sexual maturation in young female mice (the so-called Vandenbergh Effect, Novotny *et al.*, 1980). Much work on this aspect was initiated and the details of the influence on sexual development of young mice or rats by the pheromones contained in the urine from male or female conspecifics have been uncovered through elaborate and fruitful work conducted by olfactory biologists. Almost all work on the acceleration and delaying of puberty in either male or female rodents has been focused on laboratory mice. The literature cited here, therefore, is exclusively on mice, particularly on the house mouse, *Mus domesticus.*

1.4-1. Puberty in females

1.4-1-1. Puberty acceleration in females

Most of the reports, as far as the influence of external pheromones on the onset of puberty of young female mice is concerned, are on the acceleration effects of pheromones. The sexual maturation of young female mice can be easily accelerated by contact between the females and adult males (Bronson and Maruniak, 1975; Drickamer, 1974a; 1975c). It is not only the physical contact but also and more importantly, the pheromones present in the urine of the adult male mice which account for the acceleration of puberty in female young mice. In the absence of male mice, females also have an earlier puberty, when provided with the male's urine

(Colby and Vandenbergh, 1974; Cowley and Wise, 1972).

As early as 1967, two separate researchers, Castro (1967) and Vandenbergh (1967) reported simultaneously that the onset of puberty in females could be accelerated by male stimuli. Vandenbergh (1967) noted that groups of female albino mice reared from 21 days of age with an adult male showed earlier vaginal opening, first oestrus, and first mating than females housed in groups without males. Female mice housed from 21 days of age in a cage which separated them by a wire-mesh screen from adult males had their vaginal opening and first oestrus accelerated almost as much as females housed with adult males (Vandenbergh, 1969). The first oestrus of young females on male-soiled bedding from 21 days of age or from birth to 21 days of age was also enhanced (Colby and Vandenbergh, 1974; Cowley and Wise, 1972; Fullerton and Cowley, 1971; Vandenbergh, 1969).

Not only can the presence of adult males or the males' urine accelerate the onset of puberty in young female conspecifics, but also the urine from pregnant and lactating females and the urine from females in oestrus can induce earlier puberty (Drickamer, 1982c; 1984b; 1986c; Drickamer and Hoover, 1979).

Twenty-one-day-old female mice exposed to the urine of pregnant or lactating females showed accelerated puberty attainment when compared with control mice exposed to water or urine from cycling females (Drickamer and Hoover, 1979), although puberty was not as early as females exposed to urine from adult males (Drickamer, 1982b). The onset of puberty in female mice exposed to the urine of singly caged females in oestrus was earlier than that of those exposed to the urine from singly caged females in dioestrus and that of the females exposed to water (Drickamer, 1982c). The urine from group-caged but socially dominant female mice in oestrus had a similar effect (Drickamer, 1986c).

It is apparent that puberty-accelerating pheromones (PAPs) exist in both bladder and avoided urine of adult male mice (Colby and Vandenbergh, 1974; Kennedy and Brown,1970; Vandenbergh, 1969). Not all males or females, however, can excrete PAPs into their urine. Urine from low-ranking or subordinate male mice, for instance, has no puberty-acceleration effect (Drickamer, 1983a; Lombardi and Vandenbergh, 1977). Prepubertal males or castrated males release no PAPs into their urine (Drickamer, and Murphy, 1978; Lombardi *et al.*, 1976; Vandenbergh, 1969). Only those males which are dominant in a deme or presumably living alone can produce urine possessing PAPs (Drickamer, 1986a). There are, nevertheless, no seasonal changes, no density effects and no differential kinship effects associated with PAPs in excreted urine under constant environmental conditions; Table 1.4-1 (Drickamer, 1986a).

PAPs in adult male urine are nonvolatile (Drickamer, 1986b; Vandenbergh *et al.*, 1975; Vandenbergh *et al.*, 1976), but in urine from singly caged pregnant female or lactating females they are volatile (Drickamer, 1979; 1986d) and remain effective for three days after exposure to the air; Table 1.4-2 (Drickamer, 1986d). PAPs should, therefore, be different compounds or posses different constituents in the urine from different sources.

The acceleration of the onset of puberty in young female mice does not require a lengthy exposure to PAPs, nor does the amount of the urine, as the vehicle of PAPs, has to be large. A three-day treatment of adult male mouse urine to female mice under the age of 29-days (Colby and Vandenbergh, 1974), or a three-day treatment of urine from pregnant or lactating female mice to the females prior to day 29 of age (Drickamer,1984e), or three-day treatment of urine from singly caged female in oestrus to the test mice prior to day 29 of age is sufficient to cause the

Table 1.4-1: The Influences of urine from different donor mice on the puberty of young female mice

Urine Donor	Urine Source	Effect	Period ineffective	References
adult male	bladder & excreted	acce-lerate	winter	Vandenbergh,1969; Kennedy &Brown, 1970; Colby & Vandenbergh, 1974
prepubertal males & grouped females	excreted	delay		Drickamer, 1982b
singl-caged females in oestrus	excreted	acce-lerate		Drickamer, 1982c
all females regardless of aging or caging dense	bladder	delay		McIntosh & Drickamer, 1977
grouped females	bladder excreted	delay	mid-summer	Castro, 1967; Vandenbergh *et al*, 1972; Colby & Wise, 1972; Drickamer, 1982a
socially dominant females in oestrus	excreted	acce-lerate		Drickamer, 1985 Drickamer, 1986c
socially subordinate females in oestrus	excreted	delay		Drickamer, 1985 Drickamer, 1986c

(continue-1; Table 1.4-1)

Urine Donor	Urine Source	Effect	Period ineffective	References
pregnant or lactating females in last 2/3 days	bladder	acce-lerate	winter	Drickamer, 1983b
pregnant and simutaneously lactating females	excreted	acce-lerate		Drickamer, 1983b
pregnant or lactating females + grouped non-reproductive females	excreted	delay		Drickamer, 1982b
grouped females regardless of previous reproductive history	excreted	delay		Drickamer, 1982c
singly-caged females with shortened phtoperiod or in food depreivation	excreted	delay		Drickamer, 1984d

earlier attainment of puberty in young female mice (Drickamer,1986b), similarly, a

2-hr/day of exposure to adult male mouse's urine, or 1-hr/day of exposure to intact adult male mouse (Drickamer, 1983a) is also sufficient to induce early puberty.

Table 1.4-2: The characteristics of female puberty acceleration or delaying chemosignals

Donor	PAP*	PDP*	Volatility	Respon-sive organs	Bio-chemical nature	Effective in air
adult males	+	-	relatively non-volatile	testes	protein[1]	
singly-caged preg./ lact. females	+	-	rather volatile	adrenal	?	1-3 days[2]
group-caged females	-	+	slightly volatile	adrenal	?	5-7 days[3]

*: PAP: puberty acceleration pheromone. PDP: puberty delay pheromone.

[1] Vandenbergh *et al.*, 1975;

[2] Drickamer, 1986b;

[3] Coppola and Vadenbergh, 1985, Drickamer, 1986b.

In addition, a supply of only 0.03cc/day of urine from pregnant or lactating females , or as little as 0.001 cc/day of urine from singly caged females in oestrus, or even less, a minimum 0.0001cc/day of male urine has been shown to

bring about puberty acceleration in young female mice (Table 1.4-3), (Drickamer, 1982b; 1983c; 1984c; 1986b).

1.4-1-2 Puberty delay in females

Although most of the work on puberty acceleration was conducted in the 1970s (Vandenbergh, 1979) research into puberty delay in young female mice caused by urinary cues yielded rather promising results a decade later. Young female mice housed in groups have shown delay in attaining first vaginal oestrus (Cowley and Wise, 1972; Drickamer, 1974b; 1977; Vandenbergh *et al.*, 1972). Females exposed to urine from group-caged females also experienced delay to the same extent as the group-caged mice (Drickamer, 1982c). These results indicate that the puberty-delaying pheromones (PDPs) are excreted by group-caged females into their urine.

PDPs are also present in bladder urine of all female mice regardless of their age or caging density (McIntosh and Drickamer, 1977). Both bladder urine and excreted urine from female mice of all ages caged at sufficient density for at least a 10-day period (Drickamer, 1983c), or bladder urine from female mice within 10 days of being separated from groups and then caged individually (Coppola and Vandenbergh 1985) can delay the puberty of young female mice. Dominant females in groups, however, do not release PDPs either in oestrus or dioestrus, but do release PAPs if they are in oestrus, as mentioned above (Drickamer,1986c). Neither will puberty delay occur in young female mice if they are exposed to the urine collected in mid-summer days from group-caged female mice (in Table 1.4-1, Coppola and Vandenbergh 1985; Drickamer, 1982a).

Table 1.4-3: The efficient exposure length or dosage of PAPs or PDPs in inducing puberty acceleration or delay

Treatment	Urine source	References
	Acceleration	
3-day prior to day 29 of age	adult male	Colby & Vandenbergh, 1977
2-hr/day	adult male	Drickamer, 1983a
3-day prior to day 29 of age	pregnant or lactating females	Drickamer, 1984e
3-day prior to day 29 of age	singly-caged females in oestrus	Drickamer, 1986b
0.0001cc/day	adult male	Drickamer, 1982b; 1984c
0.001cc/day females in oestrus	singly-caged	Drickamer, 1986b
0.03cc/day lactating females	pregnant or	Drickamer, 1982b; 1983c
	Delay	
4-7 days prior to day 25 of age	bladder of females	Drickamer, 1977
1-hr/day	grouped females	Drickamer, 1983c
0.0001cc/day	grouped females	Drickamer, 1982b; 1984c

The sexual development of female mice can be postponed when they are exposed to urine containing PDPs for a 4-7 day period, prior to day 25 of age (Drickamer, 1977), and 1-hr/day exposure to the substances will be sufficient (Drickamer, 1983c). The puberty of test female mice can even be delayed with as small a dosage as 0.0001cc/day of urine from group-caged females; Table 1.4.3 (Drickamer, 1982b; 1984c). The direct contact of the test mice with the urine was found not to be critical for the phenomenon of puberty delay to occur (Drickamer and Assmann 1981).

After intensive study on the delaying effects of different combinations of urines from mice in different situations, Drickamer (1982b) showed that PDPs were so strong, or so effective, that they took precedence over other substances provided simultaneously. When young females, for instance, were treated simultaneously with three substances among which only one contained PDPs they were delayed from attaining sexual maturity. The other two substances containing PAPs such as the urine from singly caged female mice in oestrous and the urine from pregnant or lactating female mice could no longer induce puberty acceleration in young female mice (Drickamer, 1982b).

Unlike the PAPs in adult male mice, PDPs in the urine from group-caged female mice are slightly volatile, and will not lose their effectiveness until 5-7 days after exposure to the air (Coppola and Vandenbergh, 1985; Drickamer, 1986b).

1.4-2 Puberty in males

Considerably less is known about the susceptibility of puberty in male mice to olfactory stimuli (Vandenbergh, 1983), partly because it is difficult to determine precisely when male sexual maturation occurs (Brown, 1985). The published

literature implies that stimuli from adults of either sex can influence the rate of sexual maturation in males.

1.4-2-1 Puberty acceleration in males

It was reported by Fox (1968), and replicated by Vandenbergh (1971), that when young male mice were housed 4-5 in a cage and accompanied by an adult female from the time of weaning (at 21-days) to 56-days, the growth rate of testes, epididymides and seminal vesicles was faster than those caged in groups but without the company of females. Adult female cohabitation, however, could not produce larger accessory sex organs than control males which were caged singly (Bediz and Whitsett, 1979; Maruniak, Coquelin, and Bronson, 1978; Fox, 1968).

Unlike the peaceful mood a male may have towards a cohabiting young female, adult females are aggressive toward cohabiting young individuals of both sexes (Ayer and Whitsett, 1980). It was suggested by Fox (1968) and Svare *et al.* (1978) that the aggressive behaviour could account for the failure of inducing larger accessory sex organs in males between 36 and 50 days of age caged with adult females in comparison with those caged alone. Svare *et al.*(1978) found that seminal vesicle growth in the young males was inhibited by the aggressive adult female (23 out of 30 males were severely wounded). They then separated the adult female from the test males by a wire mesh barrier, which prevented the attack from the aggressive females and subsequently resulted in the accelerated growth of seminal vesicles though plasma testosterone levels and testes weights remained unchanged. This indicates that the cohabitation of adult females is able to facilitate maturation growth in young males provided that the females are not aggressive towards them (Vandenbergh, 1983).

While it is true that adult females have the capacity to accelerate the sexual maturation of young males, the data available on this matter are not as numerous as those on the matter of female puberty acceleration by urinary cues from males.

1.4-2-2 Puberty delay in males

In contrast to the fact that female stimuli generally accelerate the sexual development of juvenile males, male stimuli inhibit maturation in wild house mice (Vandenbergh, 1971). When albino mice were reared with adult females their testicular development was most rapid. The mice reared with adult males, on the other hand, had their testicular and accessory gland development delayed. Young male prairie deer mice (Bediz and Whitsett, 1979) or albino mice (McKinney and Desjardins, 1973) housed with adult male conspecifics from weaning (21-day-old) have lower weights of testes, seminal vesicle, epididydimes and preputial glands and lower testosterone levels than found in males housed without adult males. Male gerbils *(Meriones unguiculatus)* living with their fathers have delayed scent-gland growth and inhibited reproductive behaviour (Agren, 1981; Swanson and Lockley, 1978). Swanson (1980) further reported that the males living with their father until six months of age had fully developed seminal vesicles but did not reproduce.

Not only the existence of adult males but also the odour from adult males was found to have an impact on sexual development young males. Lecyk (1967) reported that after 50 days adjacent to cages occupied by sexually active adult conspecifics, juvenile male voles (*Microtus arvalis*) had lighter testes and fewer spermatozoa than control males. Lawton and Whitsett (1979) showed that prairie deer mice raised from 22-56 days of age on bedding soiled by adult males had lower testes and seminal vesicle weights than juveniles reared on clean bedding . The decrease in seminal vesicle weight was also induced by applying urine from

adult male deer mice in groups onto the nares of juveniles from 22 to 56 days of age. In these males, however, the testes remained intact.

In contrast to the results presented above, Terman noted (1968) that when exposed to bedding soiled by large groups of mice (47 individuals in a cage), male juvenile prairie deer mice in bisexual pairs were found to have heavier testes and seminal vesicles at 100 days of age than the mice provided with clean bedding. In male California voles (*Microtus californicus*) bedding from mothers delayed maturation in males (both androgen level and seminal vesicle weights), whereas bedding from father induced large seminal vesicles and the bedding from unrelated adult males had no effect on sexual maturation of the voles (Rissman *et al.*, 1984).

Furthermore group housing can retard the growth and development of young males as of young females. The weights of testes, epididymides and seminal vesicles at 35-42 days of age were significantly lighter in group-caged male deer mice than in singly caged controls (Bediz and Whitsett, 1979). The minimum group size to induce the retardation of sexual maturation in prairie deer mice was 9-11 in a cage. Males caged 4 in a group were not affected.

1.4-3. Sensitivity of recipient to the PAPs and PDPs

Urine samples collected in every month from the same mouse do not necessarily have same effects in influencing sexual maturation. There are seasonal changes in the effectiveness of both PAPs and PDPs.

In winter, for example, neither bladder urine nor excreted urine from adult male mice or from pregnant or lactating females can accelerate the puberty of young females (Drickamer, 1984a;1987). The delay of puberty by the urinary pheromones from group-caged female mice occurred during fall, winter and early spring

(September, October, November, December, January, February, March, April), but not during late spring and summer (May, June, July, August; Drickamer, 1984a). Uteri from peri-pubertal females were heavier in the summer moths (May to September) than during the winter months (November to February; Vandenbergh *et al.*, 1975).

It was also found that the puberty in laboratory strains of *Mus musculus* arrived significantly earlier in winter than in summer under constant laboratory conditions (Drickamer, 1977). Male mice born in March and May had slightly heavier tests than those born in July, September and November. Seminal vesicles, coagulation glands and preputial glands were heavier when males matured in spring than in autumn and winter. Female mice born in March and May reached puberty earlier than those born in other times of the year (Kruczek and Marchlewska-Koj, 1988). Albino rats born in spring reached puberty 5 days earlier than the rats born in autumn (Donovan and van der Werff ten Bosch, 1959).

All these results suggest that the seasonal changes of the effectiveness of PAPs or PDPs are largely due to seasonal shifts in sensitivity of the test young female mice but not to any change in the nature of the pheromonal substances released by donor mice (Drickamer, 1984a; 1987).

Except for seasonal changes, the effectiveness of accelerating or delaying pheromones in sexual development of young female mice is also altered by the deprivation of food supply and by the shortening of photoperiod (Drickamer, 1984d). The breeding and the hierarchy status of the odour donor also play an important role (Drickamer, 1986c, Table 1.4-4).

Table 1.4-4: The production and release of PAPs or PDPs

Urine source	PAPs	PDPs	References
prepubertal male mice	-	-	Lombardi *et al.*, 1976; Drickamer *et al.*, 1978
socially dominant adult males	+	-	Lombbardi & Vandenbergh,1977;
socially subordinate adult males	-	-	Drickamer, 1983a
adult male with shortened photoperiod	-	-	Drickamer, 1984d
castrated adult males	-	-	Lombardi *et al.*, 1976; Drickamer *et al.*, 1978
adrenalectomized adult males	+	-	Drickamer, 1983a
virgin females	-	+	Cowley & Wise, 1972
psudopregnant females	+	-	Cowley & Wise, 1972
early pregnant and lactating females	-	-	Drickamer, 1983b
late pregnant and lactating females	+	-	Drickamer, 1983b
ovariectomzed females	-	-	Drickamer *et al.*, 1978
adrenalectomized females	-	-	Drickamer & McIntosh, 1980

Note: The urinary substance is not effective in producing puberty-acceleration when it is collected from male mice or supplied to test female mice at the onset of the light portion of the daily cycle (Drickamer, 1982a).

1.4-4 Sources of the PAPs and PDPs

The relation of PAPs and PDPs to reproductive organs has also been studied. Urine from prepubertal or castrated males does not possess the puberty-accelerating ability (Vandenbergh, 1969; Colby and Vandenbergh, 1974). The injection of testosterone in castrated males restores the presence of the chemosignal in the urine (Lombardi and Whitsett, 1976). These results indicate that the release of PAPs has some relationship to the testes or testis-related hormones. Adrenalectomy does not affect the releasing of female-PAPs in male urine (Drickamer, 1983a), but it causes the absence of PDPs in the excreted urine of grouped female mice or the bladder urine of all female mice (Drickamer and McIntosh 1980). If group-caged and adrenalectomized female mice are given a therapy of hydrocortisone or corticosterone (glucocorticoids), they will regain the ability to release PDPs into their urine (Drickamer and Shiro, 1984). Ovariectomy, on the other hand, does not alter the presence of the delay substance in either excreted or bladder urine (Drickamer *et al.*, 1978). It has also been shown that the reproductive history of a female and any associated hormone changes cannot affect the production or release of PDPs (Drickamer, 1982c).

We can now draw a clear picture of the roles each of the sexual glands plays in the release of PAPs or PDPs into urine (Table 1.4-5). There are at least two gonadal glands involved in producing or releasing PAPs and PDPs: testes in male mice, and the adrenal glands (but not the ovaries) in female mice. The hormones involved are LH and testosterone in male and LH, FSH, estrogen and prolactin in the female (Bronson, 1979).

Table 1.4-5: Urinary cues in relation to reproductive glands

	Relation to the onset of puberty		Behvioural attractiveness		Release of LH in males	
Glands	intact	removed	intact	removed	intact	removed
ovary	delay	delay	+	-	+	+
hypophysis					+	-
female adrenal	delay		+	+		
male adrenal	accelerate	accelerate	+	+		
testis	accelerate		+	+		

1.4-5. The involvement of serum hormones in the release and reception of PAPs and PDPs

It is well known that the secretion of LH and testosterone of male mice increases in the presence of urine from female mice (Macrides *et al.*, 1975; Purvis, 1978). The increment of serum LH of a male mouse can be detected within 5 minutes after the male mouse encounters a female (Coquelin and Bronson, 1979; 1980a, b), followed by a rise in plasma testosterone (Coquelin and Desjardins, 1982).

Urine from ovariectomized females can still cause the increase of serum levels of LH, but urine from hypophysectomized female has no effect. Urine from ovariectomized or hypophysectomized female mice will not be attractive to, nor cause behavioural change in, male mice (Johnston and Bronson, 1982). These

results suggest that the ovary-related pheromones contained in female urine is responsible to the behavioural changes of male mice. Pituitary-related pheromones contained in female's urine, on the other hand, is responsible for both endocrinal and behavioural changes in male mice.

It is reasonable, then, to assume that there are at least two categories of pheromones produced and released by female mice into their urine: ovary-related pheromone which modifies only the behaviours of target individuals and a pituitary-related hormone which regulates both endocrine activities and behaviours of recipients.

The hormonal responses of young females to the odour cues of males, on the other hand, includes a sequential release of LH, oestradiol, a secondary suppression of follicle-stimulating hormone (FSH), an elevation in serum prolactin and finally an elevation of serum progesterone and preovulatory release of LH (Bronson, 1974; 1976). The change of the hormone concentration will then, in turn, modulate behaviour of recipient to the odour of a donor (Bronson and Coquelin, 1980).

1.4-6. Field studies on PAPs and PDPs

The development of the research of the effectiveness of pheromones in male or female mice urine on the onset of puberty in young females has been rapid during the last two decades. At least four urinary chemosignals have so far been found to have the ability to affect the date of onset of puberty in mice. They are the urine from males, the urine from pregnant or lactating females, the urine from females in oestrus and the urine from females in group. These four chemosignals can, in turn, fall into two categories - one which accelerates (contains PAPs, the first three) and one which delays the onset of puberty of recipients (contains PDPs, the latter),

(reviewed in Drickamer, 1986).

The phenomenon of puberty delay or acceleration in mice has been reported in wild populations as well as in laboratory populations (Drickamer, 1977; 1979). The first evidence that wild female house mice living under natural conditions can also produce the puberty-delaying pheromone was described by Massey and Vandenbergh (1980). Urine collected from the wild female mice on "highway islands" (cloverleaf highway sections) when the population was in high density delayed the first oestrus in juveniles when compared with controls. Coppola (1986) verified that female house mice living in wild populations produce the puberty-delaying pheromone in response to acute increases in population density. By introducing 40 female wild house mice that had been raised in the laboratory onto a highway island on three occasions during the eight months study, Coppola was able to create acute population growth. Only the urine from the females on the highway island after the deliberate population growth delayed the attainment of first oestrus in females in laboratory. Urine collected before the artificial population explosion did not cause a significant delay. Not only can the urine from feral populations in high density delay the arrival of first oestrus in juvenile females, as noted above, but also the urine from grouped females in laboratory delays the onset of puberty (Drickamer, 1991). The population growth of *Mus domesticus* in six field enclosures each measuring 0.1 ha was slowed down with the treatment of urine from group-caged adult female mice and accelerated with the treatment of urine from adult males. Similar to the latter result of Drickamer's experiments, Massey and Vandenbergh (1981) accelerated pubertal onset among test females in the laboratory by applying the urine collected from males in natural populations. Furthermore, Fan and Vandenbergh (1991) reduced the latency and increased the duration of contact

of the test females with partner males by providing the test females with male-soiled bedding prior to pairing. The females eventually reproduced litters with an increase of about 1.4 pups per litter without the reduction in body weight of the pups at birth.

It is apparent that the the effects of PAPs and PDPs are detectable both in laboratory and in feral populations so the remaining aspects which need further research are the characterization of the components of the pheromones and the study of the applicability of the pheromones as well as the possibility of laboratory synthesis of the analogues of the effective components (Vandenbergh, 1987). These might eventually allow the development of programmes designed to control the rodent pest populations (Massey and Vandenbergh, 1980).

1.5. Biology of *Rattus rattoides*

1.5-1. *Rattus rattoides* is an agricultural pest

The rat, *Rattus rattoides,* a field species widely spread in coastal area, south of China (Fig. 1-1), causes severe damage to a wide range of crops throughout the year. It mainly eats rice, wheat, peanut, sweet potato, sugar can, some fruits (banana and lychee), different vegetables and some animals, including snails, crabs, fish, shrimps, frogs, grasshoppers and other insects (Shou, 1962; Zhan, 1979).

The rat eats the rice seeds and the seedlings in spring. As shown in Plate 1-1 the seedlings were ruined by the rat in the rice field. When the rice is in the stage of heading, the rat then cut the talks and eats the milky grains. In harvest time, the rat not only eats the grain in the field but also takes as much as possible and stores them in its burrow. Sometimes it can ruin 20% (Zhan, 1979) and even up to 70% to 80% (Shou, 1962) of the rice in the field (also see Plate 1-2). They cut wheat (Plate 1-3), dig up peanuts (Plate 1-4) and chew bananas (Plate 1-5). Its seriousness as an agricultural pest is not in doubt.

1.5-2. *The ecology of the rat*

The rats live in the burrows in the banks of crop fields, of streams, and of hills. In winter days they make nests in rice-stalks (Shou, 1962). They migrate short distances seasonally according to the abundance of foods (Zhan, 1984). In early spring the rats live in the fields where wheat and vegetables are grown. In late spring and early summer most of them move to paddy field but some remain in the vegetable fields. They stay in rice fields and the fields of sweat potatoes in autumn. In winter most of the rats migrate to the fields of sugar can, vegetables and wheat.

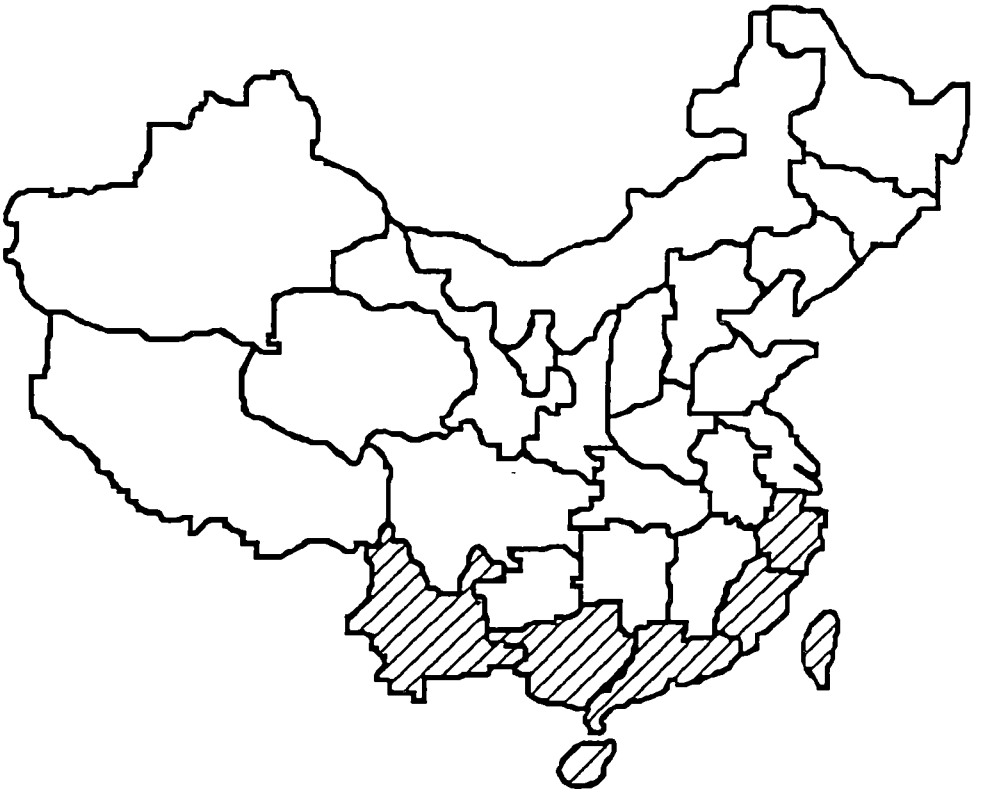


Fig. 1-1: Distribution of Rattus rattoides in southern China (stippled area).

(After Shou, 1962.)

Plate 1-1: Rice seedlings ruined by the rat.

Plate 1-2: Rice ears cut by the rat.

Plate 1-3: The damage to wheat by the rat.

Plate 1-4: Peanuts dug and chewed by the rat.

Plate 1-5: The bite into bananas.

The rats are very good swimmers so that although there are criss-cross networks of irrigation channels in the fields they can migrate with easy to wherever foods are available (Shou, 1962). They stay in the burrows all the time when the weather is bad. It was reported that the adult rats became extremely irritated after 15 - 20 minutes under the mid-day sun (2:00 pm) when the temperature was about 40.5 °C, and then died by 30 minutes (Lin and Xin, 1962). Wang and Qing (1981) reported that when the temperature rose to 35.6°C the rats dug from the burrow became feeble, panted and soon died. It was also observed that, when the temperature was 30°C - 36°C, over half the wild-caught rats in the laboratory were sick (showing a loss of appetite, lung and liver oedema, liver and abdomen tumors, intestinal pneumatosis and cough) and eventually died (personal observation). Low temperature can also kill the rats as reported by Lin and Xin (1962). The neonates died after being in a temperature of 6°C for only 7 minutes. During the field trapping it was found that the rats were dead or nearly dead in the traps when the temperature fell below 10°C (the night temperature was 4-9°C some time, personal observation).

The rats are most active during 4:00 to 6:00 am and from 6:00 to 9:00 pm in the summer but are active from 8:00-9:00 am to about 3:00 pm in winter when the temperatures are comparatively mild (Shou , 1962).

1.5-3. Growth

Neonates of the rat open eyes at the age of 11.11 ± 0.07 days and start to take food while still suckling at 15.68 ± 0.29 days, when the first molar appears (Qing *et al.*, 1981). The rats grow fastest from day 15 - 70 and slow down from day 70 - 160 (from juvenile - maturation). During this period of time (day 70 - 160) the

growth is variable among individuals due to the environmental factors. Those of the rats born in spring grow up in worm weather reach maturation and enter reproduction at this period. Those born in autumn, however, grow up in colder weather in winter and early spring enter reproduction in an elder age. When the rats mature (after 160 days) their growth slow down and the body weights even decrease in some individuals (Qing *et al.*, 1981).

1.5-4. Reproductive capability

Gestation period of the rat is 21 - 24 days (Zhu, 1963). The rats are found to be able to breed throughout a year with an average pregnancy rate of 30.42%. The average litter size is 6.14 ± 1.9 ranging from 2-13 (Qing and Wang, 1981; Shou, 1962). The observations on 41 females in the laboratory show that the reproductive probability is 1-6 litters per annum averaging 2.92 ± 0.22 (Qing and Wang, 1981). The pregnancy rate changes with season. The percentages of pregnant rats caught in the field are comparatively low in spring and summer, it increases in autumn and reaches its peak in September - October with pregnancy rate at about 70% (Qing and Wang, 1981). Zhan (1981) reprorted that the rat did not breed in winter and early spring (from November - February) when the temperature was comparatively low and food was not as abundant as in other months in the area. He observed reproductive peaks of the rat in mid-spring (April) and mid-summer (August). These variations from different researchers are probably due to geographic differences. In addition, the rats born in spring were found to breed earlier than those born in autumn (Qing *et al.*, 1981). While the population of the rat grow very fast in the field the number of the rats actually breed does not appear very high. Lin and Xin (1962) reported that only about 25% of the population have the capability of reproduction.

1.6. Aims of the research

The present study was designed to achieve the following objectives:

To investigate the growth and development of the species both before weaning (from day-1 of age to day-21) and after weaning (from day-21 to day-80).

To investigate the reproductive capability of the species in captivity.

To investigate the lengths of oestrous cycles of the females and the influence of male olfactory cues on them.

To investigate the influence of adult male and female olfactory cues on the development of ovaries and the attainment of puberty in the females.

To investigate the influence of living density and adult male olfactory cues on sexual development of the males.

