# THE EFFECT OF PHOTOPERIOD ON SOME ASPECTS OF REPRODUCTION IN A SOUTHERN AFRICAN RODENT: THE POUCHED MOUSE (*SACCOSTOMUS CAMPESTRIS*).

THESIS
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by

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## Mice

I think mice
Are rather nice.
Their tails are long,
Their faces small,
They haven't any
Chins at all.
Their ears are pink,
Their teeth are white,
They run about
The house at night.
They nibble things
They shouldn't touch
And no one seems
To like them much.
But I think mice
Are nice.

Rose Fyleman

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## Abstract

The pouched mouse, *Saccostomus campestris* is widely distributed south of the Sahara, inhabiting both sub-tropical and tropical latitudes and a number of different biotic zones, where it breeds seasonally. In North temperate latitudes, reproduction in small mammals is controlled by photoperiod however this cue becomes less influential as latitude decreases towards the equator. The aim of the present study is to establish the role of photoperiod in the environmental control of reproduction in a seasonally breeding small mammal at low latitudes in a highly unpredictable environment.

Spermatogenesis of domesticated and F1-generation pouched mice was not inhibited by short daylength while decreasing daylength significantly affected the oestrous cycle of adult domesticated female pouched mice. Photoperiod had little effect on the oestrous cycle of F1-generation females while a possible inherent circannual endogenous rhythm controlled inhibition of reproduction in these females.

Body mass of male and female juvenile pouched mice was consistently lower in short daylength and in juvenile female pouched mice the onset of fertility may be weight-dependent. The attainment of sexual maturity of domesticated and F1/F2-generation females was retarded but not halted in short daylength and females in long daylength reached puberty 7.8 – 10.2 days earlier. Short daylength also lengthened the interval between vaginal perforation and first oestrus. Puberty in juvenile males was age-dependent as both domesticated and F1/F2-generation males attained puberty at 50 days of age, although fewer males were fertile in long daylength than short daylength.

Although litter size of pouched mice was smaller in short daylength for both domesticated and wild-caught females this was not due to a reduction in the ovulation rate. Domesticated females had significantly larger litters than wild-caught females.

Male and female pouched mice have evolved different reproductive strategies as males become sexually mature at the same age irrespective of photoperiod and remain fertile throughout the year. In contrast, females tend to be more complex as juveniles delay reproductive maturity and adults become non-reproductive in short daylength. However, in the presence of a fertile male and if environmental conditions are favourable, in short daylength, females can become reproductively active within approximately 3 days. Thus, although the pouched mouse has adopted a purely opportunistic reproductive strategy, vestiges of photoresponsiveness are still present in the female. The seasonality exhibited in the wild is therefore due to the female. Pouched mice live in a highly unpredictable environment so the sole use of photoperiod to regulate reproduction would be disadvantageous.

# Chapter 1

# General Introduction

## 1.1 Introduction

The Rodentia is the largest of all mammalian orders and includes more than 1685 species, more than 50% of all mammals, in 354 genera of 34 families (Skinner and Smithers, 1990). Rodents are widely distributed throughout the southern African subregion and have adapted to most terrestrial habitats. Some rodents are active on the surface and occupy subterranean burrows (springhares, *Pedetes capensis*; fat mice, *Steatomys* spp.; gerbils, Subfamily Gerbillinae; rock mice, *Aethomys* spp.; water rats, *Dasymys incomtus*; pygmy mice, *Mus* spp.; whistling rats, *Parotomys* spp.) while others spend most of their lives underground (molerats, Family Bathyergidae). Other rodents live on the surface (cane-rats, *Thryonomys* spp.; vlei rats, *Otomys* spp.; striped mouse, *Rhabdomys pumilio*; *Mastomys* spp.; house rat, *Rattus rattus*; Brown rat, *Rattus norvegicus*) and still others are arboreal (squirrels, Family Sciuridae; spectacled dormice, *Graphiurus ocularis*; woodland dormice, *Graphiurus murinus*; climbing mice, *Dendromus* spp.; tree mouse, *Thallomys paedulcus*; woodland mouse, *Grammomys* spp.) (De Graaff, 1981; Skinner and Smithers, 1990; Stuart and Stuart, 1996). The only areas that rodents have not colonised are Antarctica and some oceanic islands (De Graaff, 1981; Skinner and Smithers, 1990). Their wide distribution and, often, large populations have caused rodents to become general pests to man and in some areas they have caused major economic damage. Notwithstanding this, they have also become an integral part of most ecosystems (De Graaff, 1981).