Chapter 2. General methods

2.1. Field trapping

Rattus rattoides were caught in Lanqi Isle, about 80 kilometers east of the suburb of Fuzhou City, P. R. China. They were collected by live trapping with wire live traps baited with a piece of sweet potato or a crab. The rats are most active from dusk (about 6:30 - 7:30 pm) to mid-night and again at dawn (4:00 - 6:30 am) in summer and autumn (Shou, 1962). The trapping was started at 5:00 pm. The traces of the rats were spotted first and marked along the bank of the field. The freshness of the traces was determined by fresh foot prints, faeces or the lack of cobwebs and the existence of chewed ears of rice. The traps were placed in the middle of the trace or at a side of the entrance to a burrow. It was normally by 6:00 - 6:30 pm that 20 - 25 traps were placed in positions. About 90 minutes to two hours later, all traps were checked. Captured rats were removed and the traps reset. An hour or more later, this was repeated again. It was repeated for three times during each trapping night till about 10:30 pm; then the traps were left in the field and collected early next morning (5:00 to 5:30 am).

2.2. Laboratory housing

The rats were brought back to the laboratory and singly housed in standard plastic cages measuring 29 x 18 x 16 cm. Rice hulls were put into the cages as bedding. Water and food (Shanghai Animal Food Company, Shanghai, P. R. China) were available *ad libitum*. The room was adjusted to a temperature of 22 - 25°C and a light regime of 12 hrs light (6:00 am - 6:00 pm) and 12 hrs dark (6:00 pm - 6:00 am). The cages were checked daily for the water leakage because of the

shaking of the water bottles by the rats in their attempts to escape. The cages were changed and cleaned immediately if they were wet, otherwise once a week.

2.3. Breeding

Sexually mature females (vagina opened) and males (testes measured about 2 cm) were paired in bigger cages, sized 35 x 65 cm, for five days and then separated and housed singly again in smaller cages. Females not pregnant after 21 days were paired again in 10 days. From September, 1989 to April, 1992 twenty-seven of pairings were carried out. Three generations and more than 1,000 rats were born in the laboratory during the study. Neonates were weighed one day and six days after birth and thereafter at 7-day intervals. They were separated from their mothers when they were 21 days and divided randomly into groups according to the various experimental designs.

2.4. Treatment

2.4-1. Standard group: Every month at least 20 female and male weanlings were housed each with an opposite sex sibling in a cage. They were kept in the breeding room where adult females in different reproductive situations (pregnant, lactation, paired with males) were housed. These rats were designed to be a standard group serving as controls for the other treatments.

2.4-2. Singly housed group: Some of the weanlings were housed singly and kept in a room in which no individuals of the opposite sex were housed.

2.4-3. Females housed each with an adult male: Female weanlings were housed each with an adult male in the breeding room throughout the experiment.

2.4-4. Singly housed and provided with bedding soiled by adult male or female: Female or male weanlings were housed singly and provided with the

bedding from either adult male or female. They were housed in the room where only singly housed adult males or females were kept to provide the bedding for the experiments. Every morning from the beginning of the experiment, bedding (rice hulls with urine) was collected from the cages housing the adult and then about 2 cc (Drickamer, 1974) of the bedding was provided to the test rat in a corner of the cage.

2.4-5. Group housed: Female or male weanlings were housed 4-5 in a cage. All weanlings in the experiments mentioned above were weighed 9 days after weaning and then in an interval of 10 days till the end of the experiment. The testis lengths and the lengths of body and tails of the male weanlings were measured. Body and tail lengths were measured by placing the rat alongside a ruler and the testis length was measured with a Vernier callipers.

2.4. Anatomy

For the measurement of the weights of testes and epididymides and the weights of ovaries and uteri, the test individuals were killed by cervical dislocation at the given ages, or 9 days after weaning, and further individuals at 10-day intervals.

The organs were separated from the connective tissues and weighed and immediately fixed with Bouin Solution and embedded later. Histological-sections were carried out and sections cut at 8 μ were stained with haematoxylin and eosin (HE) for microscopical examination of the appearance of mature sperm in seminiferous tubules and convoluted ducts.

Because the number of the rats bred in the laboratory for the experiments was extremely limited only 3 - 8 rats were sacrificed for the examination of the organs.

2.5. Serum collection and RIA

Immediately after cervical dislocation at the ages mentioned above, the thoracic cavity was opened and the heart was cut through with scissors. A blood sample of about 5 ml was collected and centrifuged and stored at - 20° C.

RIA was carried out in Giant Panda Research Centre, Fuzhou Zoo, Fujian, P. R. China. ^{125}I Testosterone kits were purchased from ICN Biomedicals Inc., U. S.

2.6. Puberty and oestrous cycles

Test females were examined daily from 10 days after weaning for the opening of the vagina as the sign of the onset of puberty.

Vaginal smears were carried out daily at about 8:30 am for a period of 30 days in each season. Vagina lavage was carried out with a dropper. A drop of distilled water was dripped into the vagina, lavaged two to three times, and then smeared onto a clean and dry slide. The wet-mount smears were examined immediately with a light microscope and the cellular contents were judged according to the criteria of Rugh (1968) and Vandenbergh (1969). The smears were then classified as epithelial (E) smears (with more than 80% nucleated or cornified epithelial cells, as seen at proestrus and oestrus in the rat) or as leukocytic (L) smears (with more than 20% leukocytes and seen at metoestrus or dioestrus in the rat; Mandl, 1961; Champlin, 1971; Hoover and Drickamer, 1979; LeFevre and McClintock, 1988). E-smears represent follicular growth and ovulation phases (Nequin *et al.*, 1979) and therefore, represent proestrus and oestrus phases, whereas L-smears represent luteal activity (Parkes, 1929) and so represent metoestrus and dioestrus.

2.7. Statistical analysis

Data were analysed with an One Factor ANOVA test when there were more than two groups of test individuals and with paired or unpaired Student's t-test

when only two groups were involved. Contingency table analysis was also used in testing the differences of distribution patterns of oestrous cycles and the constituents of the cycle. In some analyses, data were previously Square-root or log-transformed to correct for heterogeneity of variance among different groups (Vandenbergh *et al.*, 1975). Polynominal regression was employed to analyse the correlations between age, body weight, testis weight and length, epididymis weight, and the lengths of body and tail.

2.8. Abbreviations and definitions

Am, adult male; **Ym**, young male; **Yf**, young female; **UAm**, urine (contained in the bedding) from adult male; **UAf**, urine from adult female; **BW**, body weight (g); **TW**, testis weight (g); **EW**, epididymis weight (g); **TL**, testis length (cm); **b.l.**, body length (cm); **t.l.**, tail length (cm); **G-day**, growth rate per day at given ages; **VO**, vaginal opening, or the onset of puberty; **E-length**, the period from the start of proestrus to the end of oestrus; **L-length**, the period from the start of metoestrus to the end of dioestrus.

Chapter 3 The growth and development of male Rattus rattoides in captivity

3.1 Introduction

As was described in Chapter 1.4, little work has been conducted on the growth and development of *Rattus rattoides*. The series of experiments described here sought to examine the pre-weaning growth and development of male rats under laboratory conditions when they were housed with their mothers as well as growth and development after weaning when they were housed singly.

3.2 Materials and methods

For the growth of the rats before weaning, the neonates were weighed a day after birth and at 7 day intervals till the time of weaning (21 days old). Growth rates based on 7-day intervals were recorded. Despite the controlled laboratory conditions, differences in growth rate were found between the males born in different seasons. Growth rates therefore were recorded during spring, summer, autumn and winter.

For the growth and development of the rats after weaning, the male weanlings were singly housed. The weights, the lengths of body and tail of the subject males and females and the lengths of the left side testis of the young males were measured at ten-day intervals until the males were 90 days.

Because of the shortage of the supply of test individuals, only three to eight male rats were sacrificed at intervals of 10 days from weaning (21 days) to 130 days old. Body weights and lengths, testis weights and lengths (left side), epididymis weights (left side) and tail lengths were measured and the correlation of

the above parameters were determined. Testes and epididymes were immediately fixed in Bouin Solution after weighing and dissected later. The slides were stained with Haematoxylin and Eosin (HE) and examined under the microscope for the appearance of mature sperm in seminiferous tubules and convoluted tubules.

The data were analysed with One Factor ANOVA, Repeated Measures with Grouping Factors and Pearson Correlation tests.

3.3 Results

3.3-1: Pre-weaning growth of the male rats

3.3-1-1: Body weights (g) of the males before weaning

Body weights of the males born in spring (April), summer (July), autumn (October) and winter (January) were weighed in 7-day intervals started from day 1 to day 21 and recorded as means (Table 3-1, Appendix 3-1).

Males born in January and April had significantly heavier birth weights (at day-1) than the males born in July and October. The birth weights of the males born in January were significantly heavier than those of the males born in April. At day-7, body weights of the males born in January were the heaviest and significantly different from those of the males born in other months. At day-14, males born in January and April had significant heavier body weights than those of the males born in July and October. At day-21 (weaning), body weights of the males born in October were significantly lighter than the body weights of the males born in January, April and July.

3.3-1-2: Pre-weaning growth rate (g/day) of the males

The growth rates of the males from birth day-1 to day-7, day-8 to day-14,

day-15 to day 21, and also the rate from day-1 to day-21 by seasons were compared and the differences between the growth rates of the males born in different months were also tested for significance (Table 3-2).

*Table 3-1: Body weights (g) of the males born in different months at day 1, 7, 14, and 21**

Month	n	Day-1	Day-7	Day-14	Day-21
Jan.	29	5.17 ± 0.17^{a}	11.41 ± 0.57^{a}	16.52 ± 0.60^{a}	22.69 ± 0.95^{a}
Apr.	61	4.42 ± 0.07^{b}	9.36 ± 0.226^{b}	15.07 ± 0.30^{ab}	21.89 ± 0.47^{a}
Jul.	59	4.16 ± 0.09^{c}	9.00 ± 0.31^{b}	14.11 ± 0.54^{bc}	20.67 ± 0.69^{a}
Oct.	40	3.94 ± 0.13^{c}	8.38 ± 0.43^{b}	12.43 ± 0.64^{c}	19.57 ± 0.90^{b}

* The values labelled with different superscript letters are significantly different ($P < 0.05$) by Scheffe's F-test in one factor ANOVA.

Repeated measures with grouping factors shows that there is no significant difference between the growth rate of the males born in different months ($F = 2.352$, $P > 0.05$). The test, however, shows significant difference between the growth rates of given times of the males born in the same month ($F = 83.169$, $P < 0.001$). Based on this result separate One factor ANOVA tests were carried out to check the differences between the growth rates of the males born in the same month (Table 3-3, Appendix 3-2). Among the males born in January the growth rates during the third week (day-15 to day-21) was significantly higher than those in first periods ($F = 3.53$, $P < 0.05$). In the males born in April the growth rate during the

than the mean growth rate from day-1 to day-21 (G-m, $F = 9.28$, $P < 0.001$) and G-7 ($F = 28.19$, $P < 0.001$) and second week (G-14, $F = 9.75$, $P < 0.001$). Meanwhile, G-m and G-14 were significantly higher thanG-7 ($F = 5.12$, $P < 0.05$ and $F = 4.78$, $P < 0.05$ respectively). For the males born in July, G-21 was significantly higher than G-7 ($F = 18.52$, $P < 0.001$), G-14 ($F = 11.58$, $P < 0.001$) and G-m ($F = 7.51$, $P < 0.001$). Similarly in the males born in October, G-21 was significantly higher than G-7 ($F = 12.54$, $P < 0.001$), G-14 ($F = 10.47$, $P < 0.001$) and G-m ($F = 5.23$, $P < 0.005$).

Table 3-2: Repeated measurements of growth rates (GR) at given ages before weaning of the males born in different months.

Between Subjects

Source	SS	DF	MS	F	P
Month	1.521	3	0.406	2.423	0.067
Error	39.881	185	0.168		

Within Subjects

Source	SS	DF	MS	F	P (H-F)
GR	16.567	2	8.283	83.169	0.000
GR*Month	0.580	6	0.097	0.971	0.438
Error	36.851	370	0.100		

Table 3-3: Pre-weaning growth rate (g/day) of the males born in different months

	January (n = 29)	April (n = 61)	July (n = 59)	October (n = 40)
G-7	0.71 ± 0.06^{a}	0.63 ± 0.03^{a}	0.61 ± 0.03^{a}	0.57 ± 0.04^{a}
G-14	0.8 ± 0.06^{ab}	0.81 ± 0.04^{b}	0.71 ± 0.04^{a}	0.61 ± 0.01^{a}
G-21	1.04 ± 0.11^{b}	1.10 ± 0.05^{c}	1.07 ± 0.07^{b}	0.99 ± 0.07^{b}
G-m	0.83 ± 0.05^{ab}	0.82 ± 0.02^{b}	0.78 ± 0.03^{a}	0.72 ± 0.04^{a}

*The values with different superscript letters in the same column are significantly different at different levels (see text for detail) by Scheffe's F-test in one factor ANOVA.

3.2-2. Growth and correlations

Pre-weaning correlations of the birth-weight with the weight and growth rate at day-7, day-14, day-21 and mean growth rate of the males born in different months were analysed (Table 3-4). There was no correlation between the birth-weight and the weights and growth rates at given ages of the males (n = 29) born in January. The birth-weight was correlated with the wights at day-7 ($r = 0.447$, $P < 0.005$) and day-14 ($r = 0.434$, $P < 0.05$) in the males (n = 61) born in April. All measures except the growth rates at day-14 and day-21 were highly correlated with the birth-weight of the males born in July (n = 59, Table 3-4). Among the males born in October, all the measures were highly correlated with the birth-weight (n = 40, Table 3-4).

Table 3-4: Pre-weaning correlation (r) of birth weight (W-1) with the body weight (W, g) or growth rate (G, g/day) at given ages of the males born in different months.

W-1 *vs*	January	April	July	October
W-7	0.027	0.477**	0.850***	0.780***
G-7	- 0.109	0.227	0.718***	0.552***
W-14	0.01	0.434*	0.653***	0.796***
G-14	- 0.333	0.039	0.324	0.746***
W-21	0.066	0.337	0.581***	0.855***
G-21	0.087	0.109	0.250	0.736***
G-m	- 0.114	0.220	0.499**	0.818***

G-m, growth rate from day-1 to day-21.

*: Bonferoni adjusted P < 0.05; **: Bonferoni adjusted P < 0.005

***: Bonferoni adjusted P < 0.001

Post-weaning growth curves of body weights and lengths, testis weights and lengths, the weights of epididymes and tail lengths are shown in Fig. 3-1 (also see Appendix 3-3). Body weight increased quickly from 25.17 ± 2.19 g at weaning to 100.38 ± 8.1 g when the males were 70 days of age. After that the weight did not change substantially but fluctuated around 100 grams (94.25 ± 6.14 g - 107.3 ± 6.03 g) (Fig. 3-1 a). Similar phenomena were also observed in the increment of testis weight and length, in epididymis weight, and the lengths of body and tail (Fig. 3-1 b, c, d and Appendix 3-4).

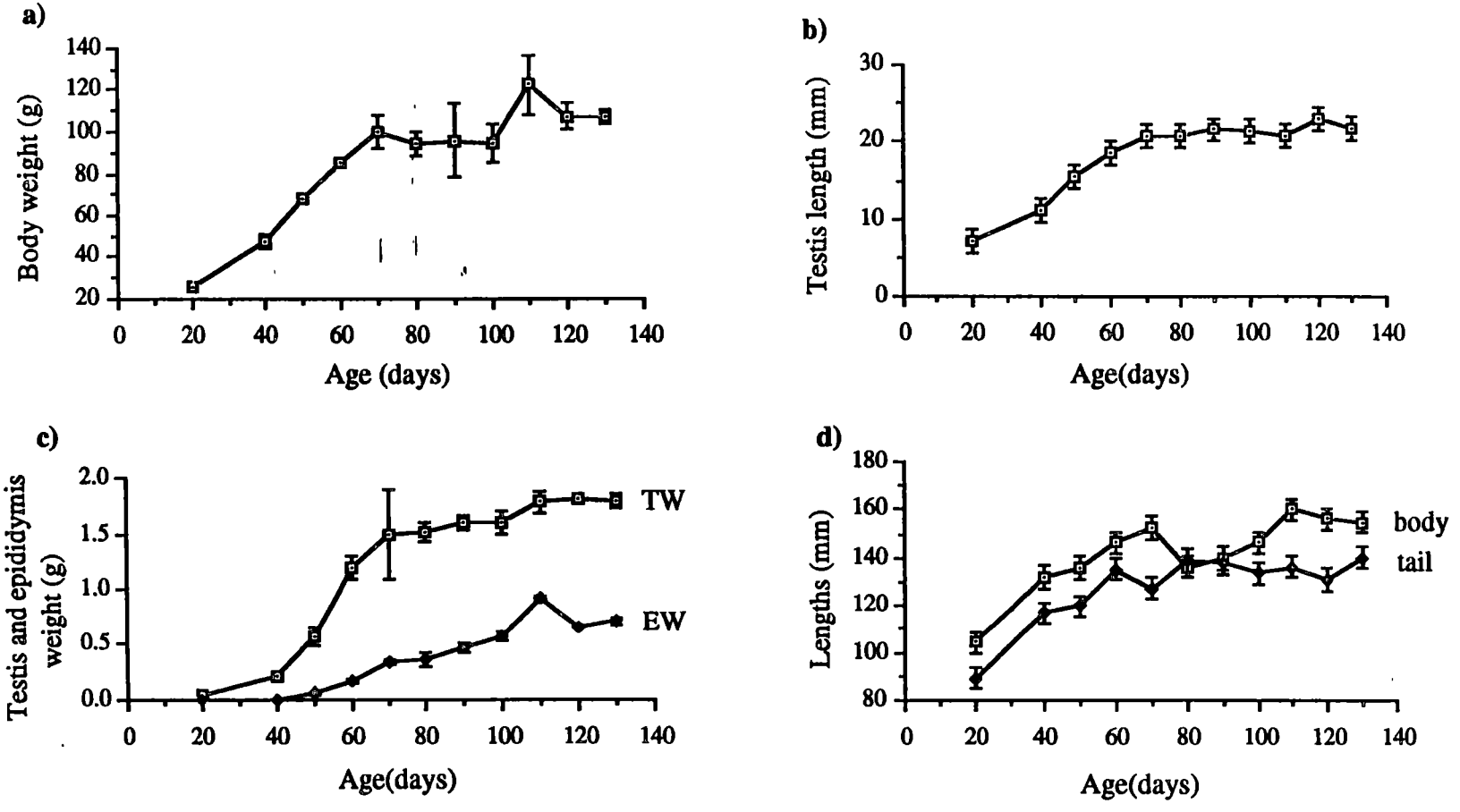


Fig. 3-1: The growth curves of body weight (a), testis length (b), the weights of testis (TW) and epididymis (EW) (c) and the lengths of body and tail (d).

Correlations among age, body weight (BW), testis length (TL), testis weight (TW), epididymis weight (EW), body length (b.l.) and tail length (t.l.) were calculated. The result shows that age is highly correlated with all aspects examined (Table 3-5, Appendix 3-4).

Table 3-5: The correlation of the growth of body weight (BW), testis length (TL), testis weight (TW), epididymis weight (EW), body length (b.l.) and tail length (t.l.) with age.

Age *vs*	Correlation coefficient (r)	Bonferoni adjusted probability
BW	0.806	$P < 0.001$
TL	0.851	$P < 0.001$
TW	0.907	$P < 0.001$
EW	0.888	$P < 0.001$
b.l.	0.785	$P < 0.001$
t.l.	0.674	$P < 0.001$

During post-weaning growth the body weight is highly correlated with testis length and weight and epididymis weight (Table 3-6, Appendix 3-5). Meanwhile, the length of testes is also highly correlated with the weights of testes and epididymides (Table 3-7, Appendix 3-6).

Table 3-6: The correlation of testis length (TL), testis weight (TW) and epididymis weight (EW) with body weight (BW).

BW *vs*	Correlation coefficient (r)	Bonferoni adjusted probability
TL	0.855	$P < 0.001$
TW	0.882	$P < 0.001$
EW	0.739	$P < 0.001$

Table 3-7: The correlation of testis weight (TW) and epididymis weight (EW) with testis length (TL).

TL *vs*	Correlation coefficient (r)	Bonferoni adjusted probability
TW	0.947	$P < 0.001$
EW	0.690	$P < 0.001$

Plasma testosterone concentrations in the males at different ages were assayed and the results are presented in Fig. 3-2 (also see Appendix 3-7). It is obvious that testosterone level was very low when the males were at the ages from 21 days to 50 days (0.04 ± 0.02 ng/ml to 0.39 ± 0.14 ng/ml). The plasma testosterone concentration increased sharply at 60 days of age and then remained at a level ranging from 2.6 ± 0.63 ng/ml to 6.44 ± 1.22 ng/ml throughout the test period.

Histological examination showed that mature sperm appeared in seminiferous tubules in testes at the age of 50 days and in the convoluted ducts of the epididymides at 60 days. The initiation of reproduction of the males was at the age of 95.33 ± 1.24 days.

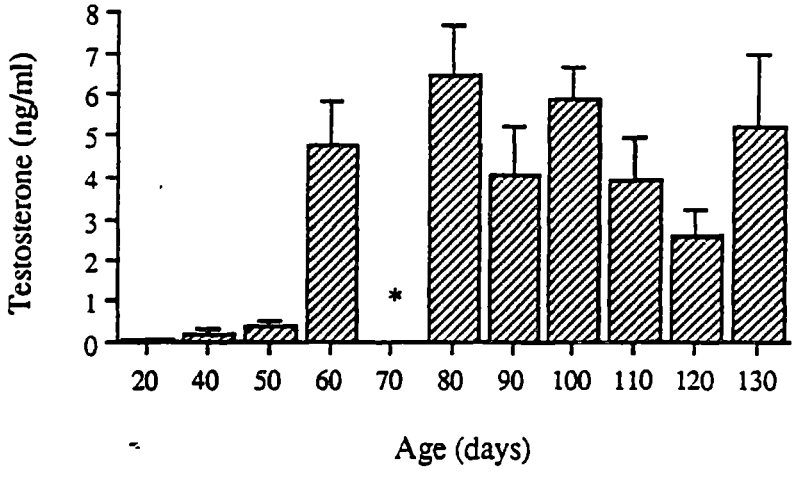


Fig. 3-5: Testesterone concentration, mean ± ISE, in the serum of singly-caged males at different ages

** No data*

3.3-3. Post-weaning growth and development of the males born in different months.

Body weights of the males born in different months recorded in 10-day intervals after weaning are shown in Table 3-8. The males born in August were

significantly heavier than those born in February at day-21 and day-30, and significantly heavier than those born in February and April at day-40. The rats born in November had significantly heavier body weights at day-21 than those born in February. From day-50 onwards the body weights of all males born in different months were not statistically different from each other (also see Appendix 3-8).

The growth and development of testes measured by lengths (cm) of the males born in different months are recorded in Table 3-9. The testis lengths at the ages day-21, day-30 and day-40 of the males born in February were significantly shorter than those of the males born in the other three months, and still significantly shorter at day-50, day-70 and day-80 than those of the males born in April. The testis lengths of the males born in August and November were not statistically different from each other at any of the examination time but they were both significantly shorter than those of the males born in April at day-40, day-70 and day-80. The testis lengths of the males born in November were significantly shorter than those of the males born in April at day-50. At day-60, testis lengths of all males were similar (also see Appendix 3-9).

3.4. Discussion:

3.4-1. Pre-weaning growth of the males

It appears that the males born heavier have heavier body weights at other measurement times before weaning (the males born in January and April, for example, Table 3-1). Neither was the body weight nor the growth rate of the males born in January, on the other hand, correlated with the birth-weight (Table 3-4). The birth-weight was correlated only with the weight at day-7 and day-14 of the males born in April (Table 3-4). All body weight at different given ages but only the

*Table 3-8: Body weights (g) of the males after weaning by season.**

Month	n	Day-21	n	Day-30	n	Day-40	n	Day-50	n	Day-60	n	Day-70	n	Day-80
Feb.	19	18.63±0.83^{a}	20	31.23±1.29^{a}	20	41.88±1.68^{a}	14	49.18±3.2^{a}	12	59.63±4.05^{a}	9	74.33±5.6^{a}	7	77.64±2.19^{a}
Apr.	24	21.92±0.84^{ab}	24	35.06±1.11^{ab}	21	44.5±1.23^{a}	23	57.67±2.63^{a}	13	65.12±3.17^{a}	17	77.21±4.04^{a}	11	93.68±4.27^{a}
Aug.	15	24.56±1.41^{b}	15	37.33±1.77^{b}	15	51.0±2.28^{b}	13	56.89±2.24^{a}	11	60.18±2.9^{a}	12	66.67±3.62^{a}	12	81.0±0.30^{a}
Nov.	13	23.35±1.24^{b}	13	33.23±1.93^{ab}	13	45.69±1.37^{ab}	12	56.0±2.74^{a}	12	61.54±2.73^{a}	12	66.58±5.23^{a}	6	81.25±5.26^{a}

* Scheffe's test in One Factor ANOVA. Values in the same column with different superscript letters are significantly different ($P < 0.05$).

*Table 3-9: Testis lengths (cm) of the males after weaning by season**

Month	n	Day-21	n	Day-30	n	Day-40	n	Day-50	n	Day-60	n	Day-70	n	Day-80
Feb.	21	0.26 ± 0.01^{a}	20	0.56 ± 0.02^{a}	21	0.70 ± 0.02^{ad}	14	0.98 ± 0.08^{a}	12	1.12 ± 0.07^{a}	9	1.33 ± 0.09^{a}	7	1.54 ± 0.09^{a}
Apr.	24	0.45 ± 0.02^{b}	24	0.74 ± 0.01^{b}	26	1.07 ± 0.03^{b}	12	1.37 ± 0.04^{b}	19	1.54 ± 0.04^{a}	18	1.88 ± 0.04^{b}	13	2.06 ± 0.02^{b}
Aug.	9	0.39 ± 0.02^{b}	14	0.82 ± 0.04^{B}	15	0.89 ± 0.06^{c}	13	1.19 ± 0.07^{ab}	11	1.33 ± 0.09^{a}	12	1.48 ± 0.09^{a}	11	1.68 ± 0.10^{a}
Nov.	12	0.38 ± 0.02^{b}	13	0.76 ± 0.03^{b}	13	0.86 ± 0.04^{cd}	12	1.10 ± 0.05^{a}	12	1.20 ± 0.09^{a}	12	1.43 ± 0.08^{a}	6	1.60 ± 0.12^{a}

*Scheffe's test in One Factor ANOVA. The values in the same column with different superscript letters are significantly different ($P < 0.05$).

growth rate at day-7 of the males born in July were highly correlated with the birth-weight (Table 3-4). In the males born in October, however, all measured weights and growth rates were highly correlated with the birth-weight (Table 3-4). There seems to be a tendency that when the whether becomes warmer, the birth-weight becomes a more important factor in postnatal growth. The rats, however, were all born in the laboratory where temperature and light were maintained consistent. Why should there be such a difference among the rats born in different months? These rats were the first or second generation of wild rats. It is possible that the rats have inherited the ability of sensing the natural rhythm although they are kept in controlled environments (Pennycuik, 1972; Drickamer and Vestal, 1973). It is therefore suggested that the birth-weight is not a factor in the growth and development of the males in winter and spring but it is highly correlated with postnatal growth in summer and autumn. The growth rates before weaning of the males born in different months, on the other hand, were not statistically different (Table 3-2). During three weeks pre-weaning development the males born in different months all grow faster in the second week than in the first week, and the fastest in the third week, or G-21 > G-14 > G-7 (Table 3-3). These results are consistant with the report by Lin and Xin (1962) that at the age of 15 days young rats start to chew the food pellets while they are still suckling. This provides extra nutrition to the growth of the young.

3.4-2. Growth and correlations

The data on growth and development of the males from 20 days of age to 130 days of age lead to the conclusions that (1) Males in this species mature in three stages. First, there is a drastic elevation of testosterone titre in blood (Fig. 3-2) at about 60 days of age. This is accompanied by the appearance of mature sperm in convoluted ducts of epididymes. Second, the growth of body and testes reaches a

fluctuating plateau between the ages of 70 - 80 days. Third, the cessation of the growth of the epididymides is coincident with the success of reproduction at about 100-110 days of age. (2) The age is highly correlated ($P < 0.001$, Table 3-5) with the increase in body weight ($r = 0.806$), testis weight ($r = 0.851$) and length ($r = 0.907$), epididymis weight ($r = 0.888$), body length ($r = 0.785$) and tail length ($r = 0.674$). (3) Body weight is highly correlated ($P < 0.001$, Table 3-6) with testis weight ($r = 0.855$), testis length ($r = 0.882$) and epididymis weight ($r = 0.739$). (4) Finally, testis length is highly correlated ($P < 0.001$, Table 3-7) with the testis weights ($r = 0.947$) and epididymis weight ($r = 0.690$).

Testis weight and length, and the lengths of body and tail increases fast in the period from weaning (21 days) to 70 days (Fig. 3-1). When the rats reaches 70 days, the growth rate slows and the rat enters a growth plateau with a fluctuation of body weight at about 100 g, testis weight of 1.5 g, testis length 2 cm, the lengths of body and tail at 15 cm and 13 cm respectively, indicating that male *Rattus rattoides* are physically mature at the age of 70 days.

Examination of endocrine and sexual gland activity further support the above results. Testosterone concentration was very low at 40 and 50 days but elevated sharply at 60-days and never fell to pre-60-day level from day 60 on (Fig. 3-5). Anatomical examination of epididymes showed that mature sperm appeared in the convoluted ducts of epididymes as well as in the seminiferous tubules of testes, at the same time as when the plasma testosterone concentration increased sharply. While testosterone concentration reached an asymptote 10 days earlier than body and testis growth did, the growth and development of epididymes was 30 days later than those aspects. The epididymes did not grow substantially until about 50 days of age (30 days later than the other features) and ceased growth at the age of 110 days (40 days later than the others) (Fig. 3-1 c).

The phenomenon of desynchronization of the growth of the epididymes and the development of testosterone concentration with the growth of body weight, testis weight and length was previously reported in albino mice by Svare *et al.* (1978). They found that the body weight of the mice increased greatly between 22 and 32 days of age, testis weight between 32 and 42 days of age, and plasma androgen levels between 32 and 52 days of age. Miller *et al.* (1977) also reported that male golden hamsters reached sexual maturity in two stages. First stage there was a rapid increase in body weight, testis weight, plasma testosterone levels, and flank-gland size; secondly there was an increase in sperm concentration and the frequency of sexual behaviours including mounting, intromission, and ejaculation. It is therefore suggested that the elevation of testosterone concentration in the blood (at about 60 days of age) is the initiating factor of sexual maturity in *Rattus rattoides*, triggering the maturity of sperm in the testes and the transportation of the sperm into epididymes. The growth of body and testes reaches an asymptote at about 70 days of age, and finally the growth of the epididymes reaches its maximum rate from 100 days to 110 days of age. This is accompanied by successful reproduction (refer to 3.2-2), the operational criterion of maturity.

The correlations between body weight and both testis weight and length, the correlations between testis length and the weights of testes and epididymes enable an estimate to be made of the weights of testes and epididymes. This is an estimate of the maturity of the rats caught in the field.

3.4-3. Post-weaning growth of the males born in different months

There were not much difference in post-weaning growth among the males born in different months. When the males were at and over 50 days old the weights were not statistically different (Table 3-8). This result and the result discussed in 3.4-1 suggest that the males born in different months may have some differences in

physical growth in early development but the difference will disappear 10 days before the males enter the first stage of maturation (3.4-2).

The development of testis length of the males born in different months, on the other hand, had significant differences except on day-60 (Table 3-9). The wide scale difference before day-60, no difference on day-60 and only the difference between April and other three months indicate again that day-60 is the turning point of the males' growth and development.

The differences among the males born in different months were unexpected since the rats were kept in the laboratory under consistent conditions. As has been mentioned in 3.4-1 that the rats might have inherited the ability to sense the seasonal changes in the nature even though they were in artificially controlled laboratory conditions.

3.4-4. Summary

During pre-weaning growth the young grow significantly faster in their third week than in the first two. Birth-weight should be considered as a factor contributing to postnatal growth at least when test rats are born in summer and autumn in the laboratory. During post-weaning growth age correlates well with the growth and development of body weight, testis weight and length, epididymis weight, and the lengths of body and tail. Body weight correlates well with testis weight and length and the weight of epididymes. Testis weight correlates well with the length of testes and the weight of epididymes.