Small mammals have exploited a wide variety of habitats many of which exhibit seasonal changes in environmental conditions, such as temperature, rainfall, humidity, food availability and / or quality (Bronson and Pryor, 1983; Bronson, 1989; Sicard *et al.*, 1993; Bronson and Heideman, 1994). In response to these seasonal changes, many small mammals reproduce seasonally. Small mammals are more readily affected by environmental change than large mammals because they have a larger surface area to volume ratio, higher relative metabolic rate, reduced ability to store fat and reduced mobility (Eisenberg, 1981; Read and Harvey, 1989; Promislow and Harvey, 1990). Food provides all the energetic needs for a mammal's metabolic processes and in order to reproduce successfully an animal needs enough food to provide energy for the other physiological processes before energy is partitioned to reproduction. Thus a change in food quantity and, or quality will affect reproduction. Ambient temperature will affect the energetic cost of thermoregulation and a drop in ambient temperature will result in an increase in the energy required to maintain homeothermy. Thus, at low ambient temperatures reproductive processes will only continue if food is plentiful (Millar, 1977; Gittleman and Thompson, 1988; Bronson, 1989). Rainfall indirectly affects the availability and quality of food, as together with temperature they determine the growth of vegetation, and so in regions with highly seasonal rainfall reproduction tends to be seasonal. Due to the short lifespan of most small mammals it is more beneficial to use an interaction of environmental factors to control reproduction

(Bronson and Pryor, 1983; Bronson, 1988; 1989; Sicard *et al.*, 1993; Bronson and Heideman, 1994).

Most studies of mammalian reproduction have been carried out on the species found in north temperate latitudes yet most mammals are found in the tropical and subtropical latitudes (Bronson, 1989). At north temperate latitudes the seasons are markedly different and the seasonal changes in environmental variables are predictable (Bronson, 1985; 1989; Bronson and Heideman, 1995). At these latitudes, most small mammals reproduce seasonally breeding during summer while the winter inhibition of reproductive activity is controlled either by photoperiod or the availability of energy (Bronson, 1985; 1989; Bronson and Perrigo, 1987; Bronson and Heideman, 1994). At temperate latitudes, the changes in photoperiod from winter to summer are pronounced and highly predictable and most mammals use these changes in daylength to interpret the seasonal time (Lee, 1970; Bronson, 1989; Bronson and Heideman, 1994). Photoperiod is therefore used as a proximate factor to prepare the body metabolically for the oncoming season and to regulate seasonal breeding (Bronson, 1989; Bronson and Heideman, 1994).

Tropical latitudes are characterised by less marked and less predictable seasonal changes in climate and less extreme changes in photoperiod and consequently one should expect different patterns of reproduction and less reliance on photoperiod as a cue for reproduction (Bronson, 1989; Bronson and Heideman, 1994). Little work has been carried out on the control of reproduction of tropical species but available data suggest that below 10° latitude, species (such as Cane mice, *Zygodontomys brevicauda*; tropical bat, *Anoura geoffroyi*; *Peromyscus aztecus*; *Elephantulus rufescens*; *Acomys wilsoni* and *A. percivali*) are generally unresponsive to changes in photoperiod (Neal, 1986; Heideman and Bronson, 1990; 1992; 1994; Bronson and Heideman, 1992; Heideman *et al.*, 1992; Demas and Nelson, 1998). There are, however, a few species (such as *Arvicanthus* spp.; *Proamys* (*Mastomys*) *natalensis*; gerbil, *Tatera nigricauda*; striped field mice, *Rhabdomys pumilio*; *Peromyscus nudipes*) that do breed seasonally (Neal, 1986; Bronson, 1989; Heideman and Bronson, 1992; 1993; Bronson and Heideman, 1994). Seasonal reproduction in the tropics may be a response to rainfall, which is markedly seasonal and which in turn affects the availability of food. For example, reproduction in *Peromyscus nudipes* is limited to the rainy seasons (Bronson, 1985; 1989; Bronson and Heideman, 1994).

In the sub-tropics, seasonal changes in daylength are great enough for mammals to use photoperiod as a cue for reproduction and some species are photoresponsive (*Arvicanthis niloticus*; rock hyrax, *Procavia capensis*; musk shrew, *Suncus murinus*) (Neal, 1986; Rissman *et al.*, 1987; Wayne and Rissman, 1991; Sicard *et al.*, 1993) while other species are not photoresponsive species (vlei rat, *Otomys*; striped field mouse, *Rhabdomys pumilio*; red flying fox, *Pteropus scapulatus*; golden mole, *Amblysomus hottentotus*; *Tatera brantsii*) (Neal, 1986; Perrin, 1986; Bronson and Heideman, 1992; O'Brien *et al.*, 1993; Jackson and Bernard, 1999). In a population there may be some variation with regard to their response to photoperiod with some individuals being photoresponsive while others remain unaffected by photoperiod (Bronson, 1989; Heideman and Bronson, 1991; Kliman and Lynch, 1992; Kerbeshian *et al.*, 1994; Nelson *et al.*, 1994; Bronson and Kerbeshian, 1995; Stanfield and Horton, 1996).

South Africa falls between 34°S and 22°S encompassing temperate and subtropical climates. Rainfall is higher in the austral summer in most parts of South Africa, although winter rains fall in the Western Cape which has a Mediterranean climate (Tyson, 1986). However, through most of South Africa, rainfall is highly unpredictable (Neal, 1986; Perrin, 1986). The seasonal variations in ambient temperature are more predictable with lower temperatures in the austral winter and higher temperatures during the austral summer. Since rainfall and ambient temperature affect food availability and quality, these will also change seasonally and the unpredictable rainfall often results in unpredictable changes in food abundance and quality.

In order to maximise their reproductive success small mammals living in these regions should be expected to have evolved a more flexible or opportunistic reproductive strategy that allows reproduction during unpredictable periods of suitable conditions. Indeed, this is the case, and some small mammals will extend their breeding period if the rainfall period is extended (Bushveld gerbil, *Tatera afra*; *T. leucogaster*; striped field mouse, *Rhabdomys pumilio*; *Myosorex varius*; *Mastomys natalensis*; *Lemniscomys griselda*; *Aethomys namaquensis*) (Rautenbach, 1982; Neal, 1986; Perrin and Swanepoel, 1987; Korn; 1989; Willan and Meester, 1987; Makundi, 1995). There are also small mammal species that breed throughout the year in South Africa (vlei rat, *Otomys irroratus*; *Aethomys chrysophilus*; *Tatera brantsii*; golden mole, *Amblysomus hottentotus*; round-eared elephant shrew, *Macroscelides proboscideus*) (Bernard *et al.*, 1994; 1996;Perrin, 1986; Korn, 1989).

The subfamily Cricetomyinae includes two genera and two species, the giant rat (*Cricetomys gambianus*) and the pouched mouse (*Saccostomus campestris*) (Skinner and Smithers, 1990). Since the genus *Saccostomus* was first described by Peters in 1846 there has been some controversy as to the number of species. In 1903 Palmer replaced *Saccostomus* Peters with *Eosaccomys* but by 1914 the name had reverted to *Saccostomus*. Until recently *Saccostomus* was thought to be a monotypic species, however, Hubert (1978) separated the genus into two species, *S. campestris* and *S. mearnsi*. This distinction was based on morphological differences and differences in the diploid chromosome number (Hubert, 1978; Keesing, 1998). The morphological features used to separate the two species were differences in ear length, tail length, upper molar tooth row and pelage colour. *Saccostomus mearnsi* had a variable diploid number (2n = 40 – 42 chromosomes) while *S. campestris* had a 2n of 46 chromosomes (Hubert, 1978). Gordon (1986), however, found that the diploid number of *S. campestris* was highly variable (2n = 28 – 50 chromosomes) (Gordon and Watson, 1986).