Male *Rattus rattoides* mature in three stages, starting on day-60 with the elevation of plasma concentration and the appearance of mature sperm in convoluted ducts of epididymes as well as in seminiferous tubes of testes. Then, this is followed by the second stage of growth deceleration in body weight, testis weight

and length, and the lengths of body and tail on day-70. Finally the third stage of maturity is seen in the decline of the rate of epididymis growth on day-110. The pattern of attainment of sexual maturation of the males includes the convergence of the males born in different months of body weight at day-50 and testis length at day-60.

Chapter 4. The growth and development of female Rattus rattoides in captivity

4.1. Introduction

This chapter describes the growth and development of female *Rattus rattoides,* both before and after weaning. This is the first report of such basic information of the growth and reproduction of females in captivity.

4.2. Materials and methods

To determine the growth before weaning females were weighed one day after birth (W-1) and again six days later (W-7), and then at 7-day intervals until weaning (day-21). Data were collected from the females born in January, April, July and October. The correlation between the body weight at given ages and the weight at birth and correlation between growth rates in the different periods and the body weight at birth were calculated.

To determine the growth after weaning, females born in March, June, September and December were used. They were weighed 9 days after weaning (at age 30 days) and then at 10-day intervals until age 80 days. Correlations of the weights at given ages with the weight at birth or at weaning were calculated.

Four to five females were sacrificed by cervical dislocation at 10-day intervals from day-20 to day-130. Body weights and the weights of ovaries and uteri were measured.

All test individuals were kept in the laboratory with controlled temperature and light regime. Date were analysed with One factor ANOVA, Repeated measures with grouping factors and Pearson correlation test.

4.3. Results

4.3-1.Pre-weaning growth of females born in different months

Females born in January had significantly heavier body weights at day-1, day-7 and day-14 than those born in July and October and females born in April had significantly heavier body weights at day-1 than those born in October (Table 4-1).

*Table 4-1: Mean body weight (W-day) at given ages of the females born in different months (one factor ANOVA)**

	W-1 (g)	W-7 (g)	W-14 (g)	W-21 (g)
January	4.69 ± 0.13^{a}	10.98 ± 0.61^{a}	17.05 ± 0.76^{a}	22.83 ± 1.07^{a}
April	4.44 ± 0.14^{ab}	$9.07 \pm 0.0.35^{ab}$	14.80 ± 0.47^{ab}	20.16 ± 0.65^{a}
July	4.08 ± 0.11^{bc}	8.75 ± 0.34^{b}	13.72 ± 0.59^{b}	20.15 ± 0.68^{a}
October	3.89 ± 0.14^{c}	8.26 ± 0.63^{b}	12.43 ± 0.89^{b}	19.09 ± 1.15^{a}
F	5.775	4.668	6.148	2.381
P	< 0.001	< 0.005	< 0.001	> 0.05

* January n = 43, April n = 52, July n = 30 and October n = 21.

The weights in the same column with different superscripts are significantly different ($P < 0.05$).

By the time of weaning (day-21) the body weights of all the females born in different months were not significantly different. The growth rates of females born in different months were not statistically different ($F = 2.237$, $P > 0.05$, Table 4-2). The growth rates in different weeks, however, were significantly different ($F =$

35.432, P < 0.001, Table 4-2). In the females born in January, the growth rates in the three weeks were not significantly different from each other (Table 4-2, Appendix 4-2). For the females born in April, growth rate in the first week (G-7) was significantly smaller than the growth rates in the second week (G-14, F = 4.229, P < 0.01) and the third week (G-21, F = 5.731, P < 0.005) (Table 4-2, Appendix 4-2). Growth rate in the third week of the females born in July was significantly greater than the rates in the first week (F = 17.891, P < 0.0001) and second week (F = 10.447, P < 0.0001) (Table 4-2, Appendix 4-2). The growth rate in the third week of the females born in July and October was significantly greater than in first week (F = 6.224, P < 0.001) and second week (F = 4.04, P < 0.01) (Table 4-2, Appendix 4-2).

The weights at the given ages were significantly correlated with birth weights in the females born in April, July and October, but not in the females born in January (Table 4-3). Pre-weaning growth rates were significantly correlated with birth weighs only in the females born in October (Table 4-3). In addition, mean pre-weaning growth rate of the females born in July was also significantly correlated with the weight at birth (Table 4-3).

4.3-2. Post-weaning growth of the females born in different months

Mean body weight at day-40 of the females born in September was significantly lighter than the weights of those born in March (F = 6.782, P < 0.005), June (F = 21.873, P < 0.001) and December (F = 5.206, P < 0.005) (Table 4-4 and Appendix 4-3). At the same time, mean body weight of the females born in June was significantly heavier than that in the females born in December (F = 7.02, P < 0.001). At age-50 days, mean body weight of females born in September was significantly lighter than those of the females born in March

*Table 4-2: Pre-weaning growth rates (g/day) in different weeks of the females born in different months**

	January (21)	April (43)	July (52)	October (30)
G-7	0.75 ± 0.07^{a}	0.60 ± 0.04^{a}	0.59 ± 0.31^{a}	0.56 ± 0.06^{a}
G-14	0.88 ± 0.05^{a}	0.82 ± 0.04^{b}	0.69 ± 0.42^{a}	0.63 ± 0.05^{a}
G-21	0.99 ± 0.15^{a}	0.85 ± 0.06^{b}	1.03 ± 0.06^{b}	0.92 ± 0.07^{b}
G-m	0.87 ± 0.05^{a}	0.75 ± 0.03^{ab}	0.76 ± 0.03^{ab}	0.70 ± 0.05^{ab}

Repeated measures

Between subjects

Source	SS	DF	MS	F	P
Month	1.090	3	0.363	2.237	0.087
Error	23.137	142	0.163		

Within subjects

Sources	SS	DF	MS	F	P (H-F)
a	6.973	2	3.487	35.432	0.000
a*month	1.378	6	0.229	2.326	0.048
Error	27.946	284	0.098		

* G-7: growth rate from day-1 to day-7, G-14: day-8 to day-14, G-21: day-15 to day-21, G-m: mean growth rate from day-1 to day-21.

The values in the same row with different superscripts are significantly different (for P-values see text).

Table 4-3: The correlation (r) of body weight (g) and growth rate (g/day) at day-7 (W-7 and G-7), day-14 (W-14, G-14), day-21(W-21, G-21) and mean growth rate (from day-1 to day-21, G-m) with the weights at birth (W-1) of the females born in different months (Pearson correlation test).

	n	W-7	W-14	W-21
January	21	0.303	0.080	0.265
April	43	0.620**	0.613**	0.490*
July	52	0.809**	0.666**	0.571**
October	30	0.799*	0.817*	0.842*

	n	G-7	G-14	G-21	G-m
January	21	0.013	0.256	0.272	0.141
April	43	0.367	0.234	0.039	0.365
September	52	0.629	0.351	0.229	0.480*
October	30	0.676**	0.737**	0.687**	0.804**

* $P < 0.01$, ** $P < 0.001$.

($F = 4.905$, $P < 0.01$) and June ($F = 6.658$, $P < 0.005$). At day-60 and day-70, the mean body weight of females born in September was significantly lighter than those of the females born in June ($P < 0.05$). By the time of day-80, however, there were no significant differences among the females born in different months (Table 4-4 and Appendix 4-3).

Table 4-4: Body weights (W-day, g) of the females born in didfferent months (one factor ANOVA).

Month n	W-30	W-40	W-50	W-60	W-70	W-80
Mar. 12		41.58 ± 5.27^{a}	51.95 ± 4.33^{a}	62.25 ± 8.86^{ab}	77.25 ± 9.38^{ab}	76.1 ± 6.85^{a}
Jun. 9		52.28 ± 3.71^{ab}	55.56 ± 3.93^{a}	67.72 ± 5.26^{a}	78.19 ± 8.27^{a}	79.83 ± 6.48^{a}
Sep. 12	16.75 ± 0.84	23.27 ± 1.63^{c}	33.91 ± 3.11^{b}	45.21 ± 3.8^{b}	51.73 ± 3.28^{b}	55.08 ± 4.27^{a}
Dec. 14	28.07 ± 0.73	36.12 ± 1.14^{a}	45.62 ± 2.1^{ab}	53.89 ± 3.79^{ab}	59.89 ± 4.53^{ab}	67.17 ± 5.69^{a}
F		22.725	8.099	4.128	4.93	2.422
P		< 0.001	< 0.001	< 0.05	< 0.01	> 0.05

Pearson analysis of the correlation of the weights at the given ages with birth weights of the females shows that the weights at the given ages were not correlated with birth weight (Table 4-5). The weights at day-40, day-60 and day-70, however, were correlated with the weight at weaning among the females born in March (Table 4-6). For the females born in September, body weights at day-30, day-40, day-50 and day-60 were correlated with the weight at weaning. Among the females born in December, the weights at day-40, day-50 ad day-60 were correlated with weaning weight. Body weights of the females born in June were not correlated with weaning weight.

Table 4-5. Pearson correlation (r) of post-weaning body weights at given ages (W-day) with birth weight (W-1)

	n	W-30	W-40	W-50	W-60	W-70	W-80
Mar.	12		0.523	0.569	0.643	0.564	0.373
Jun.	9		0.101	- 0.010	0.033	0.002	0.003
Sep.	12	0.159	- 0.163	- 0.493	- 0.369	- 0.303	- 0.175
Dec.	14	- 0.265	- 0.261	0.072	- 0.002	0.073	- 0.270

4.3-3. Sexual development of the females

The body weights, and the weights of ovaries and uteri from day-20 to day-130 of the females born in the laboratory increased steadily from day-20 to day-80 and then either decelerated and entered a growth plateau (Fig. 4-1, a) or fluctuated around the weight at day-80 (Fig. 4-1, b and c). The weights of ovaries and uteri at

the most of the given ages were not correlated with body weights (Table 4-7). The correlation was found only between the uterine weight and body weight at age-80.

Table 4-6. Pearson correlation (r) of the weights at given ages (W-day) with the weight at weaning (W-21).

	n	W-30	W-40	W-50	W-60	W-70	W-80
Mar.	12		0.704*	0.431	0.801**	0.741**	0.084
Jun.	9		0.470	0.321	0.236	0.140	0.202
Sep.	12	0.906**	0.718**	0.582*	0.576*	0.502	0.501
Dec.	14	0.051	0.560*	0.657*	0.578*	0.625*	0.628*

* $P < 0.05$, ** $P < 0.001$ (Bonferoni adjusted value).

4.3-3. Female reproduction in captivity

Wild adult females and those born and reared in the laboratory were singly-caged in the laboratory. Certain numbers of the females were paired each month with adult males for 5 days to produce offspring for experiments. Vaginal plugs were observed during the period of pairing in some but not in all of the females which gave birth 21 days later. The youngest female to give birth was 83 days of age, 33 days after attaining puberty.

The breeding rate, measured by dividing the number of the females giving birth with the number of females paired, was recorded monthly and is shown in Table 4-8. The percentage of the females impregnated on the 4th day of the 5-day duration was significantly greater than on the other days. The average breeding rate

in the year was 20.36% ranging from 9.52% in August to 29.73% in October.

Table 4-7: Pearson correlations (r) of ovarian weights (OW) and uterine weights (UW) with body weights at given ages (days).

Ages	n	OW	UW
21	7	0.470	0.713
30	4	- 0.048	0.777
40	4	0.764	0.851
50	4	0.118	0.175
60	4	- 0.462	0.717
80	4	- 0.220	- 0.960*
90	4	0.665	0.702
100	3	0.929	0.926
110	3	- 0.017	- 0.409
120	5	0.722	0.731
130	5	- 0.759	- 0.434

* $P < 0.05$ (Bonferoni adjusted value), ln-transformed data used in the analysis.

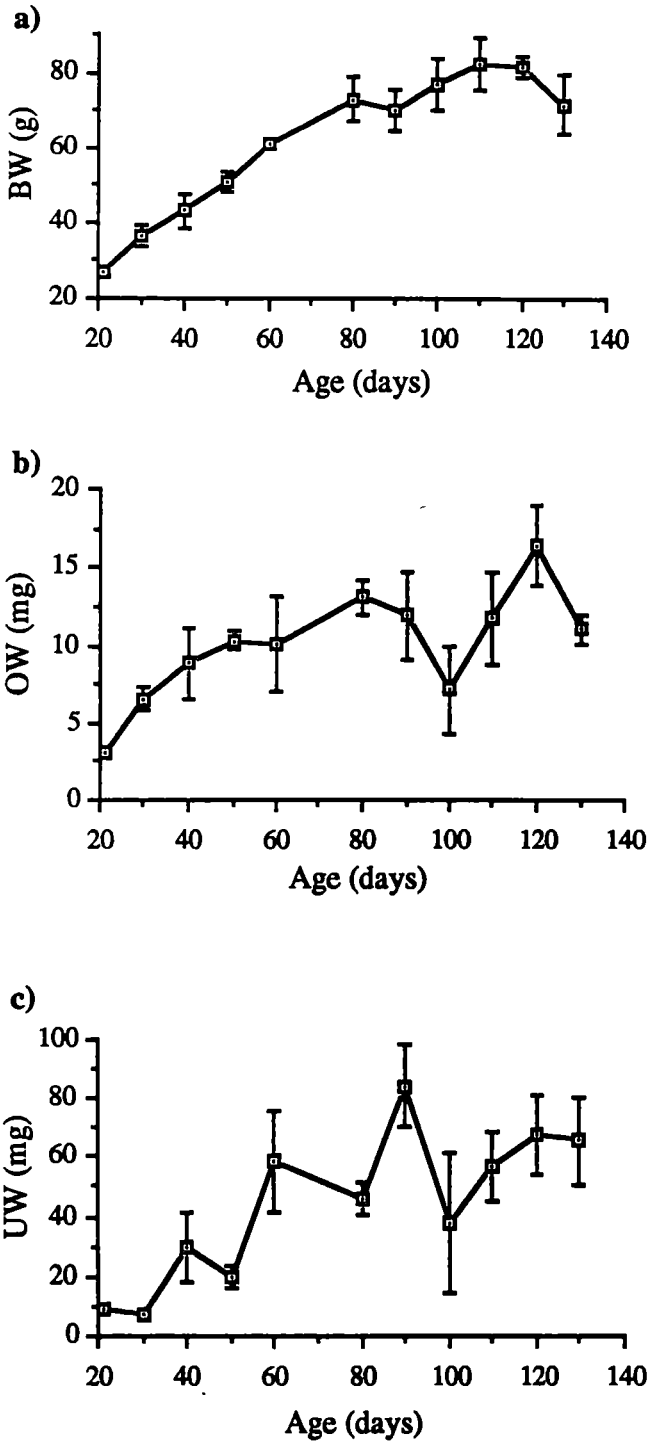


Fig. 4-1: Growth curves (mean ± SE) of body weight (BW, a), ovarian weight (OW, b) and uterine weight (UW, c) of the female Rattus rattoides

Table 4-8: Reproductive capability of the females in captivity [1]

Month	No. Paired	No. Bred	% Bred	% mated during the pairing days day-1	day-2	day-3	day-4	day-5
Jan.	122	29	23.77	44.83	17.24	13.79	20.69	3.45
Feb.	195	20	10.26	45.0	25.0	5.00	5.00	20.0
Mar.	139	30	20.58	16.67	26.67	13.33	26.67	10.0
Apr.	50	12	24.0	8.33	16.67	8.33	50.0	8.3
May	48	13	27.08	23.08	7.69	7.69	30.77	23.08
Jun.	90	17	18.89	11.76	5.88	17.65	29.41	5.88
Jul.	42	11	26.19	9.09	9.09	18.18	45.45	9.09
Aug.	42	4	9.52	0.0	25.0	25.0	25.0	25.0
Sep.	43	8	18.6	25.0	25.0	12.5	37.5	0.00
Oct.	37	11	29.73	36.36	18.18	27.27	36.36	0.00
Nov.	49	9	18.37	0.00	0.00	11.11	44.44	33.33
Dec.	49	8	16.33	0.00	12.5	50.0	12.5	25.0
mean			20.36	18.34*	15.74**	17.49*	30.32	13.6***
±SE			1.82	4.8	2.54	3.53	3.89	3.23

[1] Females were paired for six days in April and May, nine days in June, and five days in all other months. The percentage of females mated on 6th day was 8.33% in April and 7.69% in May. No females were mated beyond 6th day.

*: Significantly different from day-4 at 95%.

**: Significantly different from day-4 at 99%.

***: Significantly different from day-4 at 99.5%.

Apart from the experiments of pairing the females with males for five days and then separating them, as mentioned above, twelve pairs of the rats were kept separately in large arenas each measuring 1.5 x 0.7 m and not separated to see if there is a postpartum oestrus. Rice hulls, straw and bricks and a small cage were placed in the arena. Food and water were provided *ad libitum*. One of the females were found to be able to breed in 22 day intervals for two litters and another one bred in 22 days intervals for three litters.

The females, no matter whether before or after mating, during pregnancy or lactating, were aggressive to the males. The males, on the other hand, were apparently subordinate to the females. The female was busy with building her nest by drawing straw into the cage, heaping up the rice hulls around the cage and pulling every piece of brick onto the cage leaving only a hole as the entrance to the nest. The male, however, stayed in the corner of the arena without rice hulls or other materials. All the males were frequently attacked, which left obvious wounds in their tails. In fact, most stud males in the laboratory were severely beaten, many of them were tailless after a few pairing and a few were even killed during the pairing period. In the arena the male was always kept away from the cage by the female, which was the only access to water, so that another cage had to be put in to provide the male with water. When the female's comfortable nest was wet because of the water leakage, she immediately took over and the male's cage and expelled him from it.

In one arena, a young male and a young female from the female's first litter were kept with their parents. They both shared the nest with the mother; the father stayed outside the cage in the corner. When the mother delivered the third litter the young male was driven away from the nest and stayed with his father while the young female remained inside the cage with her mother and the neonates. The

neonates were well looked after. The males in the arenas never attacked the young weanlings.

4.4. Discussion

4.4-1: The growth of the females in the laboratory

It was described in Chapter 3 that birth weights were different in males born in different months but the difference became less significant and eventually faded at the end of the three-week pre-weaning growth period. A similar phenomenon occurs in females; there were also significant differences in body weights at the first three measurements among females born in different months but no statistical difference in body weights at the time of weaning (Table 4-1). As with males, females grow fastest in the third week of the pre-weaning period. As mentioned in Chapter 1.2-3 and Chapter 3.4-1 that at about age 15 days the first molar appears and the rats start to chew solid food while still sucking milk. This increases nutrition-intake and accelerates the growth.

During pre-weaning growth, body weights at 7-, 14- and 21-day of ages were significantly correlated with the weight at birth in females born in the months tested except January (Table 4-3). Growth rates in the same periods, on the other hand, were significantly correlated with the weight at birth only in the females born in October. These results indicate that body weight at birth is an important factor influencing the body weights at given ages during pre-weaning growth period of the females born in April, July and October. It is also an important factor influencing the growth rate during pre-weaning growth period of the females born in October but not in January, April and July.

Post-weaning body weights do not correlate with the weight at birth (Table 4-

5). The weights, however, were significantly correlated with the weights at weaning in the females born in March, September and December (Table 4-6). Body weight at weaning, therefore, is influential to post-weaning growth in the females born in the three months.

The growth of ovaries and uteri, like the growth of body weight, increases steadily from weaning to age 80 days, and then fluctuate around the weights gained by that age (Fig. 4-1). The weights of ovaries and uteri at given ages, however, were not correlated with the body weights (Table 4-7), indicating that body weight is not a factor influencing the growth of these organs. The growth of body weight and ovarian weight are quite smooth but the growth of the uteri fluctuates markedly (Fig. 4-1).

4.4-2: Reproductive capability and behaviour of the females in the laboratory

R. rattoides is an agricultural pest in southern China yet little is known about its biology, especially reproductive biology. There are only a few articles about the rat and two of them presented field data of reproduction of the rat. The pregnancy rate was obtained by dividing the total number of adult female rats caught with the number pregnant, following monthly trapping. Qing and Wang (1981) reported that the rat breeds through the year, except in December. The average pregnancy rate was 29.22%, ranging from 0% to 72.41%. Zhan (1982) notified, however, that the rats did not breed from November (late autumn) to February. He recorded the mean pregnancy rate as 24.17% in average, raging from 0% to 63.63%. The data from them and the data collected in this study are listed together for comparison in Table 4-9.

There are obvious differences between the monthly pregnancy rates in the three studies. Firstly, the data reported by Qing and Wang (1981) were obtained in

Table 4-9: Reproductive data of R. rattoides from different researchers

Sources	Xu (1991, Fujian)			Zhan (1982, Fujian)			Qing & Wang (1981, Guangdong)		
Month	No. of females paired	No. of females bred	Pregnant rate (%)	No. of females caught	No. of females pregnant	Pregnant rate (%)	No. of females caught	No. of females pregnant	Pregnant rate (%)
Jan.	122	29	23.77	-	-	0	60	18	30.00
Feb.	195	20	10.26	-	-	0	27	8	29.63
Mar.	139	30	20.58	-	-	28.54	90	34	37.78
Apr.	50	12	24.00	-	-	50.00	35	6	17.14
May	48	13	27.08	-	-	31.25	39	1	2.56
Jun.	90	17	18.89	-	-	36.36	14	2	14.29
Jul.	42	11	26.19	-	-	10.00	17	8	47.06
Aug.	42	4	9.52	-	-	63.63	23	7	30.43
Sep.	43	8	18.6	-	-	43.75	20	7	35.00
Oct.	37	11	29.73	-	-	27.27	29	21	72.41
Nov.	49	9	18.37	-	-	0	31	11	35.48
Dec.	49	8	16.33	-	-	0	36	0	0
mean			20.36			24.17			29.22

Guangdong Province about 900 kilometers south-west of Fujian Province where Zhan conducted his survey. The geographical distance may count for the difference. Secondly, those two reports are based on field investigations while the data in this thesis were obtained in the laboratory. The rats, when housed in controlled laboratory condition, bred in every month including winter when those in the field were not (Table 4-9). Finally, although the pregnancy rates in so-called reproductive peaks in Guangdong (October) and Fujian (April and August) are quite high the average rate is not much higher than the laboratory rate. Additionally, it was noted by Lin and Xing (1962) that only 25% or more of the rats caught in the fields would have reproductive capability, judging by the presence of uterine scars.

It was reported by Whitten (1956) that when grouped female mice were exposed to a male, the majority of them were stimulated to enter oestrus on the third night. An insemination peak was found in the present study on the fourth day of the 5-day pairing period, although the female rats were not grouped before pairing. This phenomenon suggests that the males are able to induce oestrus in females in four days after they encounter the females, and that pre-encounter grouping of the females is not required.

Behavioural dominance of females over males has been noticed in this species. Whenever a female is paired with a male she starts to attack him and it is always the male that will be subordinated after the conflict. Yet, the female will be fertilised, though the male experience cuts and bruises to his tail and sometimes be killed. Though preliminary, these data suggest the adults live solitary lives.

4.4-3: Summary

Female *Rattus rattoides,* like the males, can have significantly different weights at birth, but by the time of weaning there are no significant differences in

the body weights of the females born in different months. The females also grow fastest in the week. Physical growth and the growth of sexual organs are rapid from weaning to 80 days, then decelerated and fluctuated around the weights gained by then.

Body weights at given ages before weaning were significantly correlated with the weight at birth in the females born in April, July and October but not January. Body weights at given ages after weaning were not correlated with the weight at birth but at weaning in the females born in March, September and December. The development of sexual organs was not correlated with physical growth.

The females can reproduce throughout a year with two low breeding rates in February and August in the laboratory. A post-partum oestrus was observed in the females. It was also observed that males were behaviourally subordinate to females.

Chapter 5. The oestrous cycle of Rattus rattoides - a laboratory study

5.1. Introduction

The oestrous cycles of some species of rodent - mainly laboratory species - are well documented. The length of the oestrous cycle of the laboratory rat is 4-5 days (Freeman, 1988; Bronson, 1989), while that of the laboratory mouse is reported to be 4-6 days (Whitten, 1956; Marsden and Bronson, 1965; McClintock, 1983). Wild rats (*Rattus l. lutreolus*), on the other hand, were reported to have 4.8-6 days cycle lengths (Breed, 1978).

It is well established in many species that a male, or its urine, can induce shortened oestrous cycles in conspecific females. Urine from male rats can reduce a 5-day cycle into 4-day cycle in females (Hughes, 1964; Chateau *et al.*, 1972; Aron and Chateau, 1971). It can also induce reflex ovulation in females that are not cycling because of environmental factors such as inadequate food (Cooper and Hayes, 1967), or constant light (Johns *et al.*, 1978).

The laboratory female Wistar rat was found to have shorter oestrous cycles (instead of longer oestrous cycles which mice would have) when they were caged in groups after being isolated from weaning to adulthood (Roser and Chateau, 1974), or exposed to female urine (Aron, 1973). Group housing also shortened the oestrous cycles of Sprague-Dawley rats which had been living previously in a colony room (McClintock, 1981). Female rats can produce two antagonistic pheromones during oestrus and the one which shortens oestrous cycles of others (called follicular odour) is able to override the other (called ovulatory odour), which tends to prolong the cycle (McClintock, 1983 a; 1983 b).

The effect of group housing on the oestrous cycle may vary in accordance with different laboratory stains. The variance of sensitivity in different oestrous phases of the recipients may also modify the results (Champlin, 1971; van der Lee and Boot, 1955; Dewar, 1959; Ryan and Schwartz, 1977; McClintock, 1983 b). It can, therefore, be even more variable in wild species. So far there are no data on the oestrous cycle of the wild rat, *Rattus rattoides*. The purposes of the experiments designed here are firstly to observe the lengths of oestrous cycle of the wild rat in captivity, and secondly, to see if housing density and the presence of an adult male or his odour will also affect the oestrous cycle in this species, as happens in the other species mentioned.

5.2. Materials and methods

More than 250 wild rats were caught from Lanqi Isle, south of Fuzhou City, Fujian Province, China, in November 1989. Thirty-five young females were chosen to be housed singly in standard plastic cages of 29 x 18 x 16 cm with water and food (Shanghai Animal Food Company, Shanghai, China) available *ad libitum*, and in a separate room free of males' odour. The room was adjusted to a temperature of 22 - 25°C, and a light regime of 12 hrs light (6:00 am - 6:00 pm) and 12 hrs dark (6:00 pm - 6:00 am). Each of the wild rats was examined daily for the first sign of vaginal introitus.

To determine the length of the oestrous cycle, vaginal lavage was conducted among the females whose vaginae were perforated from 8:00 am to 9:00 am daily for 30 consecutive days (17th December, 1989 - 16th January, 1990). The wet-mount vaginal smears were examined immediately with a light microscope (Drickamer, 1974) and the cellular contents were judged according to the criteria of Rugh (1968) and Vandenbergh (1969) to determine the phases of oestrous cycle.

The smears were classified as epithelial (E) smears (with more than 80% nucleated or cornified epithelial cells, as seen at proestrus and oestrus in young rats), or as leukocytic (L) smears (with more than 20% leukocytes as seen at metoestrus or dioestrus in young rats), (Mandl, 1961; Champlin, 1971; Hoover and Drickamer, 1979; LeFevre and McClintock, 1988). E-smear represents follicular growth and ovulation phases (Nequin *et al.*,1979) and therefore, represents proestrus and oestrus phases; while L-smear represents luteal activity (Parkes, 1929) and so represents metoestrus and dioestrus.

In all the experiments designed here each cycle was divided into two parts: one was labelled E, containing phases of proestrus and oestrus; the other was labelled L, containing metoestrus and dioestrus. Two sets of data were collected and analysed: mean lengths of oestrous cycles, E-smears and L-smears, and the distributions of different lengths of oestrous cycles, E-smears and L-smears. Firstly the examination of the mean lengths of oestrous cycles was carried out because there were no such data in this species. The length of E-smears and L-smears were also checked for the purpose of knowing how each of the two parts contribute to the length of an oestrous cycle. Secondly the distributions of the lengths of E-smears and L-smears were examined to see if they fell into different styles in different seasons or with different treatments.

Occasionally the oestrous cycles were found to exceed 10 days. They were omitted from the analysis for the reason that their inclusion would distort the value for the mean oestrous cycle length, following the rationale of Breed (1978).

In data analysis, data collected from a group of female rats in 30-day examination were pooled and compared with the pooled data from the other groups. One factor ANOVA test, Student's t-test and contingency table analyses were used.

Experiment 5-1: This experiment was designed to assess the lengths of

oestrous cycles of the rats caught from the field. As reported in Chapter 3 and Chapter 4 that the rats could, to some extent, sense the seasonal changes although they were housed in consistent laboratory environment. It was assumed different lengths of oestrous cycles in different seasons might also be found in the rats in laboratory. The lengths of the oestrous cycles of the rat were therefore checked and recorded by seasons. The lengths of the oestrous cycles were assessed with vaginal lavage.

After 30 days of daily examination, the rats were reared without disturbance in the same room and under the same conditions until the following spring (in 1990). They were examined again for 36 days from 26th March to 30th April. Using the same procedure the females were examined in summer (10th May to 9th June, 1990) and autumn (1st September to 30th September, 1990).

Experiment 5-2: To examine if group housing will shorten or lengthen the oestrous cycle in the rat, 40 females were assigned randomly to two groups in the summer of 1990, one consisting of 4 cages each holding 5 rats and the other consisting of 20 separately caged ones. Vaginal smears were taken from all rats daily for 30 days.

Experiment 5-3: To examine if the presence of an adult male will induce the change of the length of oestrous cycles 40 female rats were divided into two groups, in the autumn of 1990, one consisting of 20 singly caged females and the other of 20 females each cohabiting with an adult male. Vaginal smears were examined daily throughout the test period.

5.3. Results

5.3-1. Oestrous cycles of the females in captivity

5.3-1-1. Oestrous cycle lengths

The mean lengths of oestrous cycles of the rats in four seasons in captivity, when examined with one factor ANOVA test (Table 5-1, also see Appendix 5-1), shows no statistically significant difference to each other ($F = 1.09$, $P > 0.05$).

The distributions of the cycles in different lengths in the seasons are analysed with contingency table (Fig. 5-1, Appendix 5-2). There was no significant difference among the distributions in different seasons, although the χ^2 value closely approached significance ($DF = 21$, $\chi^2 = 32.06$, $P = 0.0577$).

5.3-1-2. Lengths of E-smear and L-smear phases in the oestrous cycle

The oestrous cycle, as mentioned before, has been divided into two parts: the period with E-smear including proestrous and oestrous phases (E) and the period with L-smear including metoestrous and dioestrous phases (L).

The mean length of E-smears in winter was significantly shorter than in other three seasons ($F = 4.14$, $P < 0.01$, Table 5-1 and Appendix 5-1). The E-lengths in other three seasons were not statistically different (Table 5-1 and Appendix 5-1). No statistical difference was found in the mean lengths of L-smears in different seasons ($F = 1.52$, $P < 0.2074$, Table 5-1 and Appendix 5-1).