*Saccostomus campestris* is widely distributed throughout southern Africa, south of the Sahara; in Zambia, Angola, Botswana, Malawi, Zimbabwe, Mozambique and most of South Africa. *Saccostomus mearnsi* is found in central Kenya, Tanzania, Uganda and Ethiopia (Hubert, 1978; De Graaff, 1981; Meester *et al.*, 1986; Skinner and Smithers, 1990; Keesing, 1998). In some areas, however, it is not very abundant and only small numbers are trapped.

The pouched mouse being a relatively docile rodent, is easy to handle and lends itself well to laboratory experimentation (Hanney, 1965; Wrangham, 1969; Pitchford and Visser, 1970; Westlin-van Aarde, 1988). Various aspects of the biology of the pouched mouse have been studied including population dynamics (Ansell, 1960; Hanney, 1965; De Graaff, 1981; Meester *et al.*, 1986; Skinner and Smithers, 1990), thermoregulation and energetics (Perrin and Clarke, 1987; Haim *et al.*, 1988; 1991; Ellison and Skinner, 1991; 1992; Speakman *et al.*, 1992; Ellison *et al.*, 1994; Ellison, 1995; Lovegrove and Raman, 1998), development and developmental behaviour (Earl, 1978; Earl, 1980; Westlin-van Aarde, 1989b; Westlin and Dott, 1993; Westlin *et al.*, 1995), reproduction (Westlin-van Aarde, 1988; 1989a, 1989c; Westlin and Millar, 1992; Bernard and Hall, 1995; White and Bernard, 1996; 1999), behaviour (Earl and Nel, 1976; Earl, 1977; Hickman and Machiné, 1986), feeding (Pettifer and Nel, 1977; Perrin and Curtis, 1980; Bowland, 1986; Perrin and Kokkinn, 1986; Bowland and Perrin, 1989; Kerley, 1989; Ellison, 1993; Miller, 1994) and diseases (Moczon and Swiderski, 1983; Ellison and Westlin-van Aarde, 1990).

The pouched mouse is a medium sized (~ 70g) subtropical rodent and shows sexual dimorphism as males are slightly larger than the females (Skinner and Smithers, 1990). They are nocturnal and spend a large portion of the evening collecting food, which they carry in their cheek pouches, and then hoard in burrows (Pettifer and Nel, 1977; Ellison, 1993). Pouched mice have been found to travel long distances to find food with a total foraging excursion distance of 3.7km (Ellison, 1993). Although they are omnivorous they will preferentially store seeds, of which they collect a wide variety, in their burrows (Kerley, 1989; Miller, 1994). Being solitary, pouched mice are only found in pairs and family groups (lactating female and her pups) during the breeding season (Earl, 1980; De Graaff, 1981; Skinner and Smithers, 1990; Ellison, 1993). The pouched mouse is an r-strategist and is short-lived (Perrin, 1986), living for one or two reproductive season in the wild, and up to 4 years in the laboratory (Pers. obs.). In the wild, pouched mice breed seasonally in the wet, warm summer months while reproduction is inhibited in winter (Hanney, 1965; Smithers, 1971; Skinner and Smithers, 1990). In some instances, reproduction may occur throughout the year as Hanney (1965) found juveniles in all months except August/September, however reproduction peaked from February to July. Recent studies have shown that spermatogenesis and the oestrous cycle of pouched mice are not affected by photoperiod (Bernard and Hall, 1995; Tinney *et al.*, 2001) and will reproduce all year round under optimal conditions found in the laboratory (Westlin-van Aarde, 1988; Bernard and Hall, 1995; Tinney *et al.*, 2001). They have a number of large litters during the breeding season (Perrin, 1986). The females have a four-day oestrous cycle, ovulate spontaneously with no post-partum oestrus, and a lactational anoestrus (Westlin-van Aarde, 1988). They tend to be very aggressive towards conspecifics although such behaviour is more common in females. Once the females have successfully mated the female will attack the male even killing it if it is not removed from the cage (Wrangham, 1969; Pitchford and Visser, 1970; Earl, 1978; Earl, 1980; Westlin-van Aarde, 1988; 1989c; Westlin and Dott, 1993).