The distributions of E-smears of differing lengths were significantly different among the seasons ($DF = 21$, $\chi^2 = 43.57$, $P < 0.005$, Fig. 5-2 and Appendix 5-3). The distribution in spring was significantly different from summer ($DF = 7$, $\chi^2 = 14.62$, $P < 0.05$) and winter ($DF = 7$, $\chi^2 = 20.43$, $P < 0.005$), but not from autumn ($DF = 7$, $\chi^2 = 5.35$, $P > 0.05$). The distribution of E-smears in summer was significantly different from autumn ($DF = 5$, $\chi^2 = 11.41$, $P < 0.05$) and winter ($DF = 6$, $\chi^2 = 25.58$, $P < 0.001$). Furthermore, the distribution in autumn was

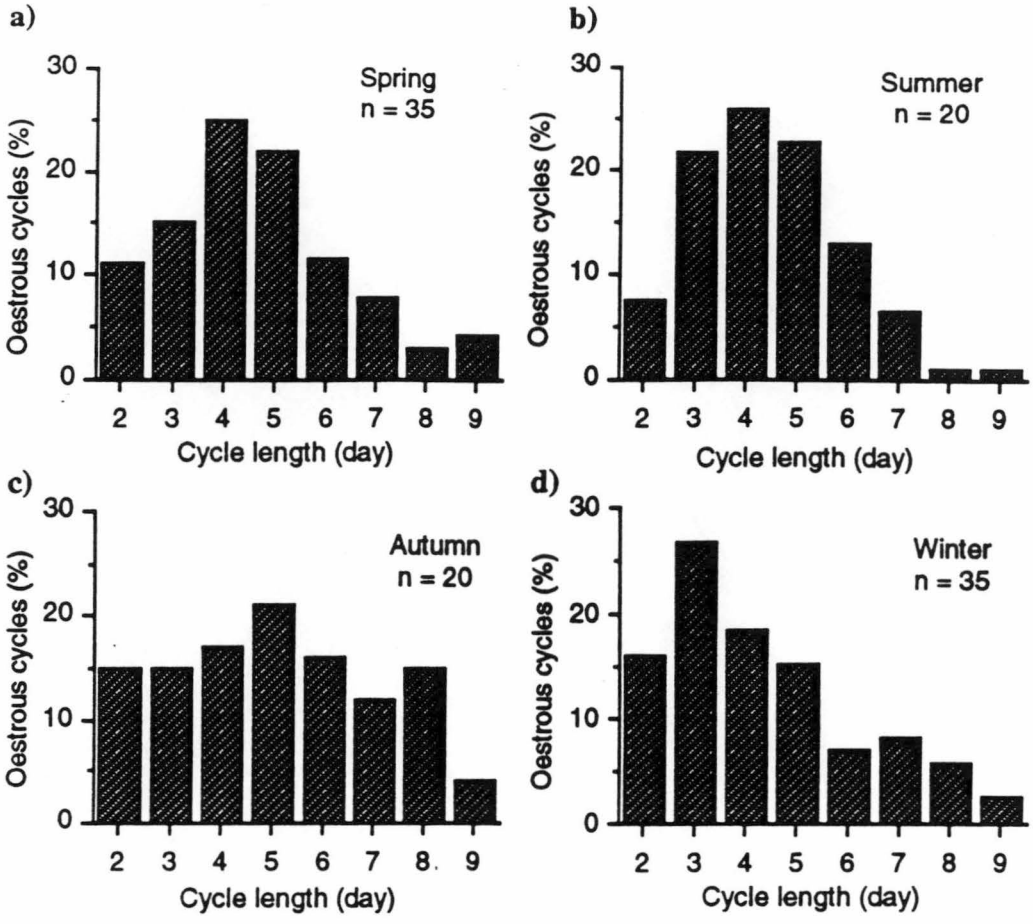


Fig. 5-1. The percentage of the oestrous cycles of differing lengths of the females in spring (a, 164 cycles), summer (b, 92 cycles), autumn (c, 100 cycles) and winter (d, 157 cycles).

n: number of rats examined in each season.

*Table 5-1. Mean lengths (day ± SE) of oestrous cycles and the constituents of E-smear and L-smear of the females in four seasons.**

Seasons	Total Cycles	Length (day)	E-smear (day)	L-smear (day)
Spring	164	4.65 ± 0.14	2.45 ± 0.11[a]	2.21 ± 0.1
Summer	92	4.41 ± 0.15	2.46 ± 0.12[a]	1.95 ± 0.12
Autumn	100	4.54 ± 0.18	2.36 ± 0.14 [a]	2.18 ± 0.13
Winter	157	4.31 ± 0.15	1.97 ± 0.11[b]	2.34 ± 0.13
ANOVA		F = 1.09	F = 4.14	F = 1.52
		P = 0.3548	P = 0.0065	P = 0.2074

*: The values with different superscript number in the same column are significantly different at 0.05 level.

Number of rats examined in spring and winter, 35; summer and autumn, 20.

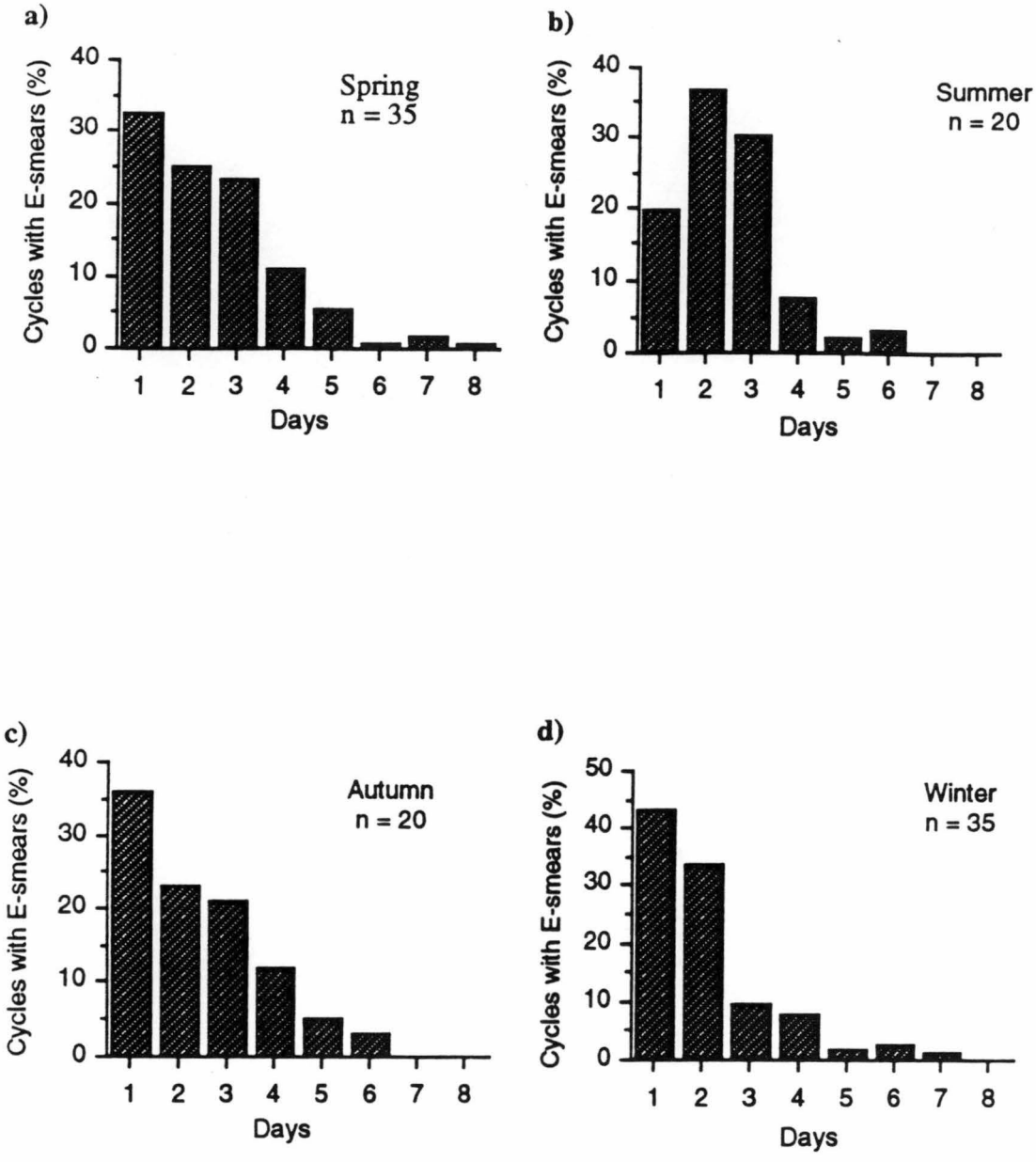


Fig. 5-2. The percentage of E-smears of differing lengths in the cycles in spring (a, 164 cycles), summer (b, 92 cycles), autumn (c, 100 cycles) and winter (d, 157 cycles).

n: the number of rats examined in each season.

significantly different from winter (DF = 6, χ^2 = 13.35, P < 0.05). Above all, the distributions of E-smears of differing lengths in spring and autumn are similar but different from those in summer and winter. The distribution pattern in summer is also different than that in winter.

On the other hand, the distributions of L-smears of differing lengths in four seasons were also significantly different (DF = 21, χ^2 = 37.19, P < 0.05, Fig. 5-3 and Appendix 5-4). The distribution patterns of L-smears of differing lengths in spring, summer and autumn, as can be seen from Fig. 5-3, were similar to each other, and different from the winter. The pattern in winter was significantly different from that in spring (DF = 7, χ^2 = 19.75, P < 0.01), and summer (DF = 7, χ^2 = 15.2, P < 0.05), but not different from autumn (DF = 7, χ^2 = 12.91, P = 0.0743, see Appendix 5-4).

5.3-2. Oestrous cycles in singly housed females and those housed in groups of five per cage.

There was no significant difference in oestrous cycle length (t = - 0.98, P > 0.05, Table 5-2) nor in E-smears length (t = 0.107, P > 0.05) between the grouped and singly caged females, but the mean length of L-lengths was significantly longer in grouped females than in singly housed (t = - 2.22, P < 0.05). Contingency table analysis of the distribution pattern of oestrous cycles of differing lengths showed that there was no significant difference between the two treatment groups (DF = 7, χ^2 = 4.4, P > 0.05, Fig. 5-4 and Appendix 5-5). There was no significant difference between single and grouped E-smear length distributions (DF = 5, χ^2 = 3.2, P > 0.05; neither was there a significant difference between single and grouped L-smear length distributions (DF = 6, χ^2 = 6.47, P > 0.05, Fig. 5-5, Appendix 5-6).

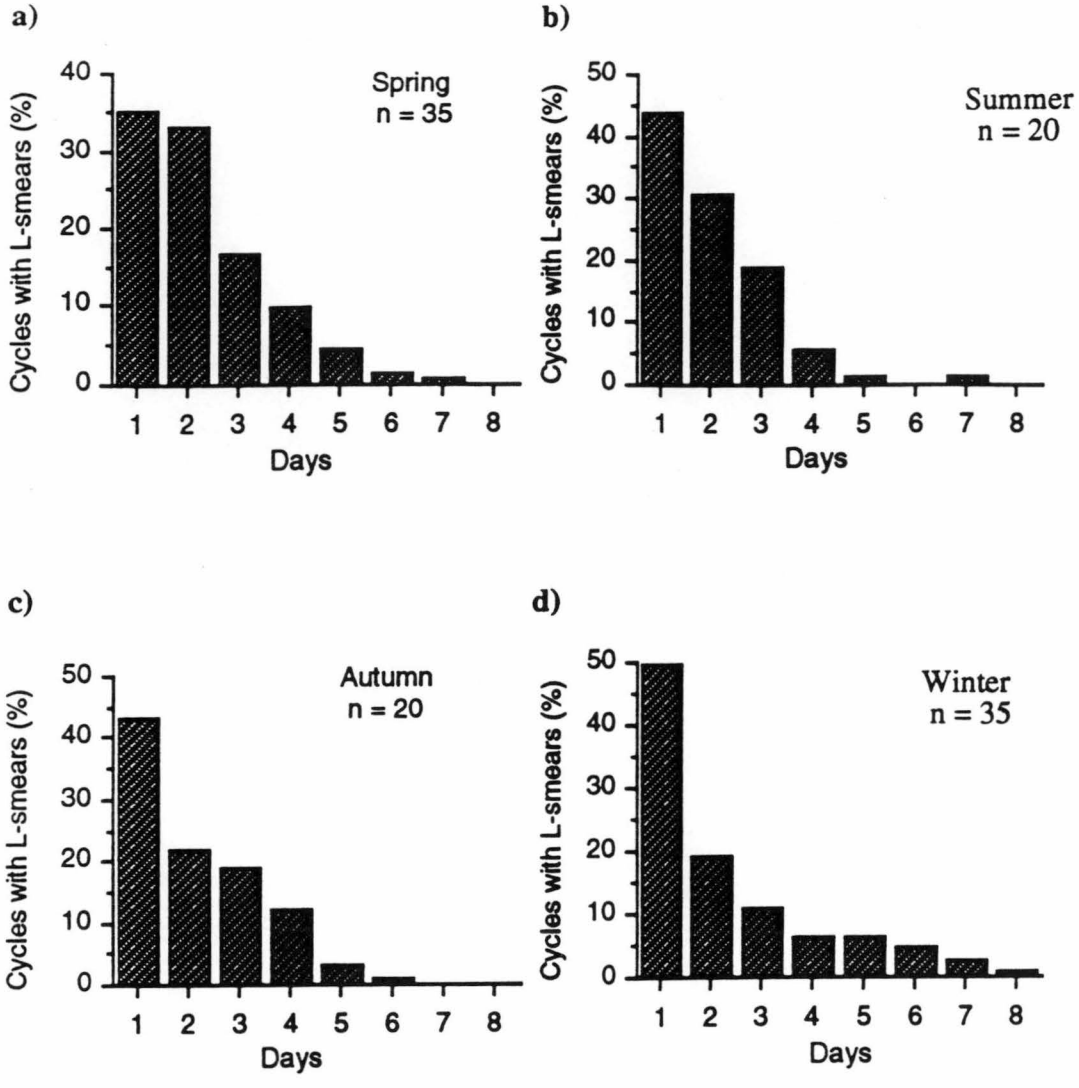


Fig. 5-3. The percentage of L-smears of differing lengths in the cycles in spring (a, 164 cycles), summer (b, 92 cycles), autumn (c, 100 cycles) and winter (d, 157 cycles).
n: the number of rats examined in each season.

*Table 5-2: Mean lengths of oestrous cycles (days ± SE) in singly housed or group housed (5 rats/ cage) females**

Treatment	No of cycles	Oestrous cycle Length (days)	E-smear (days)	L-smear (days)
Single	92	4.41 ± 0.15	2.46 ± 0.12	1.95 ± 0.12
Grouped	85	4.64 ± 0.17	2.28 ± 0.11	2.35 ± 0.14
t (2-tail)		- 0.98	0.107	- 2.22
P		0.3274	0.2851	0.0271

* Data collected from 20 females in each group during 30 days of examination.

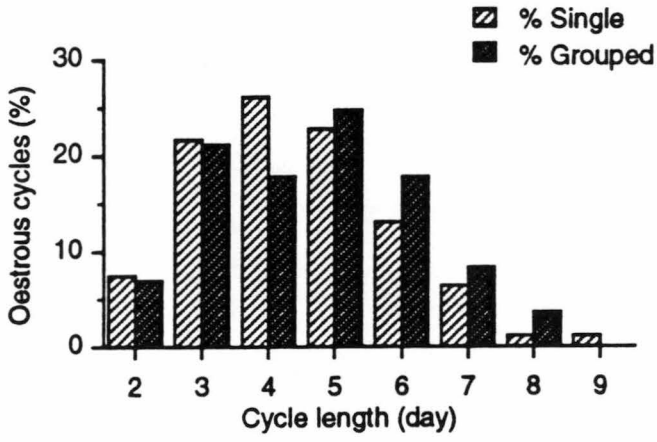
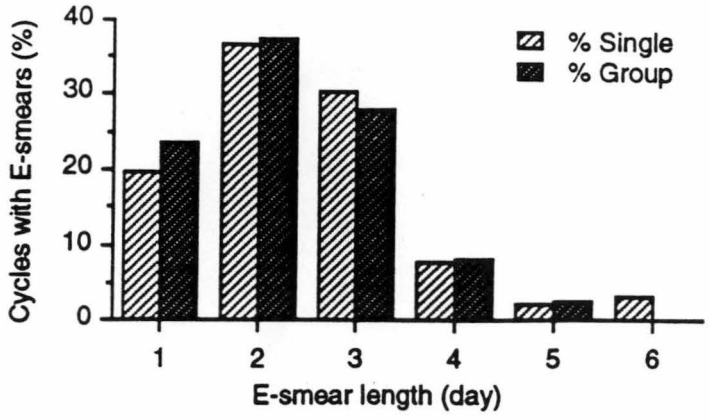


Fig. 5-4. The percentage of the oestrous cycle lengths in 20 singly housed and 20 grouped (5rats/cage) females .

a)



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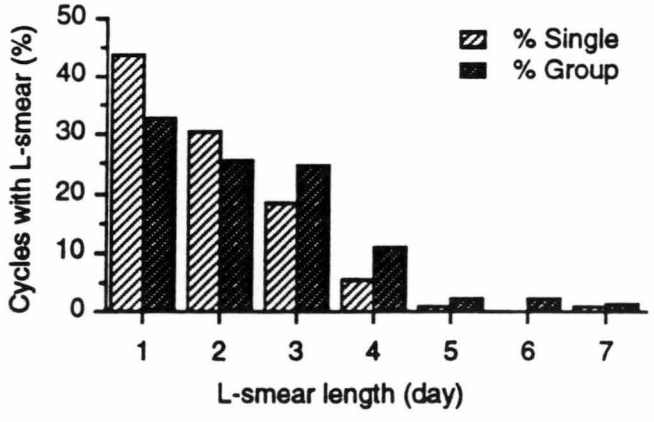


Fig. 5-5. The percentage of E-lengths (a) and L-lengths (b) in the cycles of 20 singly housed and 20 grouped (5 rats/cage) females.

5.3-3. The effects of the presence of a male on the oestrous cycle of the females

The lengths of oestrous cycles, E-smears and L-smears of the females each housed with an adult male are similar to those of singly housed controls (Table 5-3). The distribution of oestrous cycles of differing lengths was similar between the two groups (DF = 7, χ^2 = 5.27, P > 0.05, Fig. 5-6 and Appendix 5-7). Contingency table analysis show that there is no significant difference, in the distributions of either E-lengths (DF = 6, χ^2 = 7.58, P > 0.05) or L-lengths (DF = 6, χ^2 = 9.25, P > 0.05) of differing lengths between the two groups (Fig. 5-7, Appendix 5-8).

5.4. Discussion

5.4-1. The mean lengths of oestrous cycles, E-smears and L-smears and their distribution in different lengths

Pennycuik (1972) and Drickamer and Vestal (1973) have reported the existence of an innate seasonal reproductive rhythm in rats (laboratory mouse R 70 and *Peromyscys* respectively). It was therefore hypothesised that the rice field rat, *R. rattoides*, might show different lengths of oestrous cycle in different seasons although they were housed in controlled laboratory conditions. It was observed in the present study, however, that neither the oestrous cycles nor the L-lengths were significantly different between four seasons (Table 5-1 and Appendix 5-1). It seems that there is no such rhythm in cycling in females as noted in the growth and development of both sexes (see Chapter 3 and 4). The mean length of E-smears in winter, nevertheless, was significantly shorter than that in other seasons (Table 5-1 and Appendix 5-1).

*Table 5-3. Mean lengths of oestrous cycles (days ± SE) in females housed singly or housed each with an adult male (With Am)**

Treatment	No of cycles	Oestrous cycle Length (days)	E-smear (days)	L-smear (days)
Single	100	4.54 ± 0.18	2.36 ± 0.14	2.18 ± 0.13
With Am	117	4.4.3 ± 0.16	2.38 ± 0.13	2.04 ± 0.12
t (2-tail)		0.48	- 0.08	0.78
P		0.633	0.9332	0.434

* Data collected from 20 females in each group during 30 days of examination.

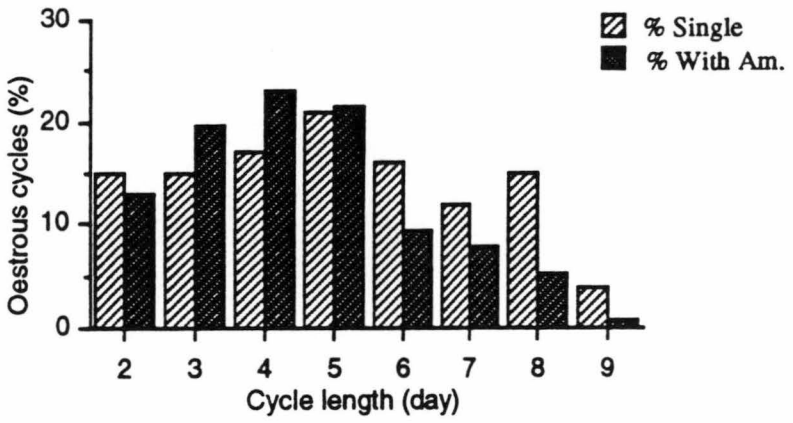


Fig. 5-6. The percentage of different oestrous cycles in 20 singly housed females and 20 females cohabiting with adult males (Am) for 30 days.

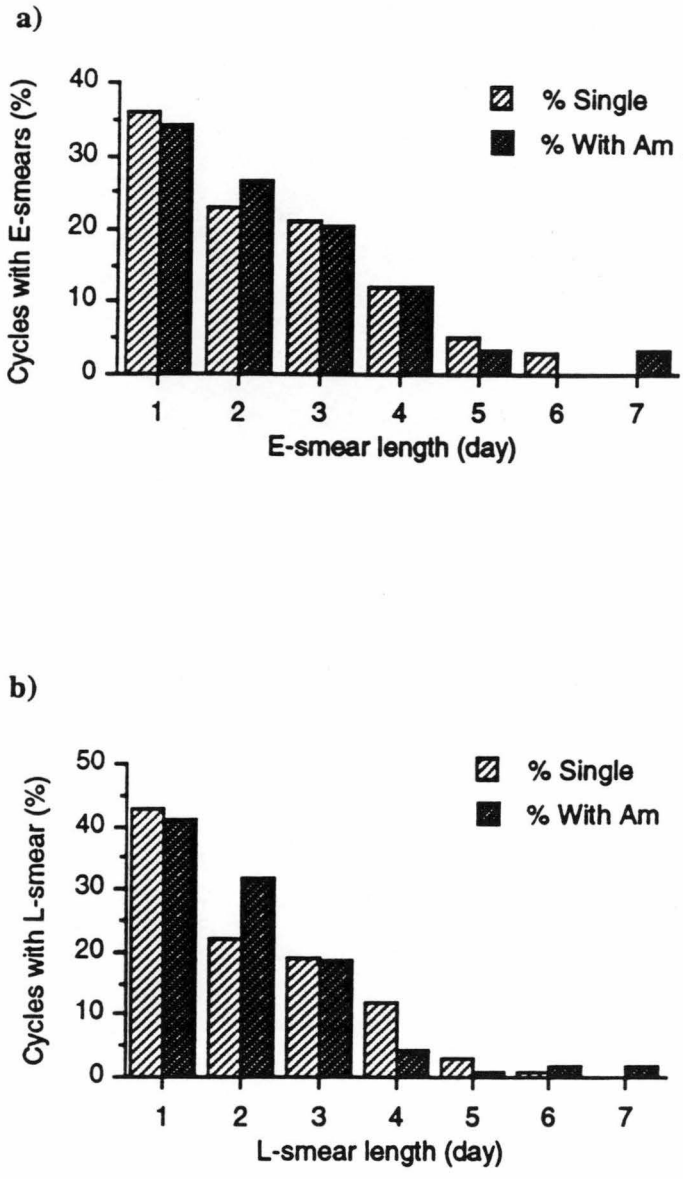


Fig. 5-7. The percentage of E-lengths (a) and L-lengths (b) in the cycles of 20 singly housed females and 20 females housed each with an adult male (Am) for 30 days.

On the other hand, the distributions of the oestrous cycles of differing lengths were not significantly different in four seasons (Fig. 5-1 and Appendix 5-2). Large proportion of cycles, as can be seen in Fig. 5-2 and Fig. 5-3, had one-day E-length and one-day L-length, the proportion decreased as the days increased, except for E-length in summer (Fig. 5-2b). The analysis of the distributions of E-smears of differing lengths (Fig. 5-2 and Appendix 5-3) and of L-smears of differing lengths (Fig. 5-3 and Appendix 5-4), however, reveals significant differences. The distribution of E-lengths in spring and autumn were very similar so that they are considered as one pattern. The distribution of E-lengths in summer and winter were different from each other and different from that in spring and autumn.

R. rattoides, according to Shou (1962) and Zhan (1982), can reproduce throughout the year, and yet there are two reproductive peaks, one in April and the other in August. It appears that when most female rats in the population are in reproductive condition the frequency distribution of E-smears in different lengths tend to be similar (ie. in spring and autumn). When comparatively smaller numbers of females in the population are in reproductive condition (summer and winter) the distributions of E-smears of differing lengths are dissimilar. The distributions of L-smears of differing lengths, on the other hand, are only of two types. Those in spring, summer and autumn are similar but they are all different from those in winter (Fig. 5-3 Appendix 5-4).

Bearing in mind that the rats had similar oestrous cycles in the four seasons in the laboratory (Table 5-1), it is reasonable to ask why the females kept in the laboratory with constant environmental conditions should still have different distribution types of E-lengths and L-lengths in the four seasons. Most of the females used in the experiments were wild caught adults (88.07%); the remainder were the first generation of the wild species in the laboratory. The seasonal rhythm,

as discussed in Chapter 3 and 4, could be genetically transmitted through the generations (Pennycuik, 1972; Drickamer and Vestal, 1973), so that the rats may still follow the old rhythm even in the controlled laboratory environment. It is also possible that apart from the temperature and day-light schedules, there might be some other environmental factors which the rats could sense, as has been suggested by Drickamer (1984).

5.4-2. Oestrous cycle lengths of singly and group housed females

It is well known that group housing can change the oestrous cycle lengths of some species of rats as well as mice. The effects, however, can be different. In the mouse, for example, grouping prolongs the cycle (van der Lee and Boot, 1955; Dominic and Pandey, 1979; Breed, 1976); while in the rat it shortens (Aron, 1973; Roser and Chateau, 1974; McClintock, 1981). In *R. rattoides* grouping did lengthen the duration of L-smear ($t = -2.22$, $P < 0.05$), but it did not affect E-lengths (Table 5-2). It slightly prolonged the oestrous cycles but the difference between the two groups was not significant ($t = -0.98$, $P = 0.3274$).

The distributions of oestrous cycles of different lengths (Fig. 5-4, Appendix 5-5), and the distributions of the lengths of E-smears and L-smears (Fig. 5-5, Appendix 5--6) were not affected by grouping. Different results may be generated if the experimental individuals are in different strains or at different ages (McClintock, 1983b). It is therefore, understandable that the response of *R. rattoides* to the treatment of grouping is different from the response seen in other species of rat or mouse. Although grouping can prolong the duration of L-smears in the cycles it does not appear to affect oestrous cycles significantly.

5.4-3. Oestrous cycles of singly housed females and the females each cohabiting

with an adult male

It was reported that the male or his urine can induce shorter oestrous cycles among female rats (Aron, 1975; Aron and Chateau, 1971; Aron *et al.*, 1966; Chateau *et al.*, 1972; Hughes, 1964). This, however, was not observed to happen in *R. rattoides*. The analysis of the data collected in the laboratory shows that neither the mean lengths of oestrous cycles, nor the mean lengths of E-smears and L-smears (Table 5-3) nor the distributions of oestrous cycles (Fig. 5-6, Appendix 5-7), and the distributions of E-smears and L-smears of differing lengths (Fig. 5-7 Appendix 5-8) were affected by the presence of adult males.

The urine of the males either contained no signal influencing conspecific females' oestrous cycles or it contained a signal which was overwhelmed by an innate rhythm of seasonal change, as suggested above. In autumn, when this experiment was carried out, the environmental parameters such as temperature and food are extremely beneficial for the reproduction of the wild rat in the field. Their higher reproductive capability in this season (Qing and Wang, 1981; Zhan, 1982) may be initiated by the change of photoperiod in the season, as generalised by Bronson (1989). The ability to sense changes of temperature and photoperiod in the wild may have been genetically incorporated so that even in the laboratory where temperature and photoperiod were under control and food and water were provided *ad libitum* the rats still expressed their highest capability of reproduction in autumn because of their endogenous rythm. Seasonal changes alter the sensitivity of mice in response to the chemosignal substances released by donors (Drickamer, 1984a; 1987). The most favourable environment in autumn might attenuate the sensitivity of the females to the possible existence of oestrus-inducing chemosignals in the urine of the males, or to the effect of the presence of the male.

It is worth mentioning here that during 30 days of test none of the 20

individually-caged females accompanied by a single adult male became pregnant. It is known that individulally-housed female voles living continuously with adult male did not enter oestrus (Richmond and Stehn, 1976). The voles entered oestrus only when the male was moved away for 8 days and re-introduced. It has been noted that diet-restricted female albino rats exhibited shorter oestrous cycles when they were exposed daily to new males behind a wire mesh barrier. When the males remained unchanged, however, the test females gradually became anoestrus (Purvis *et al.*, 1971). The induction of oestrus is, therefore, likely to depend on the presence of a novel male.

Anoestrus means that neither nucleated epithelium cells nor leukocytes are present in vaginal smears; the smears, instead, are full of mucus (Whitten, 1957; 1959). All the test rats in this experiment, however, had smears with different type of cells in varied proportions. They were thus not in anoestrus but were cycling.

Apparently, familiarity of the test females cohabiting with males did not stop cycling but did attenuated reproductive activity. Whether or not the endocrine activity of the females was also undermined is unknown and worth further study.

5.4-4. Summary

There was not significant difference in oestrous cycles in four seasons. The length of L-smears in the four seasons were also similar to each other. The length of E-smears in winter was significantly shorter than those in other seasons.

The frequency distributions of different oestrous cycle lengths are also similar in four seasons. The frequency distributions of the lengths of E-smears in spring and autumn, however, are not different form each other but they both are different from those in summer and winter. This diversity is probably relevant to the observations that the females bred well in the field throughout the year but better in

spring and autumn (Shou, 1962; Zhan, 1982). The frequency distributions of the lengths of L-smears, on the other hand, are generally similar to each other except that in winter.

Grouping of females can significantly increase the length of L-smears but not the length of E-smears of the cycle. The oestrous cycle was prolonged to some extent but not significantly when females were grouped. Why significantly longer length of L-smears in grouped females did not bring significantly longer oestrous cycles in this experiment remains unknown. The presence of an adult male and its urine showed no effect at all on the oestrous cycle length, the lengths of E-smears and L-smears.

Chapter 6. The influence of olfactory cues on the onset of puberty of female Rattus rattoides born in the laboratory

6.1. Introduction

The phenomenon of puberty-acceleration and -delay by olfactory cues has been studied extensively in the last three decades (Vandenbergh, 1967; Castro, 1967). At least four urine types have so far been found to have the ability to affect the tuning of the onset of puberty in female mice: the urine from males, the urine from pregnant or lactating females, the urine from females in oestrus and the urine from grouped females. These four types of urine and the chemosignals they contained can, in turn, fall into two categories: those which accelerate (the first three) and those which delay the onset of puberty of recipients (the latter) (reviewed in Drickamer, 1986a).

Sexual maturation, or the onset of puberty, of females can be determined by means of morphological, physiological and behavioural measurements (reviewed in Brown, 1985). Morphological measurements include the weights of ovaries and uteri; physiological measurements are plasma concentrations of LH, FSH, prolactin and oestrogen, and the age at first vaginal opening, first oestrus or first pregnancy; and the behavioural criterion is the age at first mating.

The following experiments were designed to ascertain if the presence of an adult male or his soiled bedding, the bedding soiled by adult females, or dense housing conditions play important roles in the growth and sexual development of female *Rattus rattoides*, a species in which nothing has been documented on this aspect of its biology. In the experiments conducted here the criteria of age at vaginal

opening and the weights of ovaries and uteri were used to determine the maturity of the females. Body weights were also recorded.

6.2. Materials and Methods

Two series of experiments were designed to examine the possible influence of environmental olfactory cues on the attainment of puberty in young females. One was to determine the age at the onset of puberty (vaginal opening, or VO) and the other was to examine the development of ovaries and uteri. In all experiments female weanlings (at 21-days) from different litters were randomly divided into two or three groups for different treatments. Soiled bedding from adult males or females was added daily into the cages of test rats between 8:00 and 8:30 in the morning, except for Experiment 6-10, in which the weanlings were caged either singly or in groups (4/cage) without adding any external odour stimuli.