Much of the present study, and the study of Bernard and Hall (1995) were carried out using pouched mice that had been bred in the laboratory with the original stock dating back to 1958. Laboratory conditions are more favourable than those experienced in the wild as food and water are provided *ad libitum*, making conditions optimal for reproduction. Multiple generations of breeding may have the effect of intentional or unintentional selection for the mice that breed well under laboratory conditions. In this way domestication may breed out inhibitory responses to environmental influences, and is known to advance the age of fertility onset by increasing body size (Bronson, 1989; Setchell, 1992; Bronson and Heideman, 1994). For this reason some of the experiments in the study were repeated using wild-caught pouched mice (Bronson, 1989; Bronson and Heideman, 1994). However, it must be noted that domesticated animals are less likely to be stressed under laboratory conditions and therefore the results obtained would be more reliable and uniform. There are sound theoretical reasons why reproduction in small mammals from low latitudes and unpredictable environments will not be influenced by daylength, yet few researchers have attempted to examine the role of photoperiod under controlled conditions. Thus, the primary aim of this study was to examine the role of photoperiod in the control of the oestrous cycle, spermatogenesis, litter size, ovulation rate, sex ratio and the onset of sexual maturity, in a subtropical rodent from South Africa. The pouched mouse was chosen as a model because it is reported to reproduce seasonally and is easy to handle in the laboratory. The animals used came from a laboratory bred colony and in view of the established effects of captive breeding on photoresponsiveness, several of the experiments were repeated using wild-caught, or F1-generation, animals.

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# Chapter 2

# Animal Husbandry

## 2.1 Pouched mice colony

### *2.1.1 Domesticated colony*

Ten adult, domesticated pouched mice (5 females and 5 males) (chromosome number 2n = 46) were obtained in 1992 from the Medical Research Council of South Africa (MRC) from their breeding colony in KwaZulu - Natal. The MRC colony originated from pouched mice that were live-trapped in the Nelspruit district, Mpumalanga (~25°S), in 1958. The captive stock, at MRC, were maintained in natural photoperiod until 1990 and thereafter in a constant photoperiod of 12 hours light and 12 hours darkness (12L : 12D). The MRC colony was bred from these first animals by random mating, although mating between siblings was prevented where possible. On rare occasions male offspring were not separated from their mothers until after they had become sexually mature and mating occurred. Throughout the present study these mice will be referred to as 'domesticated' pouched mice.

### *2.1.2 Wild-caught colony*

Pouched mice (female n = 4; male n = 4) were caught using Sherman live-traps in Thomas Baines Nature Reserve (33° 24' S/ 26° 40' E) and Andries Vosloo Kudu Reserve (33° 08' S/ 26°

40' E) in the Eastern Cape, South Africa in September 1994 and June 1995. The adults were initially maintained in the photoperiod experienced in the wild (September – 14L : 10D; June – 10L : 14D) before being used in the experiments. The first generation males and females bred from these wild-caught mice were used in all subsequent experiments. The pouched mouse, although widely distributed were only trapped in small numbers and thus the first generation was used as insufficient numbers of wild adults were captured. An advantage of using the first generation mice, was that all individuals were of known age and were born and reared under known conditions.

### *2.1.3 Climate*

The climate in Southern Africa for the three areas from which the animals originated, the Eastern Cape (Thomas Baines Nature Reserve and Andries Vosloo Kudu Reserve) and Mpumalanga (Nelspruit) show similar trends (Figure 2.1) (South African Meteorological Office, unpublished data). In all regions rainfall peaked in January, although Thomas Baines Nature Reserve had rainfall all year round, while in Andries Vosloo Kudu Reserve and Nelspruit there were some months in which no rain fell. Temperature showed similar trends for the three areas with maximum temperatures found in the summer months. Thomas Baines Nature Reserve and Andries Vosloo Kudu Reserve experienced the maximum temperature in January, and although maximum temperature in Nelspruit was higher in the summer months, it did not reach as high as the maximum temperatures experienced in the Eastern Cape. Minimum temperatures in the three regions dropped close to 0°C in the winter months, being lowest in July for the three areas.

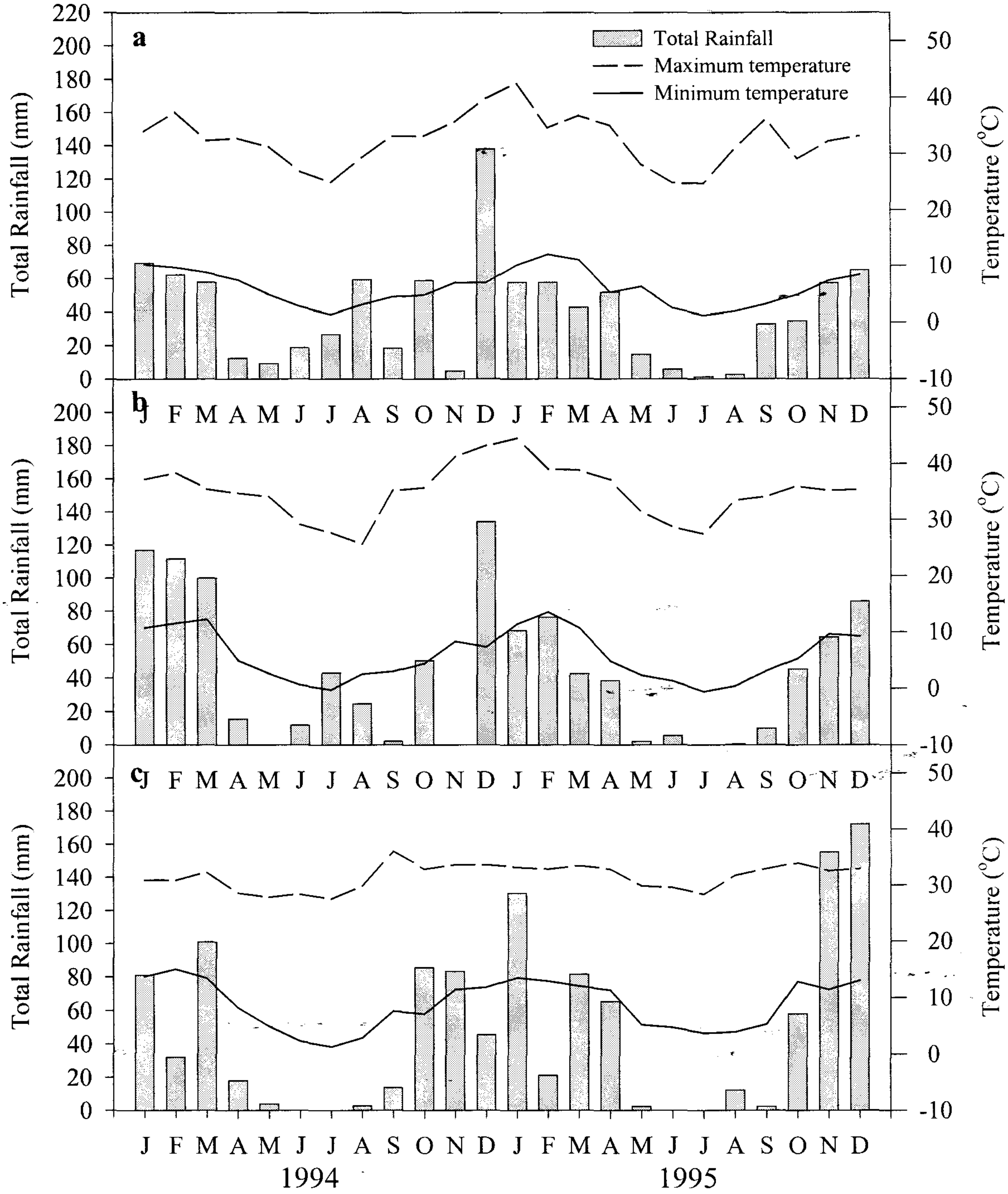

**Figure 2.1:** Comparison of monthly total rainfall, maximum monthly temperatures and minimum monthly temperatures for (a) Thomas Baines Nature Reserve (Eastern Cape, South Africa), (b) Andries Vosloo Kudu Reserve (Eastern Cape, South Africa) and (c) Nelspruit (Mpumalanga) (South African Meteorological Office, unpublished data).