Females in Experiments 6-1, 6-2, 6-3, 6-4 and 6-6 were checked daily for the first signs of vaginal introitus. The age and body weight at the onset of puberty (VO) under the influence of different olfactory stimulants was noted. In Experiments 6-5 and 6-7, the groups of test rats were sacrificed by cervical dislocation at 9 days after weaning (21 days of age) and then in 10 day intervals till the age of 50 days. Only 4 rats were used at each time because of the limited number of rats born in each month. Body weight (g) and the weights of ovaries and uteri (mg) were measured. Then, the organs were fixed in Bouin's Fluid, embedded in paraffin and later histological sections were stained with Haematoxylin-Eosin (HE) and examined for oogenesis.

Housing conditions such as room temperature, light regime and food and water schedules are as described in Chapter 2.

The data collected from all experiments with two experimental groups were

analysed with Student's t-tests whilst the data from experiments with three groups were subjected to one-factor ANOVA tests. Data were Square-root or log-transformed where it was necessary to correct for heterogeneity of variance among different groups (Vandenbergh *et al.,* 1975; Zar, 1984).

Abbreviations used in this chapter: Yf, young female; Ym, young male; Am, adult male; CAm, castrated adult male; sCAm, sham-operated adult male; UAm, bedding soiled by adult males; UAf, bedding soiled by adult females; BW, body weight; OW, ovarian weight; UW, uterine weight; VO, vaginal opening.

6.3. Results

6.3-1. Mean ages and body weights at the onset of puberty in female rats born in different months and housed with male siblings from weaning.

Experiment 6-1: This experiment examined the mean ages and body weights at the onset of puberty (VO) of females born in different months.

The average age at the onset of puberty of the females born in different months was 73.19 ± 2.18 days, ranging from 56.36 ± 1.22 to 87.67 ± 10.31 days (Table 6-1). The differences among the ages at the onset of puberty in the females born in different months were significant ($F = 4.37$, $P < 0.001$, Table 6-2). Females born in April attained puberty (56.36 ± 1.22 days) significantly earlier than females born in any other months. Females born in July attained puberty (66.28 ± 3.57 days) significantly earlier than those born in November (77.77 ± 3.62 days) and March (80.20 ± 1.94 days), but significantly later than April. Females born in May attained puberty (87.67 ± 10.31 days) significantly later than the females born in July, August (73.15 ± 6.94 days), December (69.50 ± 3.42 days) and April (Table 6-1 and Table 6-2, also see Appendix 6-1).

Table 6-1: Mean ages (days) and body weights (g) at the onset of puberty (VO) of female Rattus rattoides born in different months in the laboratory and housed with male siblings from weaning.

Birth Month	n	Age-VO±SE (days)	Weight-VO±SE (g)
May, 1990	6	87.67 ± 10.31	76.00 ± 8.27
June, 1990	14	75.50 ± 2.70	81.83 ± 5.61
July, 1990	17	66.28 ± 3.57	69.72 ± 3.47
August, 1990	13	73.15 ± 6.94	67.35 ± 3.56
November, 1990	13	77.77 ± 3.62	66.04 ± 3.15
December, 1990	10	69.50 ± 3.42	62.65 ± 3.99
February, 1991	9	71.22 ± 2.46	64.61 ± 2.35
March, 1991	10	80.20 ± 1.94	78.40 ± 7.06
April, 1991	14	56.36 ± 1.22	64.29 ± 2.20
Mean		73.19 ± 2.18	69.72 ± 2.22

Body weights at the onset of puberty in females born in different months were also significantly different ($F = 2.27$, $P < 0.05$). Average body weight at the onset of puberty was 69.72 ± 2.22 grams, ranging from 62.65 ± 3.99 to 81.83 ± 5.61 grams (Table 6-1). At the time of vaginal opening, the females born in June were

significantly heavier (81.83±5.61 g) than the females born in all other months examined except March (78.40 ± 7.06 g); females born in March were significantly heavier than those born in December (62.65 ± 3.99 g) and April (64.29 ± 2.20 g), but similar to those of females born in other months (Table 6-1 and Table 6-2, also see Appendix 6-2).

Table 6.2: Comparison of ages and body weights at vaginal opening (-VO) of female Rattus rattoides born in different months in the laboratory (one factor ANOVA test).

	May	Jun.	Jul.	Aug.	Nov.	Dec.	Feb.	Mar.	Apr.
May			**	*		*			***
	Jun.		(*)	(*)	(*)	(**)	(*)		***, (**)
		Jul.			*			*	*
			Aug.						**
				Nov.					***
					Dec.			(*)	*
						Feb.			*
							Mar.		***, (*)

Note: 1), Sqrt-transformed data used in the analysis.

Age at VO: $F = 4.37$, $P < 0.001$; Body weight at VO: $F = 2.27$, $P < 0.05$.

* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.001$.

2), Asterisk without parentheses represents significant differences in age at VO; asterisk with parentheses represents significant differences in body weight only at VO. Spaces unmarked: no significant difference.

6.3-2: The effect of the presence of an adult male (Am) on the onset of puberty (VO) of young females (Yf)

Experiment 6-2: This experiment examined the onset of puberty, by using the timing of vaginal opening as the criterion, of females each housed with an adult male compared with females housed with male siblings.

Female rats born in late May and early June were divided randomly into two groups at weaning. In test group each female was housed with an adult male (Yf+Am); the females in the control group were caged with male siblings of the same age (Yf+Ym). The mean age at the onset of puberty of the females housed with adult males was 44.9 ± 6.86 days, which was less than half of that of the females in the control group (87.67 ± 10.31 days; $t = 3.83$, $P < 0.005$; Table 6-3).

Table 6-3: Mean ages (days) and body weights (g) at the time of vaginal opening (VO) in young female (Yf) Rattus rattoides caged with adult males (Am) and in females caged with male siblings (Ym) from weaning (Student's t-test).

Treatment	n	Age-VO±SE (days)	Weight-VO±SE (g)
Yf+Ym	6	87.67±10.31	75.92±8.21
Yf+Am	10	44.9±6.86	40.2±6.77
t*		3.83	3.4
P*	(df = 14)	0.0019	0.0043

*: Square-root transformed data were used.

The mean body weight in the same test females was 40.2 ± 6.77 g at the onset of puberty while that in control females was 75.92 ± 8.21 g, which was significantly heavier than the former ($t = 3.4$, $P < 0.005$; Table 6-3).

Experiment 6-3: This experiment examined the onset of puberty (VO) in females housed with castrated adult males (CAm)*vs.* females housed with sham-operated adult males (sCAm).

In order to find out if it was the tactile stimulus from the adult male or pheromones excreted by the male which accelerated the onset of puberty of the test females and if the puberty-accelerating-pheromones in the male-soiled bedding were testes-dependent, females born in October, 1990 were subjected to one of the two treatments. One group was caged each with a castrated adult male (CAm), while the other group was caged with sham-operated adult males (sCAm). The experiment was initiated a month after the males were operated upon (Pfaff and Gregory, 1971).

As shown in Table 6-4, the females in both groups had similar body weights at the time of vaginal opening ($t = -0.33$, $P = 0.7456$). The females in the group with sham-operated males attained puberty at the age of 64.38 ± 2.55 days, which was significantly earlier ($t = -2.44$, $P < 0.05$) than that in the group with castrated males (79.18 ± 4.8 days).

6.3-3: The effect of adult male-soiled bedding (UAm) on the onset of puberty (VO) in young females (Yf).

Experiment 6-4: This experiment examined ages (days) and body weights (g) at the time of vaginal opening in the females housed with adult male-soiled bedding compared with those in the females housed with male siblings.

Young females housed with adult male-soiled bedding from weaning attained

puberty at the age of 56.94 ± 2.92 days, 10 days earlier ($t = 2.05$, $P < 0.05$), than the females caged with their male siblings which attained puberty at the age of 66.94 ± 3.99 days (Table 6-5). The body weights at the onset of puberty in females of the treated group were significantly lighter ($t = 2.12$, $P < 0.05$) than the ones in the control group (58.47 ± 3.21 g and 69.06 ± 3.89 g respectively).

Table 6-4: Mean age (days) and body weight (g) at the onset of puberty (VO) in young female (Yf) Rattus rattoides caged with castrated adult males (CAm), or caged with sham operated adult males (sCAm) from weaning (Student's t-test).

Treatment	n	Age-VO±SE	Weight-VO±SE
Yf+sCAm	8	64.38 ± 2.55	63.25 ± 5.55
Yf+CAm	11	79.18 ± 4.8	65.59 ± 4.51
t		- 2.44	- 0.33
P	(df = 17)	0.026	0.7456

Experiment 6-5: This experiment examined the increase of body weight (g), ovarian weight (mg) and uterine weight (mg) of the females treated with adult male-soiled bedding and of the females cohabited with male siblings.

In this experiment ovarian and uterine development as well as the body weight growth of the females in test and control groups were measured 9-day after weaning (at 30 days) and then in ten day intervals, at the age of 40 and 50 days. There were

no significant differences between the two groups in any of the aspects examined during the 29 days of treatment from weaning (Table 6-6).

Table 6-5: Mean ages (days) and body weights (g) at the time of vaginal opening (VO) in young female (Yf) Rattus rattoides caged alone but provided with adult male-soiled bedding (UAm) and that of females caged with male siblings (Ym) from weaning (Student's t-test).

Treatment	n	Age-VO±SE (days)	Weight-VO±SE (g)
Yf+Ym	16	66.94±3.99	69.06±3.89
Yf+UAm	18	56.94±2.92	58.47±3.21
t		2.05	2.12
P	(df = 32)	0.0485	0.0421

6.3-4: The onset of puberty in young females (Yf) treated with bedding soiled by adult females (UAf) compared to that of the females caged with male siblings (Ym).

Experiment 6-6: This experiment examined the age (days) and body weight (g) of the females housed with adult females' bedding *vs.* those of the females housed with male siblings.

Table 6-6: Mean body weights (BW, in g), and the weights of ovaries (OW, in mg) and uteri (UW, in mg) of young female (Yf) Rattus rattoides housed with adult male-soiled bedding (UAm) compared with those of females caged with male siblings (Ym) (Student's t-test).

Age (days)	Organ	Yf+UAm	Yf+Ym	t*	P*
	n	3	3		
	BW	35.83±4.13	33.83±2.95	0.34	0.7514
30	OW	5.33±0.88	8.00±1.15	- 1.87	0.1351
	UW	8.67±0.88	7.33±1.86	0.83	0.4549
	n	4	3		
	BW	41±5.12	34.67±1.3	0.95	0.3879
40	OW	6.55±1.37	4.33±0.67	1.34	0.2369
	UW	13.65±1.77	8.33±0.33	2.53	0.0526
	n	3	4		
	BW	70.83±3.24	68.0±4.04	0.55	0.6031
50	OW	9.37±2.75	7.48±1.02	0.61	0.5712
	UW	38.6±19	22.6±3.07	0.67	0.5351

* ln (x)-transformed data used in analysis.

The attainment of puberty in females housed with adult female-soiled bedding (89.45 ± 7.46 days) was significantly delayed ($t = 3.08$, $P < 0.01$) compared with

the age at the time of vaginal opening in male siblings cohabiting with females (61.9 ± 4.49). Body weights of the females treated with adult female-soiled bedding (57.38 ± 2.49) was significantly lighter ($t = -2.22$, $P < 0.05$) than those of controls (68.35 ± 4.4) at the onset of puberty (Table 6-7).

Table 6-7: Mean ages (days) and body weights (g) at the onset of puberty in young female (Yf) Rattus rattoides housed with adult female soiled bedding (UAf) compared with the females housed with male siblings (Ym) from weaning (Student's t-test).

Treatment	n	Age ± SE (days)	Weight ± SE (g)
Yf+Ym	10	61.9 ± 4.49	68.35 ± 4.4
Yf+UAf	11	89.45 ± 7.46	57.38 ± 2.49
t		3.08	- 2.22
P	(df = 19)	0.0061	0.0387

Experiment 6-7: This experiment examined the body weight (g) and the weight of ovaries and uteri (mg), measured first 9 days after weaning (at 30 days) and then at intervals of 10-day until the age of 50 days, in singly caged females and the females caged with the bedding from adult females.

The mean ovarian weight of the females housed with adult female-soiled

bedding for 9 days at the age of 30 days was 3.85 ± 0.56 mg, significantly lighter (t = - 2.866, P < 0.05) than that of singly caged females (6.53 ± 0.74 mg). Uterine

Table 6-8: The growth of ovaries and uteri in singly caged young female (Yf) Rattus rattoides and the females housed with adult female soiled bedding (UAf) from weaning (Student's t-test).

Age (days)	Organ	Yf+UAf	Yf	t	P
	n	4	4		
30	BW (g)	32.88 ± 1.33	36.63 ± 2.62	- 1.278	0.2486
	OW (mg)	3.85 ± 0.56	6.53 ± 0.74	- 2.866	0.0286
	UW (mg)	8.45 ± 1.27	7.6 ± 1.54	0.573	0.5873
	n	4	4		
40	BW (g)	49.38 ± 5.93	50.25 ± 4.29	- 0.12	0.9087
	OW (mg)	9.03 ± 0.83	10 ± 3.46	- 0.549	0.603
	UW (mg)	14.55 ± 1.61	46.65 ± 19.53	- 1.248*	0.2586*
	n	3	4		
50	BW (g)	57 ± 4.92	45.88 ± 4.89	1.567	0.178
	OW (mg)	6.37 ± 0.75	7.23 ± 1.03	- 0.626	0.559
	UW (mg)	15.9 ± 1.87	10.13 ± 0.53	3.6337*	0.015*

*: ln (x)-transformed data used in the analysis.

weight of the females housed with adult female-soiled bedding (15.9 ± 1.87 mg), however, was significantly heavier ($t = 3.6337$, $P < 0.05$) after 29 days of treatment (at 50 days) than in the controls (10.13 ± 0.53 mg, Table 6-8). No statistical differences were found in body weight between the two groups throughout the experiment.

6.4. Discussion

6.4-1. The timing of the onset of puberty as a function of month of birth

It was expected that the females born in different months in the laboratory should have similar ages and body weights at the onset of puberty because of the controlled environment. It was found by ANOVA test, however, that there were significant differences in age and body weight at the onset of puberty among the female rats born in different months. The females born in April attained puberty significantly earlier than the females born in other months; and the females born in May attained puberty significantly later than the females born in most of months. There were also significant differences among the females born in some other months (Table 6-1 and Table 6-2). Body weights at the onset of puberty in the females born in June were significantly heavier than the females born in other months except in March. On top of that the mean body weight at the onset of puberty in the females born in March was significant different from those in April and December (Table 6-1 and Table 6-2).

The females in this experiment were born to the first and second generations of wild *R. rattoides* in the laboratory and maintained under controlled conditions of photoperiod, temperature and humidity. Nevertheless some of the females still

showed significant differences to each other in the timing of the onset of puberty (Table 6-2). This is similar to the phenomenon reported in several species of *Peromyscus* (Drickamer and Vestal, 1973), in the mouse (Drickamer, 1977b; 1984; Pennycuik, 1971; Vandenbergh *et al.*, 1975) and in the rat (Donovan and van der Werff ten Bosch, 1959; Ramaley and Bunn, 1972).

No satisfactory explanation of the variation in the time of onset of sexual maturation with seasons has yet been proposed, for animals living under captive conditions with regulated environmental cues. It is, however, possible that there is an innate seasonal rhythm which causes the difference since the test subjects were all isolated from external daylight and climatic cues (Pennycuik, 1972). This rhythm may, in turn, tune one or more of the physiological processes underlying puberty in the females (Drickamer and Vestal, 1973). It is also possible that some unknown, subtle environmental cue(s) may affect the physiological rhythms (Drickamer, 1984).

6.4-2. The acceleration of the onset of puberty in females by the presence of or by olfactory cues from adult males

It is well known that the presence of an adult male mouse or the urine from the male will hasten the onset of puberty in females (Andervont, 1942; Kennedy and Brown, 1970; Vandenbergh, 1967, 1969). This phenomenon is common in some species of rats and voles as well as in mice (Vandenbergh, 1976; Hasler and Nalbandov, 1974). The present study shows that it occurs also in the rice-field rat *R. rattoides*. The young females of this species attained puberty much earlier when they were accompanied by adult males than when they cohabited only with male siblings. The time to attain puberty in young females cohabiting with adult males was about a half (44.9±6.86 days) of that when they cohabited with their male

siblings (87.67±10.31 days). This suggests that the presence of an adult male is highly effective in accelerating puberty in young female rice field rats. The result of Experiment 6-3 indicates that it was not the physical contact but the olfactory cues from the male which accelerated sexual maturation in the females (Table 6-4). It proved, at least, that physical contact with adult males alone could not induce earlier attainment of puberty in young females. The result also suggests that the pheromones in the adult male-soiled bedding were secreted by testes and excreted by the adult males into the urine. These are similar to the experimental results reported for mice by Vandenbergh (1969), Lombardi, *et al.*(1976), Drickamer and Murphy (1978).

The results of Experiment 6-4 verified the postulation made from the results of Experiments 6-2 and 6-3 that urinary cues from intact adult males alone can induce the acceleration of puberty in young females (Table 6-5). The females housed with adult male-soiled bedding attained puberty 10 days earlier than those housed with male siblings. The onset of puberty was not, however, as markedly enhanced as when the adult male was present (Table 6-3). In Experiment 6-1, when urinary cues and contact stimuli both existed there was a maximal accelerating effect, whereas when only urinary cues were present, the magnitude was not as great. This is in agreement with the fact that the puberty-accelerating effect of urinary cues from adult intact males could be magnified by contact stimulation (Drickamer, 1974a, 1975c; Bronson and Maruniak, 1975).

Experiment 6-5 was designed to determine when the enhancement of the puberty occurred. Since the accelerated ages at the onset of puberty were from 44.9 ± 6.86 days (Table 6-3) to 56.94 ± 2.92 days (Table 6-5), it was decided to check in approximately 10-day intervals from age 21 days until the age 50 days. During the 29 days of treatment, body weights and the weights of ovaries and uteri were

similar between the two groups (Table 6-6). There was, nevertheless, a trend of faster increment in the three parameters in the group treated with adult male-soiled bedding than in the control group. It is therefore suggested that in *Rattus rattoides* the acceleration of the onset of puberty is a chronic process and the difference will not be statistically significant until the test rats are under the influence of adult male cues for more than 29 days.

6.4-3. The effect of adult female-soiled bedding on the onset of puberty of young females

The attainment of puberty in young female mice, *Mus musculus* (Drickamer, 1982c) and deer mice, *Peromyscus maniculatus* (Lombardi and Whitsett, 1980) has been shown to be delayed by adult female urinary cues. This is true also in the rice-field rat, *R. rattoides*. The result of Experiment 6-6 shows that in the bedding soiled by singly caged adult females contains factors which can delay the onset of puberty in young females. Not only was the attainment of vaginal opening significantly delayed (Table 6-7), but also the physical growth significantly retarded in the females treated with adult female-soiled bedding. The body weight at the time of vaginal opening (age 89.45 ± 7.46 days) was significantly lighter in the bedding-treated females than in controls even though the treated females were about 28 days older than the controls at the time when the measurement was carried out (Table 6-7). This retardation of physical growth, however, was not found in earlier stages (from age 21 to age 50 days, Table 6-8). The effect therefore must have taken place after the females were housed with the bedding for more than 29 days.

Ovarian weights were significantly lighter in young females treated with adult female-soiled bedding than in controls after 9 days of treatment (Table 6-8), indicating that the bedding contains pheromones which will suppress the growth of

ovaries in young females. Uteri were reportedly enlarged in female voles, *Microtus ochrogaster,* by the stimulus from a male (Carter *et al.,* 1980), and the stimulatory effect could be suppressed by the presence of another female or by exposure to female urine (Getz *et al.,* 1983), implying that urinary cues from a female are suppressive to the growth of uteri (Vandenbergh, 1986). In Experiment 6-7, however, the uteri weighed significantly heavier in treated than in untreated ones at day 29 of the experiment (Table 6-8). More work needs to be carried out to explain the difference between the result in this experiments and those in previous reports.

6.4-4. Summary

In summary to the description above, it can be concluded that in *Rattus rattoides,* a), There are differences in the timing of the onset of puberty in the females born in different months in the laboratory; b), the presence of an adult male can reduce by half the time necessary for the females to attain the puberty; c), olfactory cues from adult males alone can induce the acceleration of the onset of puberty and the enhancement can be magnified by tactile stimuli from adult males; d), the bedding soiled by singly caged adult females, on the other hand, contains pheromones which can inhibit sexual maturation as well as physical growth and development of young females; e), both the puberty-accelerating effect of adult male-soiled bedding and the puberty-delaying effect of adult female-soiled bedding seem to take place only when the test female rats are subjected to the treatments for more than 29 days.

Chapter 7. The influence of olfactory cues on sexual maturation of male Rattus rattoides born in the laboratory

7.1. Introduction

As mentioned previously (section 1.4), the attainment of puberty in males has not been as thoroughly researched as in females, probably because of the lack of pivotal event that serves as a clear point of demarcation between the juvenile and adult states (Miller *et al.*, 1977). To date no work has been conducted on the sexual maturation in males of *Rattus rattoides*. The work here was to examine and determine the influence of inter-male stimuli on the growth and development of young males. Firstly the effects of housing density on the onset of puberty were examined and secondly, the effects of olfactory cues from adult males on the sexual maturation of young males were investigated.

The onset of puberty in male rats can be measured using morphological, physiological or behavioural criteria (Brown, 1985). Morphological measures include body weight, testis weight and length, epididymis weight and the weights of organs such as the prostate and preputial gland, and the diameter of seminiferous tubules, among others. Physiological measures include plasma concentrations of LH, FSH, and testosterone, and presence of sperm in penile smears and behavioural measures include age at first mounting, intromission, and ejaculation, and successful impregnation of a female. Theoretically, the more measures that are used in determining the males' maturity the more accurate will the conclusion be. In the series of experiments conducted here five measures were employed, *i.e.*, body weight, testis weight and length, epididymis weight, the appearance of mature sperm in seminiferous tubules of testes and in convoluted tubules of epididymes,

and plasma concentration of testosterone.

7.2 Materials and Methods

In all experiments only the left testis and attached epididymis of each rat were weighed and measured, and because of the limited number of rats bred in the laboratory every month, blood and organ samples were taken from only 3 -5 rats at each given age.

For the studies on the influence of housing density on testis development of young males two experiments were conducted.

Experiment 7-1: This experiment examined the effect of housing density on the development of body weight and testis length of young males. Thirty - one male weanlings born in October, 1990 were randomly divided into two groups and either caged singly or caged four to a cage.

Experiment 7-2: This experiment examined the effect of housing density on sexual maturation of the males. The increases of testis and epididymis weights and plasma concentrations of testosterone were measured. Plasma concentration of corticosterone was also measured in order to examine whether any physiological suppression occurred in grouped males. Fifty male weanlings were assigned in the same way as Experiment 7-1. Because of the limited number of rats which could be used for the experiment only 6-8, males were killed in 10-day intervals in each group.

For studies on testis development under the influence of adult male soiled bedding two experiments were conducted.

Experiment 7-3: This experiment investigated the effect of olfactory cues from adult males on the increase of body weight and testis length. Thirty - seven young males born in August, 1990 were assigned into two groups upon weaning.

Males in one of the groups were singly caged and treated with adult male-soiled bedding by adding the bedding into the cages at about 8:00 a.m. daily. The males in the control group were caged with their female siblings throughout the experiment.

Experiment 7-4: This experiment examined the effect of olfactory cues from adult males on the development of testis weight, epididymis weight, and on plasma concentrations of testosterone of the young males. Corticosterone level was also measured to see whether any suppression could be induced by the odorants from adult males. Thirty-two male weanlings born in February, 1991 were also divided into two groups as in Experiment 7-3.

In Experiments 7-1 and 7-3, body weights and testis lengths of the rats were measured at intervals of 10 days (for detail of the measurement technique see Chapter 2: General materials and methods). In Experiments 7-2, 7-4 and 7-5, test rats were sacrificed by cervical dislocation at intervals of 10 days. Immediately after cervical dislocation the thoracic cavity was opened and the heart was cut through with scissors and a blood sample of about 5 ml was collected. This procedure was finished within 2 minutes of the rat being killed. The blood was then centrifuged and stored at - 20° C. The weights of left testes and epididymes were immediately measured and recorded. The testes and epididymes were then immediately fixed with Bouin's Fluid; normal paraffin embedding and sectioning were conducted later. Sections cut at 5 μ were stained with haematoxylin and eosin (HE) and examined microscopically for the appearance of mature sperm in seminiferous tubules and convoluted ducts.

The control groups were kept in separate rooms under identical conditions. Data collected from the experiments were ln(x)-transformed where it was necessary (see Chapter 2 for detail) and analysed with an unpaired t-test.

Abbreviations: BW, body weight (g); TL, testis length (cm); TW, testis weight (g); EW, epididymis weight (g); Ym, young males; Yf, young females; Am, adult males; UAm, bedding soiled by adult males.

7.3. Results

7.3-1. The influence of group-housing (4/cage) on the development of testes in young males

Experiment 7-1: This experiment examined the effect of housing condition on the development of body weights and testis lengths of young male rats.

Twelve rats born in October, 1990 were housed in three groups upon weaning. Another 14 weanlings born in the same month were singly housed as a control group. There was no difference between the two groups either in testis weights or testis lengths when the experiment started (Fig. 7-1, Appendix 7-1 and Appendix 7-2). The growth of body weights of the males in dense groups was greatly retarded after 10 days ($t = -2.145$, $P < 0.05$) and 20 days ($t = -2.316$, $P < 0.05$) treatment. But from day 30 to the end of the experiment the body weights of the males in the two groups tended to be similar although there was a trend of being heavier in singly-housed ones. Testis development was also greatly affected by the housing density. Starting with the same mean lengths (0.34 ± 0.01 cm in grouped males and 0.34 ± 0.25 cm in singly housed ones), the testes of the males in the singly-caged group grew much faster than those in group-housed males. Testis lengths of the singly-caged males were significantly longer than that of the group-housed males from 10 days after treatment until 10 days before the end of the 70-day treatment.

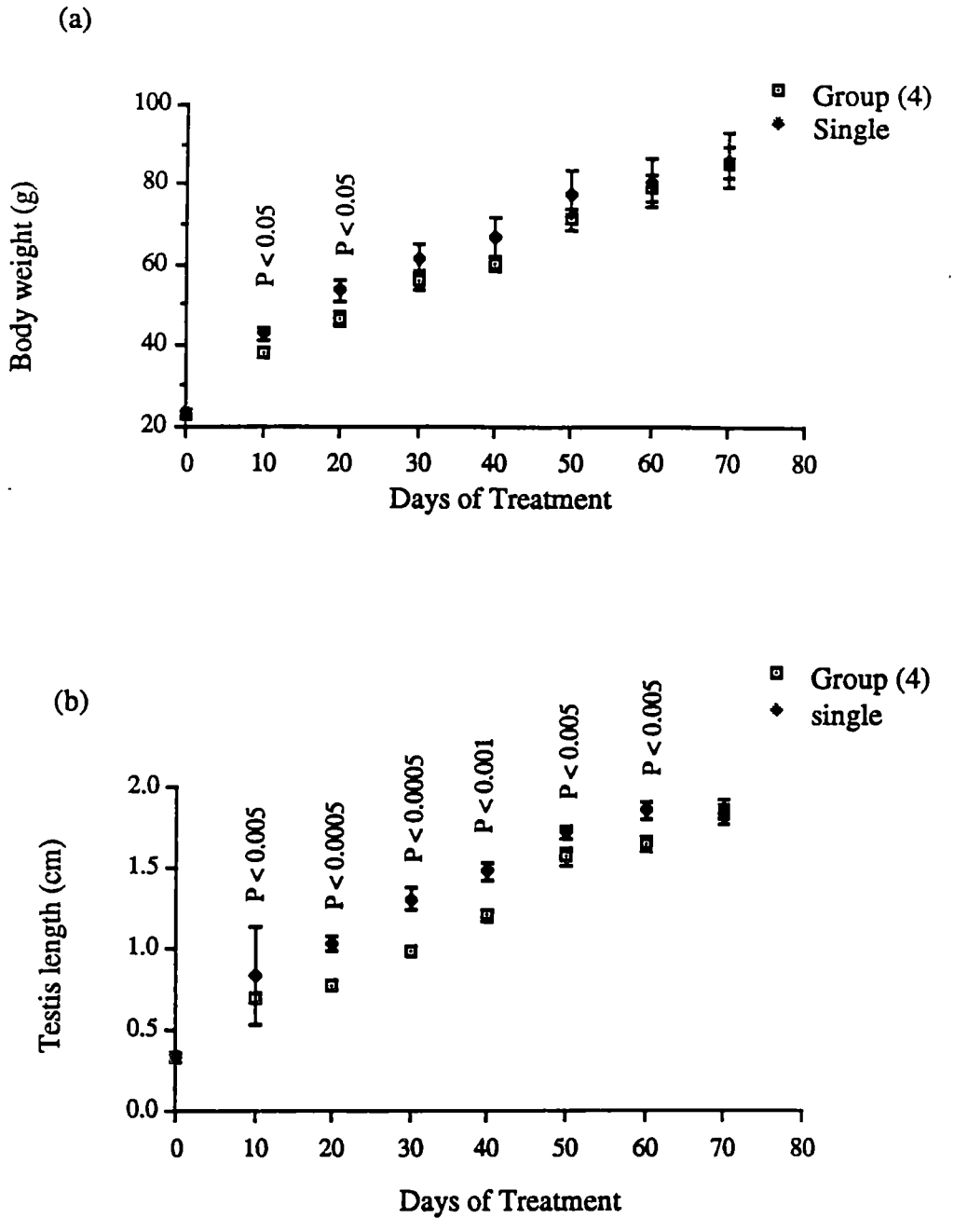


Fig. 7-1: Body weights (a) and testis lengths (b) of the males group-housed (4/cage) and the singly housed ones on different days of treatment. ln(x)-transformed data used in the analysis

The growth rates of body weight in the two groups were not significantly different except during the first 10 days of treatment ($t = -2.815$, $P < 0.01$). The growth rate of body weight was much higher in grouped males than in singly caged ones but the difference was not statistically significant (Fig. 7-2, a). It is shown in Fig. 7-2 (b) that the increment rate of testis length, on the other hand, was significantly higher in singly caged males than in grouped ones during first 20 days of the treatment (during first 10-days of the treatment: $t = 4.566$, $P < 0.0001$; during the second 10-days: $t = -3.102$, $P < 0.005$). The rate of testis length increment was still higher in singly-caged males from day 20 to day 30 of the treatment but the difference was not significant. The growth rates of the testis length from day 30 to day 40 were the same in the two groups. The rates measured on day-50 and day-70 of the treatment were higher in grouped males than in singly-caged ones ($t = 2.276$, $P < 0.05$ and $t = 2.294$, $P < 0.05$, respectively).

Experiment 7-2: The effect of housing condition on the weight increment of testes and epididymides, on plasma concentrations of testosterone and corticosterone, and on the development and transportation of sperm from testes to epididymides of young male rats.

The weanlings were under the same experimental condition as that in Experiment 7-1. At the intervals of 10 days four males in each of the counter-groups were killed. After 20-30 days of treatment the weights of testes and epididymes in group-housed males tended to grow more slowly than that in singly-housed males (Fig. 7-3 and Appendix 7-3). The weights of testes and epididymes became significantly lighter in group-caged males than in singly-caged males on day-40 of the treatment (testis: $t = 4.793$, $P < 0.005$; epididymis: $t = 4.616$, $P < 0.005$), on day-50 of the treatment (testis: $t = 6.784$, $P < 0.0005$; epididymis: $t = 4.731$, $P < 0.005$), and on day 70 of the experiment (testis: $t = 3.266$, $P < 0.05$;

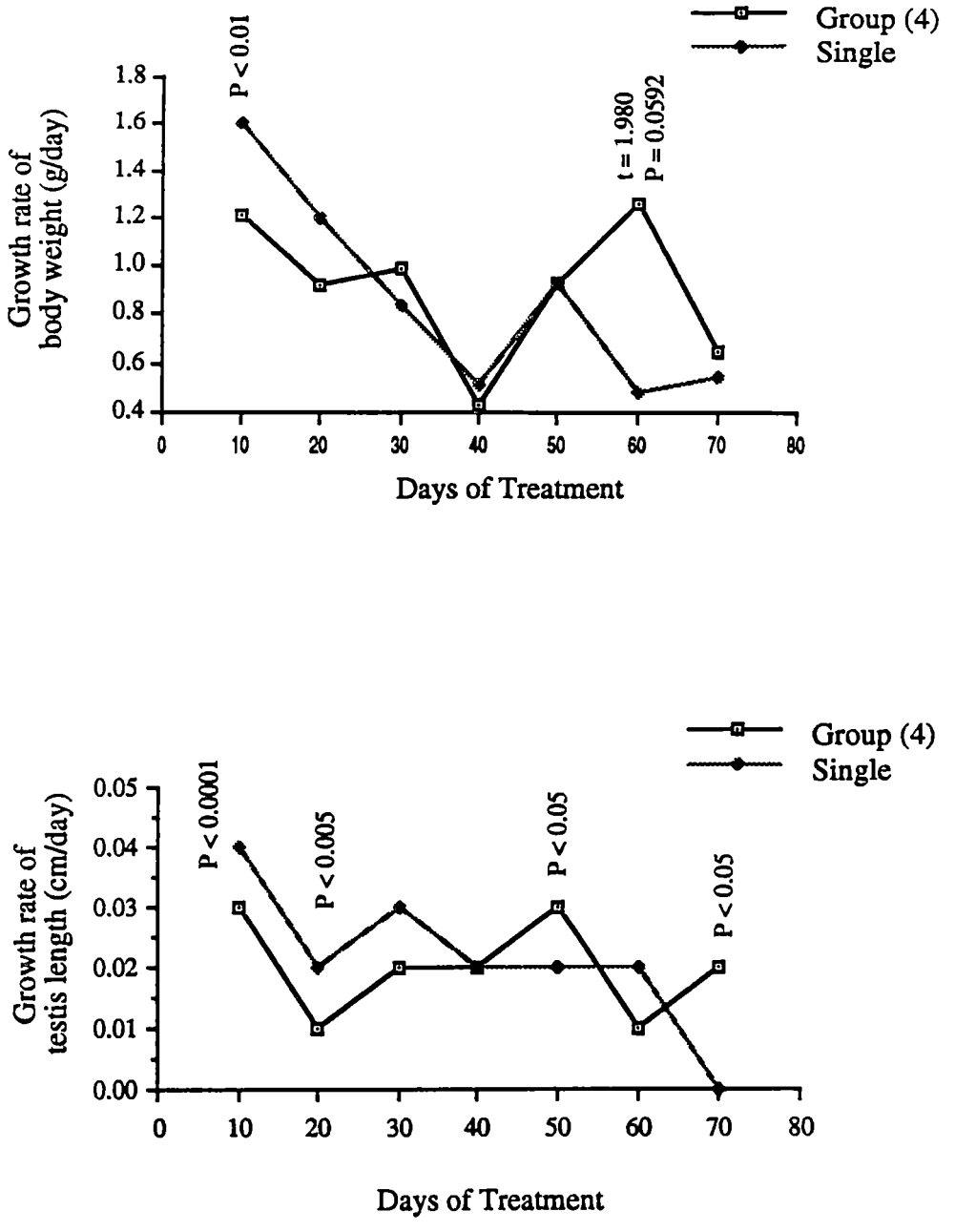


Fig. 7-2: Growth rates of body weight (g/day, a) and testis length (cm/day, b) of the males group-housed and the males singly housed. ln(x)-transformed data used in the analysis

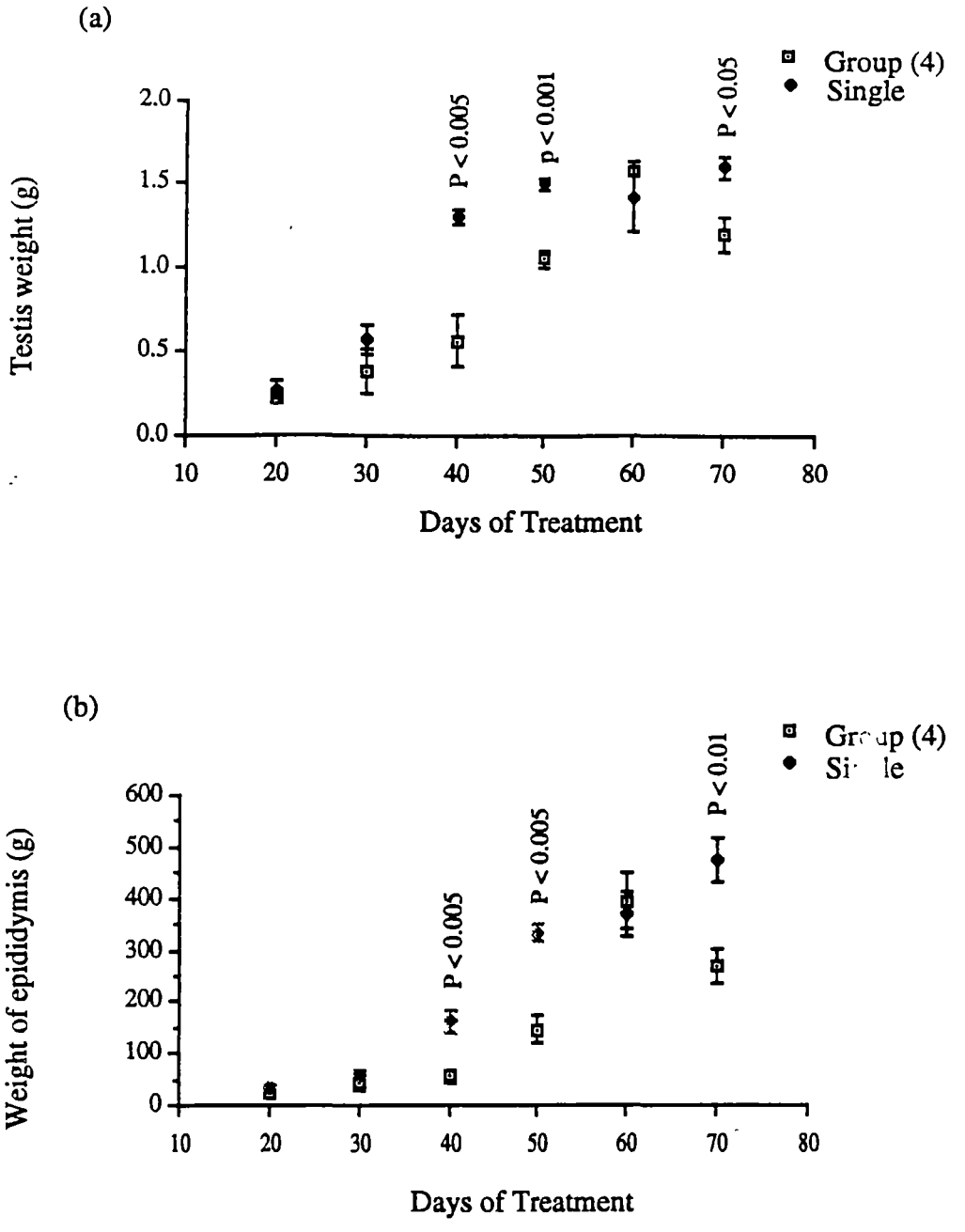


Fig. 7-3: Mean weightts of testes (a) and epididymes (b) of the males group-housed and the males singly housed. ln(x)-transformed data used in the analysis

epididymis: $t = 3.733$, $P < 0.01$). On day 60 no statistically significant difference was found between the two groups.

Plasma concentrations of testosterone and corticosterone in these two groups were tested at the same time when the weights of the above mentioned organs were measured. The values are shown in Fig. 7-4 and Appendix 7-4. Analysis based on ln(x)-transformed data showed that plasma concentration of testosterone was significantly lower in grouped males than in singly caged ones on day 40 ($t = -7.49$, $P < 0.0005$) and day 60 ($t = -4.91$, $P < 0.005$) of the treatment (Fig. 7-4, a). Corticosterone concentration remained similar between the two groups throughout the experiment (Fig. 7-4, b).

Histological observation revealed that sperm appeared in seminiferous tubules of the testes in both groups on day 30 of the experiment (50 days of age) and in the lumen of convoluted ducts of epididymides in singly caged males but not in that of the group-caged males on day 40. It was not until day 50 that the lumena of convoluted ducts of epididymides in group-caged males were observed to be full of sperm.

7.3-2. The influence of olfactory cues from adult males on the development of testis in young males.

Experiment 7-3: The influence of adult male soiled bedding on the development of body weight and testis length.

Male weanlings born in August were divided into two groups. Fifteen of them were housed with adult male-soiled bedding, another 22 were housed with female siblings as a control group. Mean body weights and testis lengths were measured at 10-day intervals, as shown in Fig. 7-5. Body weights of the males housed with

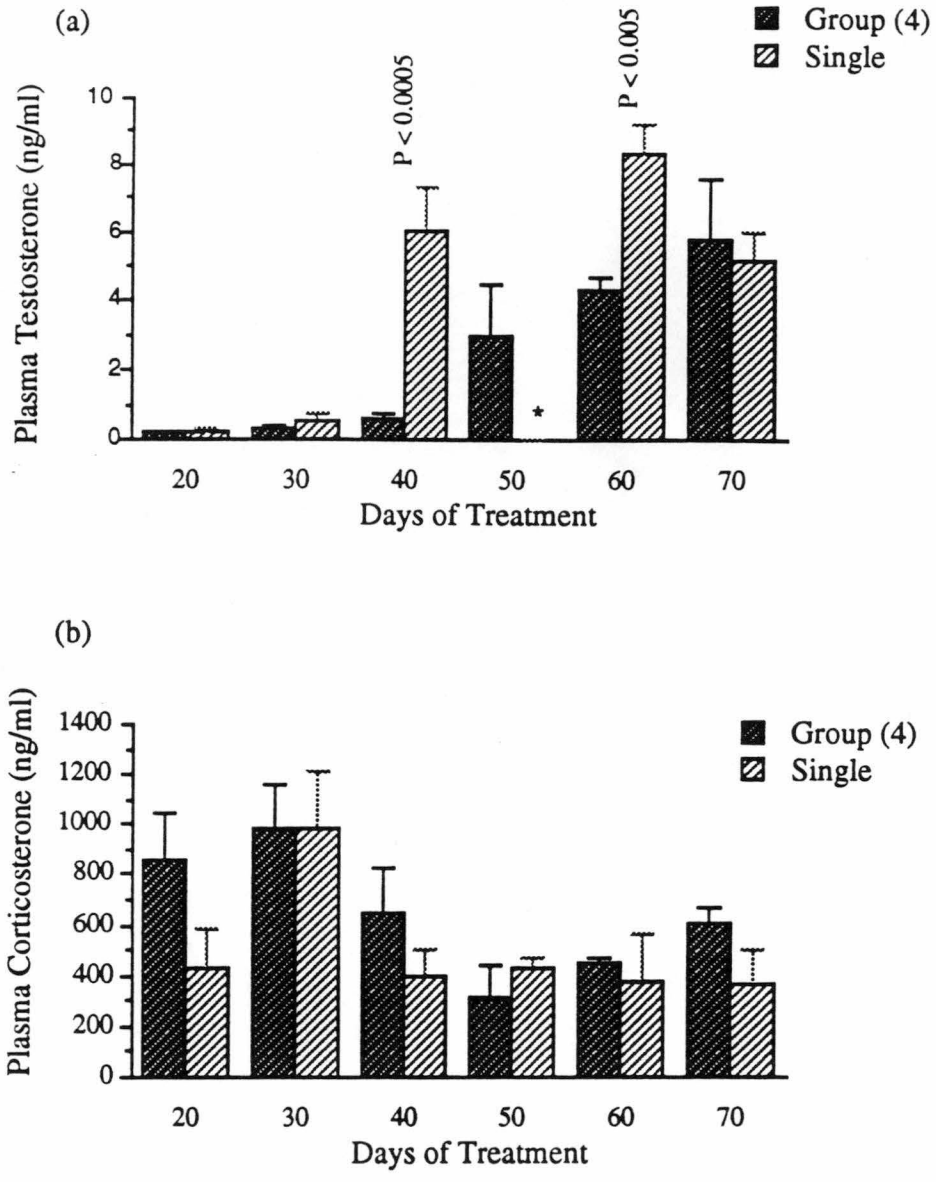


Fig. 7-4: Plasma concentrations (ng/ml) of testosterone (a) and corticosterone (b) in group-housed males and singly-housed ones. ln(x)-transformed data used in the analysis

** No data*

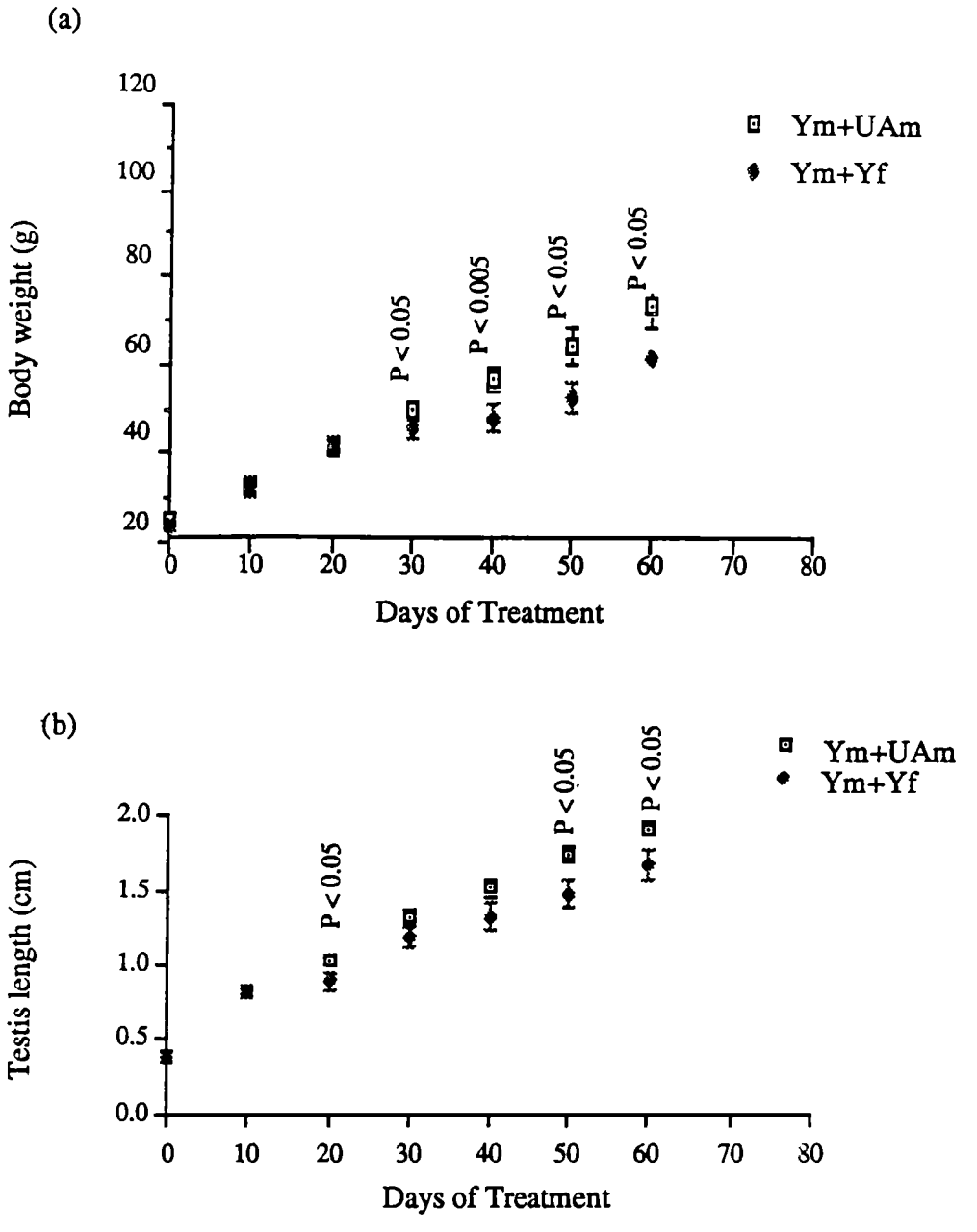


Fig. 7-5: Body weights (a) and testis lengths (b) of the males treated with adult adult males' bedding (Ym+UAm) and males housed eith female sibilings (Ym+Yf)
ln(x)-tranformed data used in the analysis

adult male-soiled bedding were significantly heavier than those in the control group after 30 days of the treatment ($t = -2.506$, $P < 0.05$). The difference was maintained until the end of the experiment (day-40, $t = -3.159$, $P < 0.005$; day 50, $t = -2.693$, $P < 0.05$; day 60, $t = -2.611$, $P < 0.05$) (also see Appendix 7-5). The testis lengths were significantly different between the two groups with males housed with male bedding having longer testes than controls measured at day-20 ($t = -2.178$, $P < 0.05$), day 50 ($t = -2.658$, $P < 0.05$) and day 60 ($t = -2.566$, $P < 0.05$) (also see Appendix 7-6). Body weights of the males treated with urine from adult males increased much faster than those of the males in the control group from day 20 to day 40 of the experiment (Fig. 7-6, a; and Appendix 7-5). The growth path of testis length, on the other hand, was not different between the groups (Fig. 7-6, b; and Appendix 7-6).

Experiment 7-4: The influence of adult male-soiled bedding on the weights of testes and epididymides, on plasma concentrations of testosterone and corticosterone, and on the transportation of mature sperm from testes to epididymides of the males.

The results of the measurements of the weights of testes and epididymides are shown in Fig. 7-7 and Appendix 7-7. No significant differences could be found between males treated with adult male-soiled bedding and those cohabiting with sibling females except the epididymides weight at day 50 of the experiment ($t = -3.163$, $P < 0.05$). Plasma testosterone concentrations (Fig. 7-8, a; and Appendix 7-8) were found to be much higher in the group treated with adult male-soiled bedding than in the control group, but ln(x)-transformed data analysis (paired t - test) shows no statistical significance, either on day 40 ($t = 1.44$, $P = 0.2009$) or day 50 ($t = 1.66$, $P = 0.1477$) of the experiment. Corticosterone level (Fig. 7-8, b; and Appendix 7-8) was higher on day 30 of the experiment in males cohabiting with

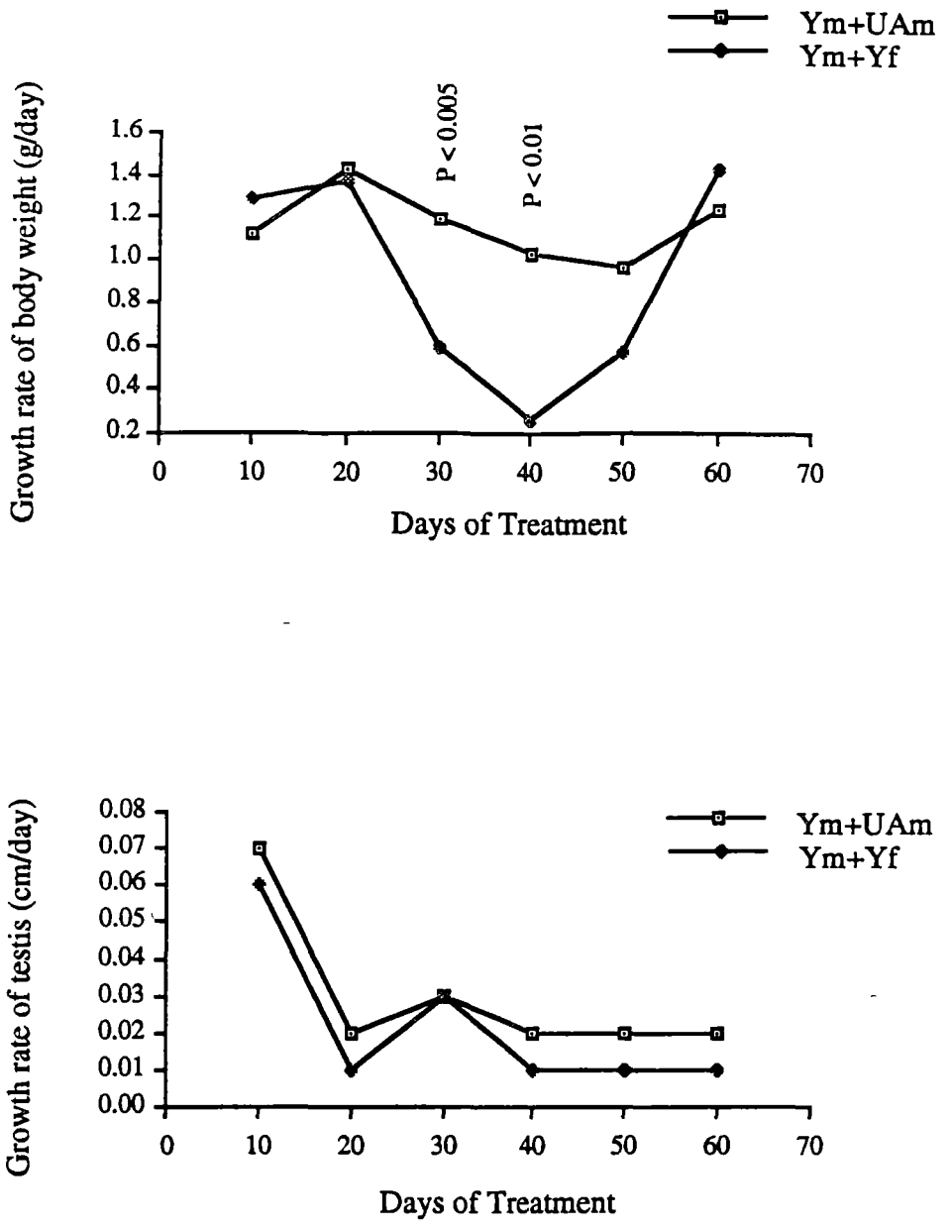
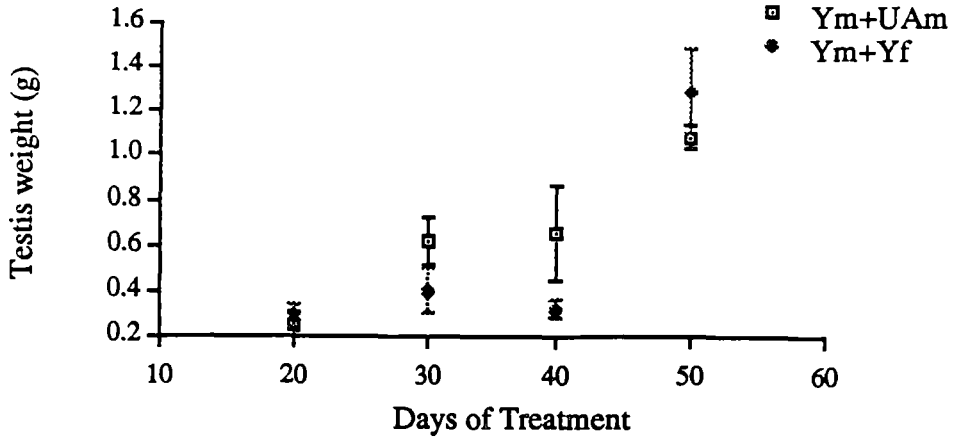


Fig. 7-6: Growth rates of the body weight (a) and testis length (b) in males treated with adult males' bedding (Ym+UAm) and males housed with female siblings (Ym+Yf).

(a)



(b)

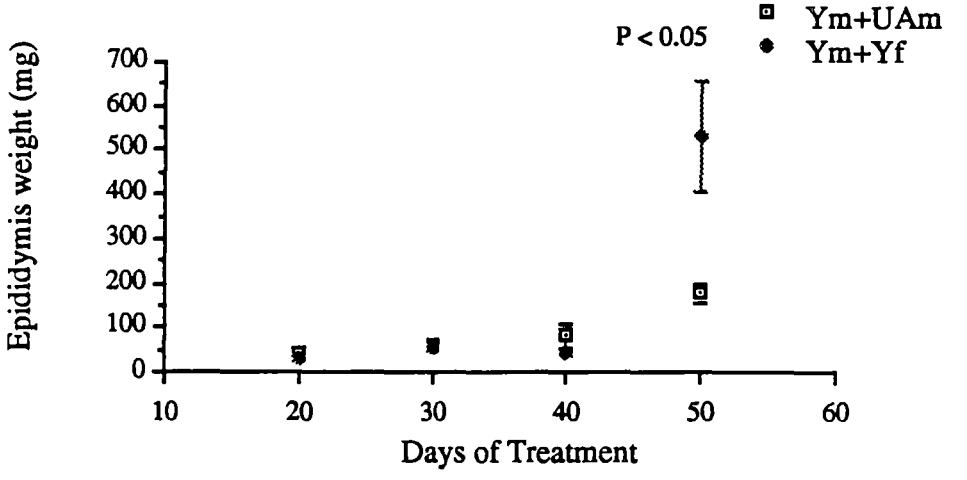


Fig. 7-7: Testis weights (a) and epididymis weights (b) of the males treated with adult male-soiled bedding (Ym+UAm) and the males housed with female siblings (Ym+Yf).
ln(x)-transformed data used in the analysis

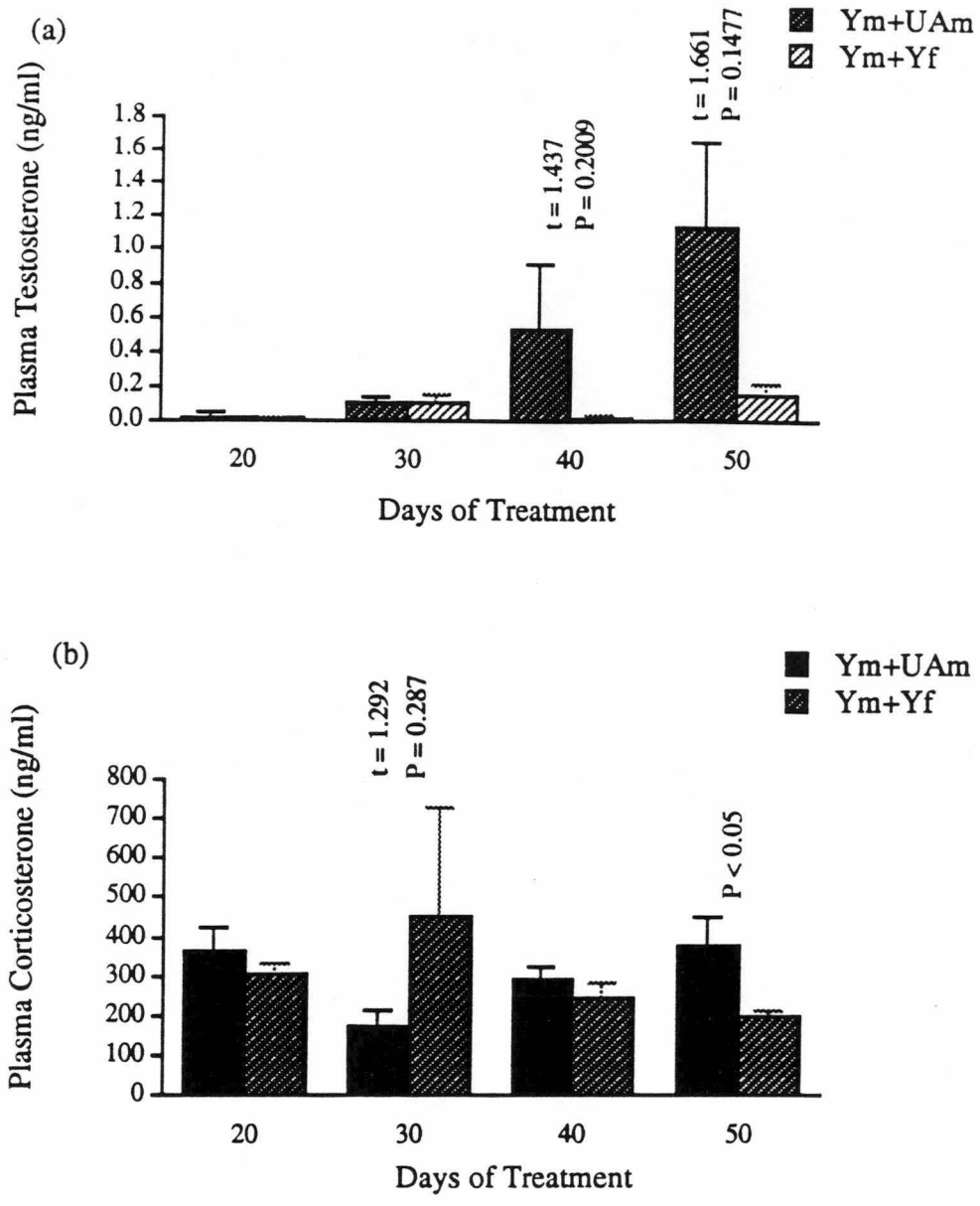


Fig. 7-8: Plasma concentrations (ng/ml) of testosterone (a) and corticosterone (b) in the males treated with adult male-soiled bedding (Ym+UAm) and the males housed with female siblings (Ym+Yf).
ln(x)-transformed data used in the analysis

sibling females than in males treated with adult male-soiled bedding. The difference was not statistically significant (day 30, t = - 2.37, P = 0.1408). On day 50, plasma corticosterone level was significantly higher in treated males than in the controls (t = 3.083, P< 0.05). Sperm appeared in the seminiferous tubules of the testis (day 30) and in the lumina of convoluted ducts of the epididymis (day 50) at the same time in both male bedding-treated group and the control group.

7.4. Discussion

7.4-1. The impact of group-housing on the growth and development of testes and spermatogenesis in males.

It is generally recognised that sexual development of young male rodents can be suppressed by a high living density (Bediz and Whitsett, 1979), and *Rattus rattoides* proves no exception. Physical growth and testis and epididymis development were depressed in group-caged males compared with that of singly caged ones. The males recovered from physical growth retardation and gained a normal growth path after 30 days in the dense group (Fig. 7-1, a). This ability implies that the males can adapt to a suppressive environment after a period of acclimatisation. Testes appear to be the organ most vulnerable to the effect of density, especially the testis length. From the beginning to the end of the 70-day experiment, the testis length was significantly shorter in group-housed than in singly caged males (Fig. 7-1, b). The increase of testis length was delayed at least by 10 days by group-housing from first measurement (10 days after the experiment started) until the end of the treatment. The increment in the rate of testis length in singly caged males was the highest in the first 10 days of the experiment. It then declined gradually and fell to 0 on day 70 of the experiment (age 90 days). The

increment rate of testis length in grouped males did not follow this pattern. During the last 10 days of the treatment, when the growth rate of testis length in singly-housed males fell to 0, the rate in grouped males increased. This resulted in nearly the same testis length in the two groups on day 70 of the experiment. The data show that a longer time is needed for the depression on the weights of testes and epididymides to take place than for the depression of body weight and testis length (Fig.7-3).

Spermatogenesis was reportedly to be inhibited in males living at high population density (Christian, 1971). It was not delayed, however, in these experiments. Instead, mature sperm were found 10 days later in the convoluted ducts of epididymides of grouped males than in singly caged ones. The delay of appearance of sperm in convoluted ducts of the epididymis can be considered to be one of the symptoms; the others such as the growth retardation of epididymides and the depression of plasma testosterone concentration, caused by group-housing. The serum concentration of testosterone in grouped males, unlike that in singly caged ones, did not rise dramatically at age 60 days (40 days of treatment) but rose smoothly from age 70 days (50 days of treatment). It was not until at the age of 90 days (70 days of treatment) that plasma testosterone concentration in grouped males reached the same level as in singly caged males.

It can be concluded that sexual maturation, as revealed by testis length and weight, epididymis weight, plasma testosterone concentration, and the appearance of sperm in epididymides, can be severely affected by population density.

The elevation of serum concentration of corticosterone has been considered one of the indicators of physiological suppression (Leshner, 1980; von Holst 1986). In this experiment, however, plasma corticosterone concentration in grouped

males was not significantly elevated (Fig. 7-4, b). Why the elevation of corticosterone level was not an index to the physical retardation in this species is unknown.

7.4-2. The influence of adult male-soiled bedding on testis development of young males

Stimuli from adult males have been reported to be able to delay the growth and sexual development of juvenile male house mice (*Mus musculus*, McKinney and Desjardins, 1973; Svare *et al.*, 1978; Vandenbergh, 1971), in prairie deer mice (*Peromyscus maniculatus bairdii*, Bediz and Whitsett, 1979), and in voles (*Microtus arvalis*, Lecyk, 1967). Two possibilities for the inhibition of sexual maturation have been advanced (Bediz and Whitsett, 1979). The first is that the sexual maturation is delayed because of aggressive behaviour of the cohabiting adult male toward the young. This was proved true in deer mice (Bediz and Whitsett, 1979). The second is that the olfactory cues emitted by the adult males can suppress the sexual development of young males. This was verified by Lawton and Whitsett (1979).

The role played by the olfactory cues from adult males in *Rattus rattoides* was different from those mentioned above. After 30 days of being treated with adult male-soiled bedding the body weight of juveniles became significantly heavier, instead of lighter, than those in the control group (Fig. 7-5, a). The growth rate of body weight was significantly accelerated from day 30 to day 40 (Fig. 7-6, a). Furthermore after being treated with bedding soiled by adult males for 20, 50, and 60 days the testes were significantly longer than those of control males (Fig. 7-5,b). Additionally a trend of increment was also observed in testis weight (Fig. 7-7, a), epididymis weight (Fig. 7-7, b), and in plasma testosterone (Fig. 7-8, a),

although the trend was not statistically significant. It appears, therefore, as if olfactory cues from adult male-soiled bedding can accelerate the growth and sexual development of juvenile males when compared with controls in which young males were reared with female siblings.

The treatment with adult male soiled bedding did not cause any change of plasma concentration of corticosterone until day 50 of the experiment (Fig. 7-8). This result suggests that no suppression occurred among the juvenile males with the treatment of adult odorants until they were 70 days. This further supports the above conclusion that adult male odorants enhance the growth and development of juvenile males.

7.4-3. Summary

It was observed that in young male *Rattus rattoides* (1) group-housing greatly retarded the growth of body weight, testis weight and length, and epididymis weight; (2) group-housing did not delay spermatogenesis but it did delay the appearance of sperm in convoluted ducts of the epididymides; (3) The premature increase of plasma testosterone concentration observed in singly caged males was delayed at least 10 days in group-housed males.

It is postulated from the results of the experiments that in *R. rattoides* adult male soiled bedding contains a pheromone (or a group of pheromones) which can significantly accelerate the physical growth and the increment of testis length in young males; and can also induce a trend of faster growth in the weights of testes and epididymes in comparison with singly caged controls.

Chapter 8 The effect of odorous cues on the reproductive biology of R. rattoides.

Rattus rattoides is an important agricultural pest in southern China. It damages crops seriously throughout the year. For many years farmers have been trying to protect their crops merely by the use of poisonous baits. Research into pest control hence has mainly focused on rat population dynamics and movement in the fields, and on a description of reproductive capability from dead-trapped samples (Lin and Xing, 1962; Qing and Wang, 1981; Wang and Qing, 1981; Zhan, 1982).

This study investigated the reproductive biology of the rat in the laboratory. The average reproductive rate of the rat in the laboratory was 20.36±1.82%. This is similar with the rates reported by Zhan (1982) from field survey. A post-partum oestrus was detected in the rat. This can explain why the rat is a serious problem to crops although only about 25% or more of the rats in the population have reproductive capability (Lin and Xin, 1962). With an environment where the foods are abundant in the fields throughout the year, the rice-field rat can quickly build up its population to compensate for the limited reduction caused by poisoning carried out every year by farmers.

Apart from the above described innate potential in reproduction external olfactory cues from adult males and females also appears to play a role in the reproduction of this species, as summarized in Table 8-1.

The attainment of puberty in young females was accelerated by the presence of adult male-soiled bedding as well as by the physical presence of the male, compared with the onset of maturity in young females housed with male siblings. The earlier attainment of puberty in the presence of the adult male or its soiled

*Table 8-1. Accelerate and delay of the onset of puberty in female R. rattoides in laboratory **

	Housed with Am	Housed with UAm	Housed with UAf	Housed with Ym
Accelerate	+	+		
Delay			+	
Intermidiate				+

*: For the definition of the abbreviations see Chapter 2.

bedding further enhances population growth. Observations in the indoor arena, however, revealed that young females did not stay in close proximity to their fathers but stayed near their mothers even after a new litter had been delivered by the mother (Chapter 4). It was found, however, sexual maturation of the females was substantially delayed and physical growth was severely retarded by adult female-soiled bedding (Chapter 6). Whether or not the juvenile females live with their mother in the field until sexually mature is not known and needs further investigation. In the fields the juvenile females might also live with their male siblings, for in all the control groups to the experiments the females housed with male siblings showed an intermidiate pace to the timing of the onset of puberty. These observations suggest that the odorous cues of adult males and adult females operate in opposite directions on the rate of population growth. When the environment allows, and if the density is low, adult male odour accelerates the onset of puberty in young females. When the density is high this, together with odorous

cues from adult females, retards the onset of sexual maturation. The actual population size at any given time will reflect the interaction of these opposing forces.

Olfactory cues from adult males as well as housing density can also alter growth rate and sexual maturation of young males in this species. As observed in the laboratory, growth of testes and epididymes in juvenile males (both weight and length), increase of body weight and plasma testosterone concentration were all enhanced by adult male soiled bedding (Chapter 7). The young males can benefit from cohabitation with adult males but will suffer from cohabitation with male siblings. Group-housed young males showed delayed growth in testis length and weight, in epididymis weight, and in plasma testosterone concentration. The appearance of mature sperm in convoluted ducts of epididymes was delayed by up to 10 days as a result of group housing. Strangely, however, the plasma corticosterone concentration revealed no stress-elevated level in the group-housed males (Leshner, 1980; von Holst, 1986). More work needs to be carried out to understand the relationship between stress and the growth-retarding effects of group housing in the rice-field rat.

Two aspects of oestrous cycles were checked: the oestrous cycle lengths and the distribution of oestrous cycle lengths in different seasons. The oestrous cycles of the females housed in the controlled conditions of the laboratory were not significantly different in four seasons. Nor were the differences found in the distributions of the oestrous cycles in different lengths in different seasons. From this point of view the rat does not seem to be able to sense the seasonal changes in the controlled conditions of the laboratory. The distributions of E- and L-smears in different lengths, on the other hand, show some changes in different seasons (Chapter 5). The different distributions of E-smears seems to be in accordance with

seasonal changes of reproductive activity (Zhan, 1982) in nature.

It appears that, unlike in *Mus musculus* (Whitten, 1956) or *Rattus novergicus* (McClintock, 1983), neither olfactory cues from males nor housing density is capable of changing the lengths of oestrous cycles in the rats (Chapter 5). The effects of these environmental features are restricted to growth and sexual maturation.

Based on the preliminary observation that adult females were very aggressive towards adult males (Chapter 4), it is suggested that adult females might be socially dominant in the fields and, that as an adult the female rat lives with her pups or juveniles but not with the adult male in the reproductive season.

It is also suggested from laboratory observation that in the fields adult males could live together peacefully or could accompany juvenile males but the latter would be more likely to stay with their mother until a new litter is delivered. The adult males would certainly have to stay away from adult females all the time except when the females are on heat. Even then the males would have to struggle through the process of copulation by tolerating the fiery attack of the females. It might well be that only a few adult males could be accepted eventually by the females. Adult females, on the other hand, would possibly be surrounded by neonates or juveniles in the reproductive season and probably live alone in the non-reproductive season. The aggressive behaviour shown towards adult males may be a means of protecting themselves from being mated by weak males. During reproduction only could those strongest males pass their partners strict "test" so the qualitative promotion of the population development could be guaranteed. Such suggestions are speculative and are unsubstantiated by data. This thesis has set the scene for future studies on social and behavioural aspects of reproduction in this ubiquitous and noxious rodent of south-eastern China.

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Appendix 3-1: One factor ANOVA test of body weights of the males born in four seasons at different ages before weaning

Analysis of variance at day-1

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	28.809	9.603	18.528
Within groups	185	95.883	.518	p = .0001
Total	188	124.692		

Model II estimate of between component variance = .198

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan vs. Apr	.746	.32*	7.035*	4.594
Jan vs. Jul	1.01	.322*	12.747*	6.184
Jan vs. Oct	1.234	.346*	16.464*	7.028
Apr vs. Jul	.264	.259*	1.341	2.005
Apr vs. Oct	.488	.289*	3.699*	3.331
Jul vs. Oct	.224	.291	.771	1.521

*Significant at 95%

Analysis of variance at day-7

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	168.454	56.151	9.284
Within groups	185	1118.954	6.048	p = .0001
Total	188	1287.408		

Model II estimate of between component variance = 1.089

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan vs. Apr	2.053	1.095*	4.566*	3.701
Jan vs. Jul	2.415	1.1*	6.252*	4.331
Jan vs. Oct	3.034	1.183*	8.527*	5.058
Apr vs. Jul	.362	.886	.217	.807
Apr vs. Oct	.981	.987	1.28	1.96
Jul vs. Oct	.618	.994	.502	1.227

*Significant at 95%

Continue-1: Appendix 3-1

Analysis of variance at day-14

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	318.012	106.004	8.575
Within groups	185	2287.003	12.362	p = .0001
Total	188	2605.014		

Model II estimate of between component variance = 2.036

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan vs. Apr	1.452	1.565	1.117	1.83
Jan vs. Jul	2.412	1.573 *	3.05*	3.025
Jan vs. Oct	4.092	1.692 *	7.591*	4.772
Apr vs. Jul	.96	1.267	.746	1.496
Apr vs. Oct	2.641	1.411*	4.542*	3.691
Jul vs. Oct	1.68	1.421*	1.814	2.333

*Significant at 95%

Analysis of variance of day-21

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	215.586	71.862	3.002
Within groups	185	4429.08	23.941	p = .0318
Total	188	4644.666		

Model II estimate of between component variance = 1.042

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan vs. Apr	.804	2.178	.177	.729
Jan vs. Jul	2.022	2.189	1.107	1.822
Jan vs. Oct	3.122	2.355*	2.282	2.616
Apr vs. Jul	1.217	1.763	.619	1.363
Apr vs. Oct	2.318	1.964*	1.807	2.328
Jul vs. Oct	1.1	1.977	.402	1.098

*Significant at 95%

Appendix 3-2: One factor ANOVA test of growth rates at given ages of the males born in the same month before weaning

Analysis of variance in January

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	1.67	.557	3.779
Within groups	112	16.504	.147	p = .0126
Total	115	18.175		

Model II estimate of between component variance = .014

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
GR-7 vs. G-14/Jan.	-.09	.2	.268	.896
GR-7 vs. GR-21/Jan.	-.328	.2*	3.527*	3.253
GR-7 vs. GR-mean/Jan.	-.122	.2	.492	1.214
G-14 vs. GR-21/Jan.	-.238	.2*	1.851	2.357
G-14 vs. GR-mean/Jan.	-.032	.2	.034	.318
GR-21 vs. GR-mean/Jan.	.206	.2*	1.385	2.039

*Significant at 95%

Analysis of variance in April

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	7.019	2.34	28.568
Within groups	240	19.656	.082	p = .0001
Total	243	26.675		

Model II estimate of between component variance = .037

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
GR-7 vs. GR-14/Apr.	-.196	.102*	4.78*	3.787
GR-7 vs. GR-21/Apr.	-.477	.102*	28.192*	9.197
GR-7 vs. GR-mean/Apr.	-.203	.102*	5.121*	3.92
GR-14 vs. GR-21/Apr.	-.28	.102*	9.755*	5.41
GR-14 vs. GR-mean/Apr.	-.007	.102	.006	.133
GR-21 vs. GR-mean/Apr.	.273	.102*	9.282*	5.277

*Significant at 95%

Continue 1: Appendix 3-2

Analysis of variance in July

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	6.972	2.324	20.651
Within groups	232	26.108	.113	p = .0001
Total	235	33.079		

Model II estimate of between component variance = .037

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
GR-7 vs. GR-14/Jul.	-.096	.122	.81	1.559
GR-7 vs. GR-21/Jul.	-.46	.122*	18.517*	7.453
GR-7 vs. GR-mean/Jul.	-.167	.122*	2.44	2.706
GR-14 vs. GR-21/Jul.	-.364	.122*	11.582*	5.895
GR-14 vs. GR-mean/Jul.	-.071	.122	.439	1.147
GR-21 vs. GR-mean/Jul.	.293	.122*	7.513*	4.747

*Significant at 95%

Analysis of variance in October

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	4.323	1.441	15.403
Within groups	156	14.593	.094	p = .0001
Total	159	18.915		

Model II estimate of between component variance = .034

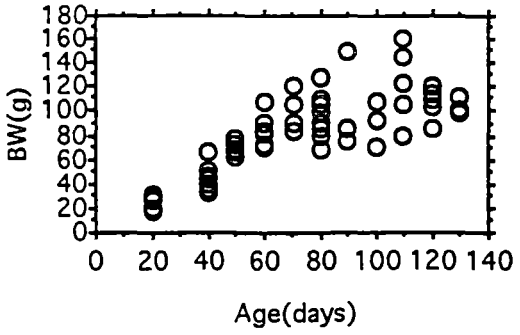
Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
GR-7 vs. GR-14/Oct.	-.036	.135	.094	.53
GR-7 vs. GR-21/Oct.	-.42	.135*	12.542*	6.134
GR-7 vs. GR-mean/Oct.	-.149	.135*	1.572	2.171
GR-14 vs. GR-21/Oct.	-.383	.135*	10.468*	5.604
GR-14 vs. GR-mean/Oct.	-.112	.135	.898	1.641
GR-21 vs. GR-mean/Oct.	.271	.135*	5.234*	3.963

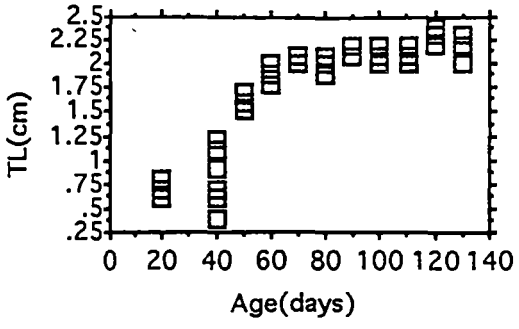
*Significant at 95%

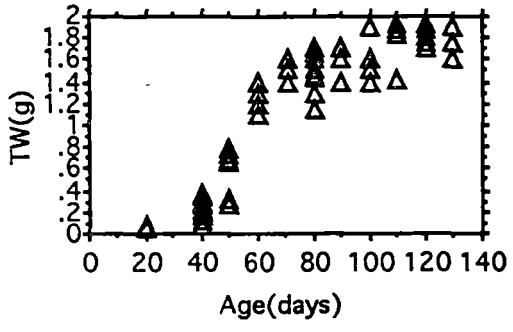
Appendix 3-3: Mean values of body weigt (BW), testis length (TL), testis weigt (TW), epididymis weigt (EW), body length (b.l.) and tail length (t.l.) at different ages.

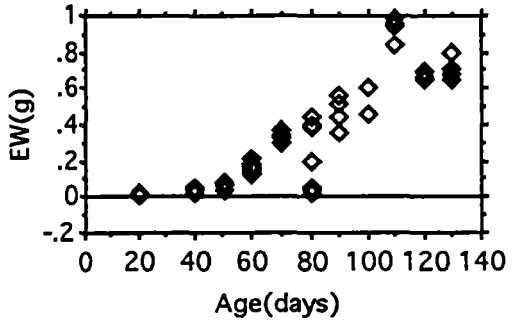
Age (days)	n	BW ± SE (g)	TL ± SE (cm)	TW ± SE (g)	EW ± SE (g)	b.l. ± SE (cm)	t.l. ± SE (cm)
20	6	25.17 ± 2.19	0.7 ± 0.03	0.05 ± 0.004	0.01 ± 0.001	10.42 ± 0.33	8.92 ± 0.15
40	8	47.0 ± 0.33	1.10 ± 0.03	0.22 ± 0.03	0.03 ± 0.003	13.19 ± 0.28	11.69 ± 0.33
50	6	68.42 ± 2.1	1.55 ± 0.03	0.57 ± 0.09	0.06 ± 0.01	13.58 ± 0.2	12.0 ± 0.41
60	3	85.33 ± 2.4	1.87 ± 0.03	1.2 0 ± 0.1	0.16 ± 0.02	14.62 ± 0.24	13.5 ± 0.54
70	4	100.38 ± 8.1	2.08 ± 0.03	1.5 ± 0.4	0.33 ± 0.02	15.25 ± 0.32	12.75 ± 0.25
80	4	94.25 ± 6.14	2.08 ± 0.03	1.52 ± 0.09	0.36 ± 0.06	13.62 ± 0.24	13.88 ± 0.43
90	4	95.88 ± 17.73	2.15 ± 0.03	1.6 ± 0.07	0.47 ± 0.04	14.0 ± 0.41	13.75 ± 0.32
100	4	94.62 ± 8.98	2.12 ± 0.05	1.6 ± 0.11	0.56 ± 0.04	14.62 ± 0.24	13.38 ± 0.59
110	5	122.5 ± 14.28	2.08 ± 0.04	1.78 ± 0.09	0.93 ± 0.02	16.0 ± 0.45	13.6 ± 0.43
120	5	107.3 ± 6.03	2.3 ± 0.04	1.81 ± 0.04	0.66 ± 0.01	15.6 ± 0.29	13.1 ± 0.4
130	4	106.38 ± 3.45	2.17 ± 0.06	1.79 ± 0.07	0.71 ± 0.03	15.5 ± 0.29	14.0 ± 0.2

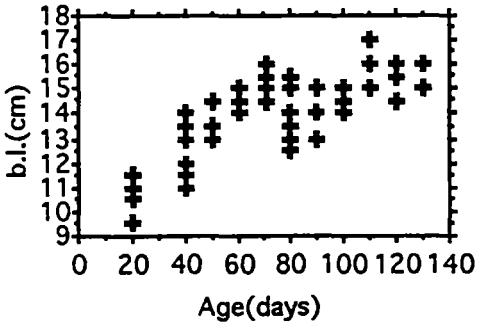
Appendix 3-4: Scattergram view of the correlation of body weight (BW), testis length (TL), epididymis weight (EW), body length (b.l.) and tail length (t.l.) with age.

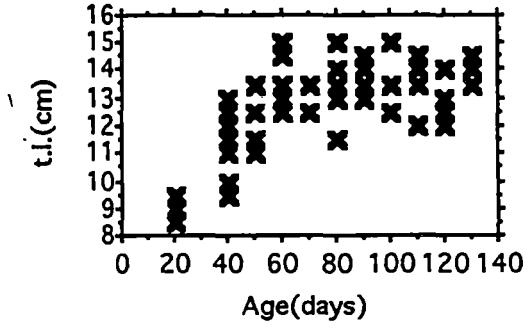




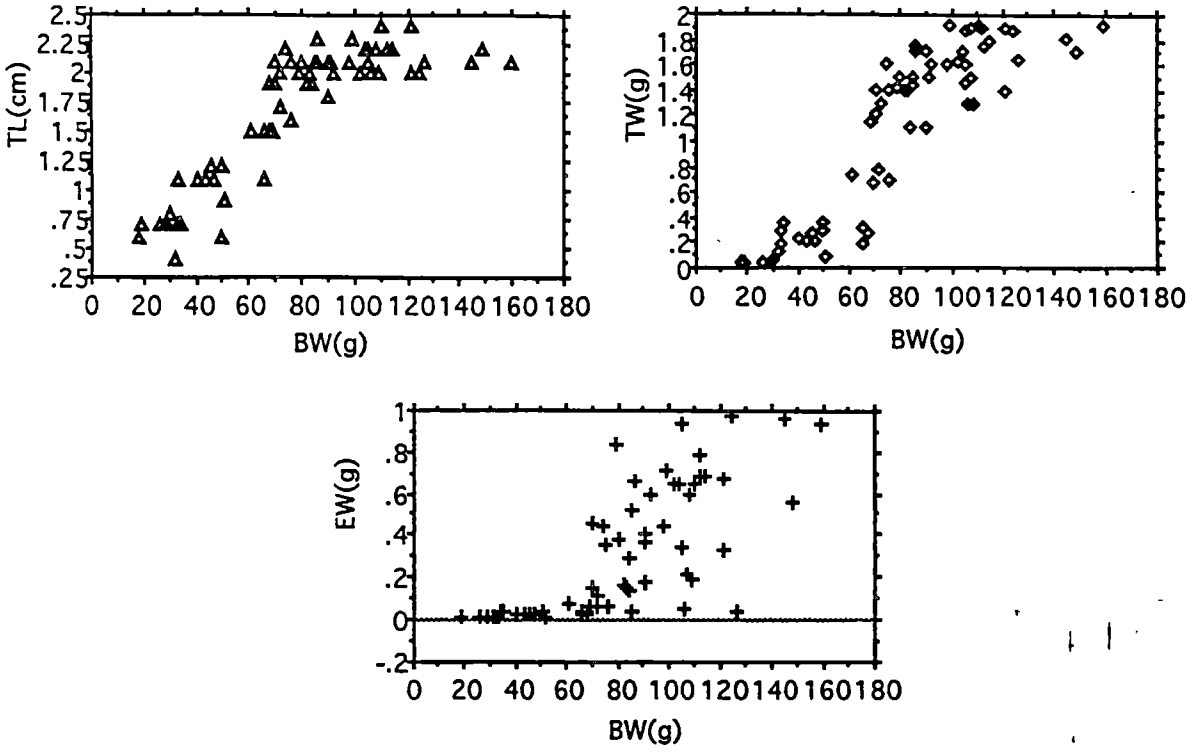




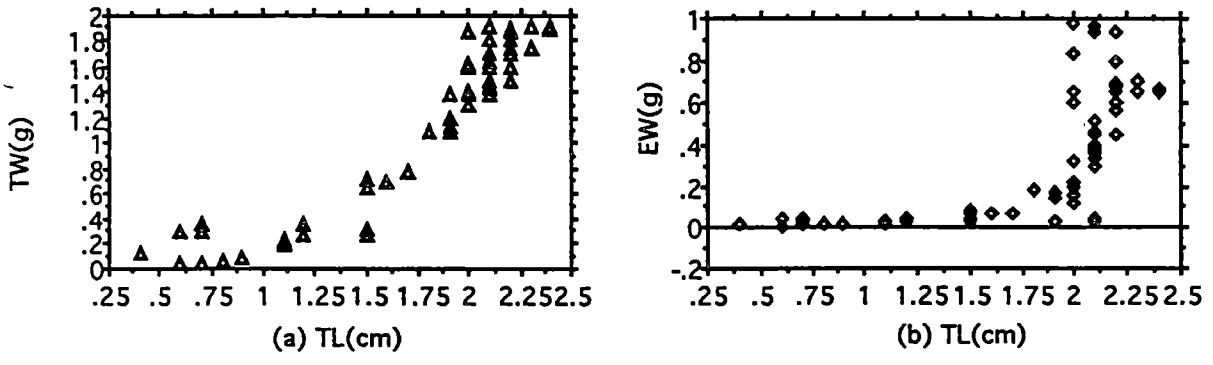




Appendix 3-5: Correlation of testis length (a), testis weight (b) and the weight of epididmis (c) withbody weight (BW).



Appendix 3-6: Correlation of the weights of testes (a) and epididymes (b) with testis length .



Appendix 3-7: Testosterone concentration of males at different ages.

NO	cpm	ng/ml		cpm	ng/ml		cpm	ng/ml		cpm	ng/ml
Testosterone			Age-20			Age-80			Age-120		
Standard curve			F1-207	4217	0.10	F1-250	1217	5.43	F1-186	2475	1.00
NSB	274		F1-205	4867	0.01	F1-238	1445	3.72	F1-190	2393	1.10
NSB	308		F1-209	4499	0.05	F1-248	1075	7.13	F1-191	1533	3.26
B°	4747		F1-206	4783	0.02	F1-252	947	9.46	F1-188	2073	1.61
B°	5327		n		4			4.00	F1-189	1359	4.26
1	4207	0.1	mean		0.04			6.44	F1-174	1347	4.35
2	4357	0.1	SE		0.02			1.22	n		6
3	3603	0.25							mean		2.60
4	3531	0.25							SE		0.63
5	2987	0.5									
6	2913	0.5	Age-40			Age-90			Age-130		
7	2615	1	F1-245	4751	0.02	F1-219	1451	3.69	F1-172	1001	8.35
8	2293	1	F2-47	3229	0.42	F1-224	1655	2.74	F1-175	1113	6.61
9	1927	2.5	F1-246	3405	0.34	F1-233	1809	2.23	F1-179	3639	0.25
10	1735	2.5	F1-247	4383	0.07	F1-234	1051	7.50	F1-176	1183	5.78
11	1293	5	n		4			4.00	n		4
12	1343	5	mean		0.21			4.04	mean		5.25
	861	10	SE		0.10			1.19	SE		1.75
	917	10									
			Age-50			Age-100					
r = - 0.99719			F2-179	3311	0.38	F1-216	1291	4.77			
Slope (m) =			F2-180	2717	0.76	F1-212	1273	4.92			
-1.716			F2-181	3457	0.32	F1-232	1207	5.53			
			F2-182	4321	0.08	F1-224	1009	8.21			
Intercept (b) =			n		4			4.00			
-0.156			mean		0.39			5.86			
			SE		0.14			0.80			
			Age-60			Age-110					
			F1-237	1201	5.59	F1-200	1651	2.76			
			F1-240	1083	7.02	F1-199	1483	3.51			
			F2-51	1345	4.36	F1-202	2015	1.72			
			F1-253	1847	2.13	F1-196	1429	3.82			
			n		4	F1-201	1029	7.86			
			mean		4.77	n		5.00			
			SE		1.04	mean		3.93			
						SE		1.05			

Appendix 3-8: One factor ANOVA test of the body weight of the males born in different months at the given ages

Analysis of variance at day-21

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	349.042	116.347	5.999
Within groups	65	1260.652	19.395	p = .0011
Total	68	1609.693		

Model II estimate of between component variance = 5.692

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb. vs. Apr.	-3.519	2.748*	2.18	2.557
Feb. vs. Aug.	-5.935	3.004*	5.189*	3.946
Feb. vs. Nov.	-4.721	3.134*	3.018*	3.009
Apr. vs. Aug.	-2.416	2.974	.878	1.623
Apr. vs. Nov.	-1.202	3.104	.199	.774
Aug. vs. Nov.	1.214	3.333	.176	.727

*Significant at 95%

Analysis of Variance Table-30

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	378.459	126.153	3.402
Within groups	65	2410.331	37.082	p = .0228
Total	68	2788.79		

Model II estimate of between component variance = 5.229

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb. vs. Apr.	-4.394	3.8*	1.778	2.309
Feb. vs. Aug.	-6.108	4.154*	2.875*	2.937
Feb. vs. Nov.	-2.006	4.333	.285	.925
Apr. vs. Aug.	-1.714	4.112	.231	.833
Apr. vs. Nov.	2.388	4.292	.412	1.111
Aug. vs. Nov.	4.103	4.609	1.054	1.778

*Significant at 95%

Continue-1: Appendix 3-8

Analysis of variance at day-40

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	788.944	262.981	5.957
Within groups	71	3134.242	44.144	p = .0011
Total	74	3923.187		

Model II estimate of between component variance = 11.968

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb. vs. Apr.	-2.296	3.887	.462	1.178
Feb. vs. Aug.	-9.238	4.479*	5.639*	4.113
Feb. vs. Nov.	-3.93	4.676	.937	1.676
Apr. vs. Aug.	-6.942	4.296*	3.462*	3.223
Apr. vs. Nov.	-1.635	4.501	.175	.724
Aug. vs. Nov.	5.308	5.021*	1.481	2.108

*Significant at 95%

Analysis of variance at day-50

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	589.635	196.545	1.865
Within groups	47	4954.11	105.407	p = .1485
Total	50	5543.745		

Model II estimate of between component variance = 7.158

Analysis of variance at day-60

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	57.785	19.262	.123
Within groups	50	7853.849	157.077	p = .9463
Total	53	7911.634		

Model II estimate of between component variance = -10.404

Continue-2; Appendix 3-8

Analysis of variance at day-70

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	867.759	289.253	1.048
Within groups	47	12968.486	275.925	p = .38
Total	50	13836.245		

Model II estimate of between component variance = 1.069

Analysis of variance at day-80

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	711.154	237.051	1.708
Within groups	34	4718.424	138.777	p = .1838
Total	37	5429.579		

Model II estimate of between component variance = 10.711

Appendix 3-9: One factor ANOVA test of testis length at different ages born different months

Analysis of variance at day-21

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.441	.147	20.591
Within groups	62	.442	.007	p = .0001
Total	65	.883		

Model II estimate of between component variance = .009

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.197	.05 *	20.31*	7.806
Feb vs. Aug	-.132	.067*	5.108*	3.915
Feb vs. Nov	-.118	.061*	4.955*	3.856
Apr vs. Aug	.065	.066	1.303	1.977
Apr vs. Nov	.079	.06*	2.342	2.651
Aug vs. Nov	.014	.074	.046	.373

*Significant at 95%

Analysis of variance at day-30

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.706	.235	20.755
Within groups	67	.76	.011	p = .0001
Total	70	1.466		

Model II estimate of between component variance = .013

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.182	.064*	10.676*	5.659
Feb vs. Aug	-.266	.074*	17.176*	7.178
Feb vs. Nov	-.207	.076*	9.875*	5.443
Apr vs. Aug	-.084	.072*	1.83	2.343
Apr vs. Nov	-.024	.073	.143	.655
Aug vs. Nov	.06	.082	.71	1.46

*Significant at 95%

Continue-1: Appendix 3-9

Analysis of variance at day-40

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	1.6	.533	15.986
Within groups	71	2.369	.033	p = .0001
Total	74	3.969		

Model II estimate of between component variance = .027

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.368	.107*	15.745*	6.873
Feb vs. Aug	-.182	.123*	2.893*	2.946
Feb vs. Nov	-.157	.129*	1.972	2.432
Apr vs. Aug	.186	.118*	3.302*	3.148
Apr vs. Nov	.212	.124*	3.875*	3.409
Aug vs. Nov	.025	.138	.044	.363

*Significant at 95%

Analysis of variance at day-50

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2.193	.731	11.067
Within groups	58	3.83	.066	p = .0001
Total	61	6.023		

Model II estimate of between component variance = .044

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.469	.174*	9.673*	5.387
Feb vs. Aug	-.214	.198*	1.554	2.159
Feb vs. Nov	-.121	.202	.481	1.201
Apr vs. Aug	.256	.179*	2.737	2.866
Apr vs. Nov	.348	.183*	4.816*	3.801
Aug vs. Nov	.092	.206	.268	.897

* Significant at 95%

Continue-2: Appendix 3-9

Analysis of variance at day-60

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	3.529	1.176	.682
Within groups	44	75.898	1.725	p = .5678
Total	47	79.427		

Model II estimate of between component variance = -.046

Analysis of variance at day-70

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2.412	.804	11.726
Within groups	46	3.154	.069	p = .0001
Total	49	5.566		

Model II estimate of between component variance = .06

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.543	.217*	8.44*	5.032
Feb vs. Aug	-.15	.232	.563	1.299
Feb vs. Nov	-.1	.232	.25	.866
Apr vs. Aug	.393	.199*	5.286*	3.982
Apr vs. Nov	.443	.199*	6.716*	4.489
Aug vs. Nov	.05	.215	.073	.468

*Significant at 95%

Analysis of variance at day-80

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2.021	.674	11.017
Within groups	31	1.895	.061	p = .0001
Total	34	3.916		

Model II estimate of between component variance = .072

Continue-4: Appendix-9

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Feb vs. Apr	-.584	.244*	7.966*	4.888
Feb vs. Aug	-.139	.244	.45	1.162
Feb vs. Nov	-.057	.281	.058	.415
Apr vs. Aug	.445	.215*	5.95*	4.225
Apr vs. Nov	.527	.256*	5.885*	4.202
Aug vs. Nov	.082	.256	.142	.652

*Significant at 95%

Appendix 4-1: One Factor ANOVA test of the body weight of the females born in different months in the laboratory

Analysis of Variance Table (W-1)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	10.849	3.616	5.775
Within groups	141	88.285	.626	p = .0009
Total	144	99.134		

Model II estimate of between component variance = .085

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan. vs. Apr.	.255	.418	.484	1.205
Jan. vs. Jul.	.614	.405*	2.998*	2.999
Jan. vs. Oct.	.797	.445*	4.179*	3.541
Apr. vs. Jul.	.359	.325*	1.592	2.186
Apr. vs. Oct.	.542	.374*	2.741*	2.867
Jul. vs. Oct.	.184	.359	.341	1.012

* Significant at 95%

Analysis of Variance Table (W-7)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	101.594	33.865	4.688
Within groups	142	1025.752	7.224	p = .0037
Total	145	1127.345		

Model II estimate of between component variance = .757

Continue 1 (Appendix 4-1)

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan. vs. Apr.	1.906	1.415*	2.366	2.664
Jan. vs. Jul.	2.23	1.374*	3.433*	3.209
Jan. vs. Oct.	2.72	1.512*	4.216*	3.556
Apr. vs. Jul.	.324	1.095	.114	.584
Apr. vs. Oct.	.813	1.264	.539	1.272
Jul. vs. Oct.	.489	1.218	.21	.794

* Significant at 95%

Analysis of Variance Table (W-21

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	291.757	97.252	6.148
Within groups	142	2246.228	15.819	p = .0006
Total	145	2537.984		

Model II estimate of between component variance = 2.313

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jan. vs. Apr.	2.245	2.093*	1.499	2.121
Jan. vs. Jul.	3.326	2.033*	3.488*	3.235
Jan. vs. Oct.	4.621	2.237*	5.558*	4.084
Apr. vs. Jul.	1.081	1.621	.58	1.319
Apr. vs. Oct.	2.376	1.87*	2.102	2.511
Jul. vs. Oct.	1.294	1.803	.672	1.42

* Significant at 95%

Analysis of Variance Table (W-21)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	180.571	60.19	2.381
Within groups	142	3589.124	25.276	p = .0721
Total	145	3769.695		

Model II estimate of between component variance = .992

Appendix 4-2: One Factor ANOVA test of the growth rates of the females born in different months in the laboratory

Analysis of Variance Table (January)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	.632	.211	1.345
Within groups	85	13.314	.157	p = .2651
Total	88	13.946		

Model II estimate of between component variance = .002

Analysis of Variance Table (April)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	1.612	.537	6.775
Within groups	168	13.327	.079	p = .0002
Total	171	14.939		

Model II estimate of between component variance = .011

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
G-7 vs. G-14	-.218	.12*	4.299**	3.591
G-7 vs. G-21	-.252	.12*	5.731***	4.146
G-7 vs. G-mean	-.144	.12*	1.884	2.378
G-14 vs. G-21	-.034	.12	.103	.555
G-14 vs. G-mean	.074	.12	.491	1.214
G-21 vs. G-mean	.107	.12	1.043	1.769

** $P < 0.01$, *** $P < 0.005$

Analysis of Variance Table (July)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	5.542	1.847	19.595
Within groups	204	19.232	.094	p = .0001
Total	207	24.774		

Model II estimate of between component variance = .034

Continue 1 (Appendix 4-2)

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
G-7 vs. G-14	-.104	.119	.995	1.728
G-7 vs. G-21	-.441	.119*	17.891***	7.326
G-7 vs. G-mean	-.165	.119*	2.497	2.737
G-14 vs. G-21	-.337	.119*	10.447***	5.598
G-14 vs. G-mean	-.061	.119	.339	1.009
G-21 vs. G-mean	.276	.119*	7.02*	4.589

*** P < 0.0001

Analysis of Variance Table (October)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2.251	.75	6.999
Within groups	116	12.438	.107	p = .0002
Total	119	14.69		

Model II estimate of between component variance = .021

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
G-7 vs. G-14	-.071	.167	.235	.84
G-7 vs. G-21	-.365	.167*	6.224***	4.321
G-7 vs. G-mean	-.146	.167	.999	1.731
G-14 vs. G-21	-.294	.167*	4.04**	3.481
G-14 vs. G-mean	-.075	.167	.265	.891
G-21 vs. G-mean	.219	.167*	2.236	2.59

** P < 0.01, *** P < 0.001

Appendix: 4-3: One factor ANOVA test of the body weight at given ages after weaning of the females born in different months in the laboratory

Analysis of Variance Table (W-40)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	4496.97	1498.99	22.725
Within groups	36	2374.667	65.963	p = .0001
Total	39	6871.638		

Model II estimate of between component variance = 146.977

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Mar. vs. Jun.	-10.694	8.682*	2.081	2.498
Mar. vs. Sep.	18.317	8.237*	6.782**	4.511
Mar. vs. Dec.	5.468	8.13	.62	1.364
Jun. vs. Sep.	29.011	7.264*	21.873***	8.101
Jun. vs. Dec.	16.162	7.143*	7.02***	4.589
Sep. vs. Dec.	-12.849	6.595*	5.206**	3.952

** P < 0.005, *** P < 0.001

Analysis of Variance Table (W-50)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2931.746	977.249	8.099
Within groups	40	4826.453	120.661	p = .0002
Total	43	7758.199		

Model II estimate of between component variance = 78.412

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Mar. vs. Jun.	-3.606	10.202	.17	.714
Mar. vs. Sep.	18.042	9.507*	4.905**	3.836
Mar. vs. Dec.	6.335	9.339	.627	1.371
Jun. vs. Sep.	21.647	9.791*	6.658***	4.469
Jun. vs. Dec.	9.94	9.628*	1.452	2.087
Sep. vs. Dec.	-11.707	8.888*	2.363	2.662

** P < 0.01, *** P < 0.005

Continue-1(Appendix 4-3)

Analysis of Variance Table (W-60)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2921.863	973.954	4.128
Within groups	36	8493.917	235.942	p = .0129
Total	39	11415.78		

Model II estimate of between component variance = 75.694

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Mar. vs. Jun.	-5.472	16.42	.152	.676
Mar. vs. Sep.	17.042	15.578*	1.641	2.219
Mar. vs. Dec.	8.365	15.377	.406	1.103
Jun. vs. Sep.	22.514	13.738*	3.683*	3.324
Jun. vs. Dec.	13.838	13.51*	1.439	2.077
Sep. vs. Dec.	-8.676	12.472	.664	1.411

* Significant at 95%

Analysis of Variance Table (W-70)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	4687.962	1562.654	4.93
Within groups	35	11094.047	316.973	p = .0058
Total	38	15782.01		

Model II estimate of between component variance = 131.539

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Mar. vs. Jun.	-.938	19.522	.003	.098
Mar. vs. Sep.	25.517	18.074*	2.739	2.866
Mar. vs. Dec.	17.365	17.84	1.302	1.976
Jun. vs. Sep.	26.454	16.499*	3.533*	3.255
Jun. vs. Dec.	18.303	16.243*	1.745	2.288

* Significant at 95%

Continue-2 (Appendix 4-3)

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Sep. vs. Dec.	-8.151	14.47	.436	1.144

Analysis of Variance Table (W-80)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	2655.455	885.152	2.422
Within groups	33	12061.535	365.501	p = .0835
Total	36	14716.99		

Model II estimate of between component variance = 57.223

Appendix 5-1: One foactor ANOVA test of the oestrous cycles in the females born in different seasons

Analysis of Variance Table of the cycles

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	10.069	3.356	1.085
Within groups	509	1574.04	3.092	p = .3548
Total	512	1584.109		

Model II estimate of between component variance = .002

Analysis of Variance Table of E-length

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	22.607	7.536	4.141
Within groups	509	926.27	1.82	p = .0065
Total	512	948.877		

Model II estimate of between component variance = .046

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Spring vs. Summer	-.011	.345	.001	.065
Spring vs. Autumn	.085	.336	.082	.497
Spring vs. Winter	.471	.296*	3.254*	3.124
Summer vs. Autumn	.097	.383	.082	.495
Summer vs. Winter	.482	.348*	2.469	2.721
Autumn vs. Winter	.385	.339*	1.663	2.233

* Significant at 95%

Analysis of Variance Table of L-length

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	8.959	2.986	1.524
Within groups	509	997.548	1.96	p = .2074
Total	512	1006.507		

Model II estimate of between component variance = .008

*Appendix 5-2. Distribution of oestrous cycle lengths in four seasons**

Season	2-day	3-day	4-day	5-day	6-day	7-day	8-day	9-day	Total cycles
Spring	18	25	41	36	19	13	5	7	164
	(20.78)	(33.25)	(36.76)	(31.01)	(17.26)	(15.03)	(6.07)	(3.84)	
Summer	7	20	24	21	12	6	1	1	92
	(11.66)	(18.65)	(20.62)	(17.4)	(9.68)	(8.43)	(3.41)	(2.15)	
Autumn	15	17	21	16	12	15	4	0	100
	(12.67)	(20.27)	(22.42)	(18.91)	(10.53)	(9.16)	(3.7)	(2.34)	
Winter	25	42	29	24	11	13	9	4	157
	(19.89)	(31.83)	(35.19)	(29.69)	(16.53)	(14.38)	(5.81)	(3.67)	
Total	65	104	115	97	54	47	19	12	

* : Number of rats examined in spring and winter, 35; summer and autumn, 20.

The values in the brackets are expected values.

DF = 21, $\chi^2 = 32.06$, P = 0.0577

*Appendix 5-3. Distribution of E-smears of differing lengths in four seasons**

	No. of rats	1-day	2-day	3-day	4-day	5-day	6-day	7-day	8-day	Total cycles
Spring	35	53	41	38	18	9	1	3	1	164
		(55.95)	(48.27)	(32.61)	(15.66)	(6.07)	(3.52)	(1.6)	(0.32)	
Summer	20	18	34	28	7	2	3	0	0	92
		(31.38)	(27.08)	(18.29)	(8.79)	(3.41)	(1.97)	(0.9)	(0.18)	
Autumn	20	36	23	21	12	5	3	0	0	100
		(34.11)	(29.43)	(19.88)	(8.79)	(3.41)	(1.97)	(0.9)	(0.18)	
Winter	35	68	53	15	12	3	4	2	0	156
		(53.56)	(46.21)	(31.22)	(15)	(5.81)	(3.37)	(1.53)	(0.31)	

DF = 21, χ^2 = 43.57, P = 0.0027

Spring *vs* Summer DF = 7, χ^2 = 14.62, P = 0.0.0411 Summer *vs* Autumn DF = 5, χ^2 = 11.41, P = 0.0.0438

Spring *vs* Autumn DF = 7, χ^2 = 5.35, P = 0.6173 Summer *vs* Winter DF = 6, χ^2 = 25.58, P = 0.0003

Spring *vs* Winter DF = 7, χ^2 = 20.43, P = 0.0047 Autumn *vs* Winter DF = 6, χ^2 = 13.35, P = 0.0379

*The values in brackets are expected values.

*Appendix 5-4: Distributions of L-smears in different lengths in four seasons**

	No. of rats	1-day	2-day	3-day	4-day	5-day	6-day	7-day	8-day	Total cycles
Spring	35	57	54	27	16	7	2	1	0	164
		(69.69)	(42.84)	(25.58)	(13.75)	(6.71)	(3.2)	(1.92)	(0.32)	
Summer	20	40	28	17	5	1	0	1	0	92
		(39.1)	(24.03)	(14.35)	(7.71)	(3.77)	(1.79)	(1.08)	(0.18)	
Autumn	20	43	22	19	12	3	1	0	0	100
		(42.5)	(26.12)	(15.59)	(8.38)	(4.09)	(1.95)	(1.17)	(0.19)	
Winter	35	78	30	17	10	10	7	4	1	157
		(66.72)	(41.01)	(24.48)	(13.16)	(6.43)	(3.06)	(1.84)	(0.37)	

DF = 21, χ^2 = 37.19, P = 0.016.

Spring *vs* Summer DF = 6, χ^2 = 5.98, P = 0.4253

Spring *vs* Autumn DF = 6, χ^2 = 5.12, P = 0.5291

Spring *vs* Winter DF = 7, χ^2 = 19.75, P = 0.0061

Summer *vs* Autumn DF = 6, χ^2 = 6.5, P = 0.3696

Summer *vs* Winter DF = 7, χ^2 = 15.2, P = 0.0335

Autumn *vs* Winter DF = 7, χ^2 = 12.91, P = 0.0743

*The values in brackets are expected values.

*Appendix 5-5. Distribution of oestrous cycles of differing lengths in singly caged and grouped females**

	2-day	3-day	4-day	5-day	6-day	7-day	8-day	9-day	Total
Single	7	20	24	21	12	6	1	1	92
	(6.76)	(19.75)	(20.27)	(21.83)	(14.03)	(6.76)	(2.08)	(0.52)	
Grouped	6	18	15	21	15	7	3	0	85
	(6.24)	(18.24)	(18.73)	(20.17)	(12.97)	(6.24)	(1.92)	(0.48)	

*: The values in the brackets are expected values.

Twenty females in each group.

DF = 7, $\chi^2 = 4.4$, P = 0.7328.

*Appendix 5-6. Distribution of E-smears and L-smears of differing lengths in singly housed or grouped (5 rats/cage) females**

	1-day	2-day	3-day	4-day	5-day	6-day	7-day	Total
E-smears:	DF = 5, χ^2 = 3.2, P = 0.6689							
Single	18	34	28	7	2	3	0	92
	(19.75)	(34.31)	(27.03)	(7.28)	(2.08)	(1.56)		
Grouped	20	32	24	7	2	0	0	85
	(18.25)	(31.69)	(27.97)	(6.72)	(1.92)	(1.44)		
L-smears:	DF = 6, χ^2 = 6.47, P = 0.3728							
Single	40	28	17	5	1	0	1	92
	(35.34)	(28.99)	(19.75)	(7.28)	(1.56)	(1.04)	(1.04)	
Grouped	28	22	21	7	2	2	1	85
	(32.66)	(24.01)	(18.25)	(6.72)	(1.44)	(0.96)	(0.96)	

*: The values in brackets are expected values.

Twenty females in each group.

*Appendix 5-7. Distribution of oestrous cycles of 20 singly caged females and 20 females caged with adult males (With Am)**

	2-day	3-day	4-day	5-day	6-day	7-day	8-day	9-day	Total cycles
Single	15	17	21	16	12	15	4	0	100
	(13.82)	(18.43)	(22.12)	(18.89)	(10.6)	(11.06)	(4.61)	(0.46)	
With Am	15	23	27	25	11	9	6	1	117
	(16.18)	(21.57)	(25.88)	(22.11)	(12.4)	(12.94)	(5.39)	(0.54)	

*: The values in the brackets are expected values.

DF = 7, $\chi^2 = 5.27$, P = 0.627.

*Appendix 5-8. Distribution of E-smears and L-smears in the cycles of the females housed singly or each cohabiting with an adult male**

	1-day	2-day	3-day	4-day	5-day	6-day	7-day	Total
E-smears:	DF = 6, χ^2 = 7.58, P = 0.2709							
Single	36	23	21	12	5	3	0	100
	(35.02)	(24.88)	(20.74)	(11.98)	(4.15)	(1.38)	(1.84)	
With Am	40	31	24	14	4	0	4	117
	(40.98)	(29.12)	(24.26)	(14.02)	(4.85)	(1.62)	(2.16)	
L-smears:	DF = 6, χ^2 = 9.25, P = 0.1601							
Single	43	22	19	12	3	1	0	100
	(41.94)	(27.19)	(18.89)	(7.83)	(1.84)	(1.38)	(0.92)	
With Am	48	37	22	5	1	2	2	
	(49.06)	(31.81)	(22.11)	(9.17)	(2.16)	(1.62)	(1.08)	117

*: The values in brackets are expected values.

Twenty females in each group.

Apendix 6-1: One factor ANOVA test of the **ages** *at vaginal opening in the females born in different months in the laboratory*

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	8	6805.033	850.629	4.146
Within groups	96	19696.814	205.175	p = .0003
Total	104	26501.848		

Model II estimate of between component variance = 55.86

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
May vs. Jun	12.167	14.218	.361	1.699
May vs. Jul	21.389	13.405*	1.254	3.168
May vs. Aug	14.513	14.034*	.527	2.053
May vs. Nov	9.897	14.034	.245	1.4
May vs. Dec	18.167	14.684*	.754	2.456
May vs. Feb	16.444	14.987*	.593	2.178
May vs. Mar	7.467	14.684	.127	1.009
May vs. Apr	31.31	13.875*	2.508*	4.48
Jun vs. Jul	9.222	10.597	.373	1.728
Jun vs. Aug	2.346	11.383	.021	.409
Jun vs. Nov	-2.269	11.383	.02	.396
Jun vs. Dec	6	12.175	.12	.978
Jun vs. Feb	4.278	12.539	.057	.677
Jun vs. Mar	-4.7	12.175	.073	.766
Jun vs. Apr	19.143	11.187*	1.443	3.397
Jul vs. Aug	-6.876	10.35	.217	1.319
Jul vs. Nov	-11.491	10.35*	.607	2.204
Jul vs. Dec	-3.222	11.215	.041	.57
Jul vs. Feb	-4.944	11.609	.089	.846
Jul vs. Mar	-13.922	11.215*	.759	2.464

* Significant at 95%

Continue-1 (Appendix 6-1)

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jul vs. Apr	9.921	10.133	.472	1.944
Aug vs. Nov	-4.615	11.153	.084	.821
Aug vs. Dec	3.654	11.961	.046	.606
Aug vs. Feb	1.932	12.331	.012	.311
Aug vs. Mar	-7.046	11.961	.171	1.169
Aug vs. Apr	16.797	10.952*	1.159	3.044
Nov vs. Dec	8.269	11.961	.235	1.372
Nov vs. Feb	6.547	12.331	.139	1.054
Nov vs. Mar	-2.431	11.961	.02	.403
Nov vs. Apr	21.412	10.952*	1.883	3.881
Dec vs. Feb	-1.722	13.065	.009	.262
Dec vs. Mar	-10.7	12.717	.349	1.67
Dec vs. Apr	13.143	11.773*	.614	2.216
Feb vs. Mar	-8.978	13.065	.233	1.364
Feb vs. Apr	14.865	12.149*	.738	2.429
Mar vs. Apr	23.843	11.773*	2.02	4.02

* Significant at 95%

Appendix 6-2: One factor ANOVA analysis of body weight at vaginal opening of the females born in different months

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	8	4148.476	518.559	2.413
Within groups	96	20626.372	214.858	p = .0203
Total	104	24774.848		

Model II estimate of between component variance = 26.284

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
May vs. Jun	-5.833	14.549	.079	.796
May vs. Jul	6.278	13.717	.103	.909
May vs. Aug	8.654	14.362	.179	1.196
May vs. Nov	9.962	14.362	.237	1.377
May vs. Dec	13.35	15.027	.389	1.764
May vs. Feb	11.389	15.336	.272	1.474
May vs. Mar	-2.4	15.027	.013	.317
May vs. Apr	11.714	14.199	.335	1.638
Jun vs. Jul	12.111	10.845*	.614	2.217
Jun vs. Aug	14.487	11.649*	.762	2.469
Jun vs. Nov	15.795	11.649*	.906	2.692
Jun vs. Dec	19.183	12.459*	1.168	3.057
Jun vs. Feb	17.222	12.831*	.887	2.664
Jun vs. Mar	3.433	12.459	.037	.547
Jun vs. Apr	17.548	11.447*	1.158	3.043

* Significant at 95%

Continue-1 (Appeidix 6-2)

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Jul vs. Aug	2.376	10.591	.025	.445
Jul vs. Nov	3.684	10.591	.06	.69
Jul vs. Dec	7.072	11.477	.187	1.223
Jul vs. Feb	5.111	11.88	.091	.854
Jul vs. Mar	-8.678	11.477	.282	1.501
Jul vs. Apr	5.437	10.369	.135	1.041
Aug vs. Nov	1.308	11.414	.006	.227
Aug vs. Dec	4.696	12.24	.073	.762
Aug vs. Feb	2.735	12.618	.023	.43
Aug vs. Mar	-11.054	12.24	.402	1.793
Aug vs. Apr	3.06	11.208	.037	.542
Nov vs. Dec	3.388	12.24	.038	.55
Nov vs. Feb	1.427	12.618	.006	.225
Nov vs. Mar	-12.362	12.24*	.502	2.005
Nov vs. Apr	1.753	11.208	.012	.31
Dec vs. Feb	-1.961	13.37	.011	.291
Dec vs. Mar	-15.75	13.013*	.722	2.403
Dec vs. Apr	-1.636	12.048	.009	.27
Feb vs. Mar	-13.789	13.37*	.524	2.047
Feb vs. Apr	.325	12.432	3.375E-4	.052
Mar vs. Apr	14.114	12.048*	.676	2.326

* Significant at 95%

Appendix 7-1: Mean body weight of group-housed (4/cage) and singly-housed males

		Mean body weights				Growth rate			
Treatment	df	Grouped	Single	t-Value	P	Grouped	Single	t-value	P
Weaning	24	23.25 ± 1.07	23.56 ± 0.63	- 0.262	0.7954				
10-day	24	38.08 ± 1.31	42.61 ± 1.60	- 2.145	0.0423	1.21 ± 0.09	1.6 ± 0.10	- 2.815	0.0096
20-day	24	46.33 ± 1.65	53.39 ± 2.44	- 2.316	0.0294	0.92 ± 0.10	1.20 ± 0.15	- 1.515	0.1427
30-day	24	56.25 ± 2.33	61.75 ± 3.66	- 1.221	0.2339	0.99 ± 0.28	0.84 ± 0.14	0.523	0.6058
40-day	24	60.50 ± 1.80	66.86 ± 5.03	- 1.116	0.2756	0.43 ± 0.28	0.51 ± 0.19	- 0.261	0.7965
50-day	24	71.67 ± 2.72	78.00 ± 5.92	- 0.919	0.3671	0.93 ± 0.12	0.93 ± 0.12	0.00	
60-day	24	79.25 ±3.37	80.86 ± 6.20	- 0.217	0.8299	1.26 ± 0.39	0.48 ± 0.16	1.980	0.0592
70-day	24	85.71 ± 4.00	86.25 ± 6.89	- 0.065	0.9487	0.65 ± 0.19	0.54 ± 0.10	0.505	0.6180

Appendix 7-2: Mean testis lengths of group-housed (4/cage) and singly-housed males

		Mean testis lengths				Growth rate			
Treatment	df	Grouped	Single	t-Value	P	Grouped	Single	t-value	P
Weaning	24	0.34 ± 0.02	0.34 ± 0.02	- 0.902	0.376				
10-day	24	0.69 ± 0.02	0.83 ± 0.03	- 3.654	0.0013	0.03 ± 0.00	0.04 ± 0.02	4.566	0.0001
20-day	24	0.78 ± 0.03	1.03 ± 0.05	- 4.384	0.0002	0.01 ± 0.00	0.02 ± 0.00	- 3.102	0.0049
30-day	24	0.99 ± 0.03	1.31 ± 0.07	- 4.24	0.0003	0.02 ± 0.00	0.03 ± 0.00	- 1.396	0.1756
40-day	24	1.21 ± 0.04	1.48 ± 0.05	- 3.828	0.0008	0.02 ± 0.00	0.02 ± 0.00	0.809	0.4266
50-day	24	1.57 ± 0.05	1.72 ± 0.04	- 2.287	0.0313	0.03 ± 0.00	0.02 ± 0.00	2.276	0.0321
60-day	24	1.65 ± 0.04	1.86 ± 0.05	- 3.214	0.0037	0.01 ± 0.01	0.02 ± 0.01	- 0.515	0.6114
70-day	24	1.83 ± 0.06	1.86 ± 0.06	- 0.369	0.7154	0.02 ± 0.01	0.00 ± 0.00	2.294	0.0308

Appendix 7-3: Testis development in group-caged young males (4/cage) from 21 days of age to 90 days of age compared with that of singly caged males.

	df.	Single	Grouped (4)	t-Value	P
Age-40 (20 days of treatment)					
TW (g)	6	0.27 ± 0.05	0.22 ± 0.02	0.938	0.3843
EW (mg)	6	32.55 ± 5.93	24.0 ± 3.84	0.962*	0.3732
Age-50 (30 days of treatment)					
TW (g)	8	0.57 ± 0.09	0.38 ± 0.13	1.248	0.2472
EW (mg)	8	59.15 ± 7.23	41.15 ± 11.05	1.568*	1.555*
Age-60 (40 days of treatment)					
TW (g)	6	1.3 0 ± 0.04	0.56 ± 0.15	4.793	0.003
EW (mg)	6	161 ± 20.37	56.35 ± 12.62	4.616*	0.0036
Age-70 (50 days of treatment)					
TW (g)	6	1.50 ± 0.04	1.05 ± 0.05	6.784	0.0005
EW (mg)	6	332 ± 16.52	145 ± 27.23	4.731*	0.0032*
Age-80 (60 days of treatment)					
TW (g)	6	1.42 ± 0.21	1.58 ± 0.03	- 1.453	0.1965
EW (mg)	6	367.5 ± 43.48	395.75 ± 55.3	- 0.400*	0.7019*
Age-90 (70 days of treatment)					
TW (g)	6	1.6 ± 0.07	1.2 ± 0.1	3.266	0.0171
EW (mg)	6	475 ± 43.55	267 ± 34.07	3.733*	0.0097*

*: ln(x)-transformed data used in analysis.

*Appendix 7-4: Plasma concentrations (ng/ml) of testosterone and corticosterone in group-caged and singly caged males**

Treatment	df.	Groped (ng/ml)	Single (ng/ml)	t-Value	P
		Plasma concentration of testosterone			
20 days	6	0.19 ± 0.06	0.19 ± 0.08	0.322	0.7581
30 days	8	0.27 ± 0.14	0.53 ± 0.21	- 0.905	0.3919
40 days	6	0.61 ± 0.14	6.08 ± 1.20	- 7.492	0.0003
60 days	6	4.36 ± 0.32	8.37 ± 0.85	- 4.913	0.0027
70 days	6	5.85 ± 1.77	5.22 ± 0.84	0.120	0.9083
		Plasma concentration of corticosterone			
20 days	6	857.40 ± 191.96	428.39 ± 160.24	1.49	0.1868
30 days	8	983.68 ± 178.77	983.78 ± 225.18	0.229	0.8244
40 days	6	652.08 ± 168.46	398.72 ± 104.84	1.257	0.2553
50 days	6	309.84 ± 127.66	424.08 ± 46.60	- 1.393	0.2129
60 days	6	453.48 ± 20.92	371.36 ± 189.11	1.097	0.3149
70 days	6	602.77 ± 64.00	361.53 ± 135.78	1.667	0.1466

*: ln(x)-transformed data used in analysis.

Appendix 7-5: Mean body weights (g) of the males housed with adult males' bedding (Ym + UAm) and the males housed with female siblings (Ym + Yf).

		Mean body weights				Growth rate			
Treatment	df	Ym+UAm	Ym+Yf	t-Value	P	Ym+UAm	Ym+Yf	t-value	P
Weaning	35	25.68 ± 0.68	24.56 ± 1.41	- 0.786	0.4372				
10-day	35	36.70 ± 0.76	37.33 ± 1.77	0.365	0.7176	1.12 ± 0.07	1.29 ± 0.11	1.36	0.1824
20-day	35	50.50 ± 1.36	51.00 ± 2.28	0.200	0.8423	1.43 ± 0.07	1.37 ± 0.13	- 0.374	0.7108
30-day	31	63.53 ± 1.57	56.88 ± 2.24	- 2.506	0.0177	1.19 ± 0.08	0.60 ± 0.20	- 3.063	0.0045
40-day	29	73.63 ± 2.72	60.18 ± 2.90	- 3.159	0.0037	1.03 ± 0.16	0.26 ± 0.22	- 2.769	0.0099
50-day	29	83.24 ± 4.31	66.67 ± 3.62	- 2.693	0.0116	0.97 ± 0.21	0.57 ± 0.22	- 1.254	0.2203
60-day	29	96.83 ± 4.42	81.00 ± 0.30	- 2.611	0.0142	1.24 ± 0.18	1.43 ± 0.38	0.521	0.6062

Appendix 7-6: Mean testis lengths(cm) of the males housed with adult males' bedding (Ym + UAm) and the males housed with female siblings (Ym + Yf).

		Mean testis lengths				Growth rate			
Treatment	df	Ym+UAm	Ym+Yf	t-Value	P	Ym+UAm	Ym+Yf	t-value	P
Weaning	25	0.39 ± 0.02	0.39 ± 0.02	0.046	0.9638				
10-day	34	0.81 ± 0.03	0.82 ± 0.04	0.244	0.8084	0.07 ± 0.01	0.06 ± 0.01	- 1.153	0.2566
20-day	35	1.03 ± 0.03	0.89 ± 0.06	- 2.178	0.0362	0.02 ± 0.00	0.01 ± 0.01	- 1.166	0.2514
30-day	31	1.32 ± 0.05	1.19 ± 0.07	- 1.495	0.145	0.03 ± 0.00	0.03 ± 0.00	0.923	0.3634
40-day	29	1.52 ± 0.06	1.33 ± 0.09	- 1.846	0.0751	0.02 ± 0.00	0.01 ± 0.01	- 1.323	0.1965
50-day	29	1.74 ± 0.05	1.48 ± 0.09	- 2.658	0.0127	0.02 ± 0.00	0.01 ± 0.01	- 1.504	0.1437
60-day	28	1.92 ± 0.04	1.68 ± 0.10	- 2.566	0.0159	0.02 ± 0.00	0.01 ± 0.01	- 1.000	0.3254

Appendix 7-7: Testis weights (TW, g) and epididymis weights (EW, g) in young males housed with adult male soiled bedding (Ym + UAm) from 21 days of age to 80 days of age compared with that of the males caged with female siblings (Ym + Yf).

	df.	Ym + UAm	Ym + Yf	t-Value	P
Age-40 (day 20 of the experiment)					
TW (g)	5	0.246 ± 0.55	0.294 ± 0.53	- 0.629	0.5468
EW (mg)	5	41.06 ± 9.04	31.72 ± 5.28	0.621*	0.5518
Age-50 (day 30 of the experiment)					
TW (g)	6	0.62 ± 0.1	0.4 ± 0.14	1.311	0.2378
EW (mg)	6	60.28 ± 7.83	42.4 ± 13.04	1.35*	0.2248*
Age-60 (day 40 of the experiment)					
TW (g)	6	0.65 ± 0.21	0.32 ± 0.04	1.51	0.1818
EW (mg)	6	80.8 ± 24.11	42.45 ± 5.48	1.39*	0.2152*
Age-70 (day 50 of the experiment)					
TW (g)	6	1.09 ± 0.05	1.30 ± 0.19	- 1.066	0.3275
EW (mg)	6	177.5 ± 19.31	531 ±124.55	- 3.163*	0.0195*

*: ln(x)-transformed data used in analysis.

*Appendix 7-8: Plasma concentrations (ng/ml) of testosterone and corticosterone in males housed with adult males' bedding (Ym + UAm) and the males housed with female siblings (Ym + Yf).**

Treatment	df.	Ym+UAm (ng/ml)	Ym+Yf (ng/ml)	t-Value	P
		Plasma concentration of testosterone			
20 days	5	0.02 ± 0.02	0.01 ± 0.00	0.904	0.4073
30 days	6	0.11 ± 0.03	0.10 ± 0.05	0.697	0.5119
40 days	6	0.53 ± 0.38	0.02 ± 0.01	1.437	0.2009
50 days	6	1.15 ± 0.51	0.15 ± 0.07	1.661	0.1477
		Plama concentrations of corticosterone			
20 days	4	362.89 ± 63.45	301.25 ± 29.54	0.74	0.5006
30 days	3	171.04 ± 41.95	449.24 ± 275.00	1.292	0.287
40 days	4	290.52 ± 31.56	247.79 ± 34.41	0.941	0.4001
50 days	4	375.64 ± 75.16	196.30 ± 13.29	3.083	0.0368

*: ln(x)-transformed data used in the analysis.