## 2.2 Animal Maintenance

### *2.2.1 Cages*

All adult animals were housed individually (so as to avoid aggressive interactions) in plastic laboratory mouse cages of three different sizes: 30 cm x 22 cm x 24 cm, 40 cm x 24 cm x 12 cm and 40 cm x 40 cm x 30 cm. Due to logistical and practical reasons the cages used differed in size due to availability. Personal observations throughout the study showed that cage size did not affect reproduction. The cage size may have affected aggression and for this reason most females with litters were housed in the largest size cages and the young were removed as soon after weaning as possible.

The cages were covered with a metal mesh cover. Bedding in the cages consisted of untreated pine wood shavings spread over the bottom of the cage, sand spread along the back of the cage, and shredded paper was supplied for nesting material. Sand was placed in the cages to allow the pouched mice to sandbath (Earl, 1978; Westlin-van Aarde, 1988). Cages were cleaned once a week.

### *2.2.2 Feeding*

Commercial rabbit pellets (Epol Complete Rabbit Pellets No. 4750, Premier Food Industries Ltd, Johannesburg, S.A.) and water were given *ad libitum* with additional supplementary food in the form of sunflower seeds and apple being supplied once weekly.

### *2.2.3 Constant Environment Rooms*

Experiments were carried out under two light regimes: long daylength and short daylength in constant environment rooms.

1) Long daylength (LD) was set at 14 hours light and 10 hours darkness (14L : 10D), with lights on at 06h00 and off at 20h00.

2) Short daylength (SD) was set at 10 hours light and 14 hours darkness (10L : 14D), with lights on at 08h00 and off at 18h00.

The photoperiodic lengths were selected as they corresponded with the daylengths experienced in austral summer and winter in the Eastern Cape, South Africa.

Temperatures were controlled at 23 ± 2°C (SD) ($n \approx 1400$) with relative humidity levels being maintained at 41 ± 5%. Although the relative humidity level was below the 45% suggested by Ellison and Westlin-van Aarde (1990), no incidences of ringtail in the mice were noted. The light intensities in the rooms were 562 ± 41 Lux (Canon light meter).

### *2.2.4 Natural Photoperiod Room*

A further room was used for the experiment that simulated the natural gradually changing photoperiod (Figure 2.2). In the natural photoperiod room, daylight entered the room through a west-facing window, which filled one wall of the room, and in this way the mice experienced the changing photoperiod as it occurred in nature. Thus photoperiod in the room gradually increased as summer solstice approached (22nd December) and decreased as winter solstice approached (22nd June; Figure 2.1; S.A. Navy tide tables, 1996 - 1997). Due to the nature of the natural light source, the light intensities within the room varied with the weather conditions outside, however, all the mice within the room experienced similar light intensities of 0-424 Lux on a cloudy day and 0-881 Lux on a sunny day.

Temperature was kept more or less constant using a combination of heaters and an air conditioner. This proved successful for most of the year (austral spring, summer and autumn) as the temperature in the room was 23 ± 3°C. However, during winter 1996 and 1997 the temperature dropped to *c.* 17°C with a mean of 18 ± 2°C (Figure 2.2). The relative humidity was kept at 40 ± 5 % and, although this level was low, ringtail did not occur in the mice (Ellison and Westlin-van Aarde, 1990).

## 2.3 The animals

### *2.3.1 Female pouched mice*

The reproductive condition of female pouched mice was monitored using vaginal smears, which were taken at *c.* 09h00 each day. Vaginal smears were carried out using a capillary tube (with one tip rounded in a flame), dipped in water or saline (ensuring lubrication), inserted into the vagina and smeared on a slide (Hafez, 1970). For the first two years the slides were stained using Ehlrich's haematoxylin and Shorr's Stains (Humason, 1967) after which it was possible to identify the different cell types and thus the different stages of the oestrous cycle without staining the slides. The smears were viewed microscopically and classified according to the cell types present (Table 2.1) (Westlin-van Aarde, 1988).

### *2.3.2 Mating*

Vaginal smears were taken from the females that were to be mated. Once the oestrous cycle was established, *i.e.* the female showed an oestrous-type vaginal smear, she was monitored until she was in pro-oestrus (Table 2.1). On the morning of pro-oestrus the female was found to be receptive to a male and a male was introduced into the cage of the female. They were observed for a short period of time and if the female showed any aggression the male was removed. If the female was receptive to the male he remained in the cage overnight.

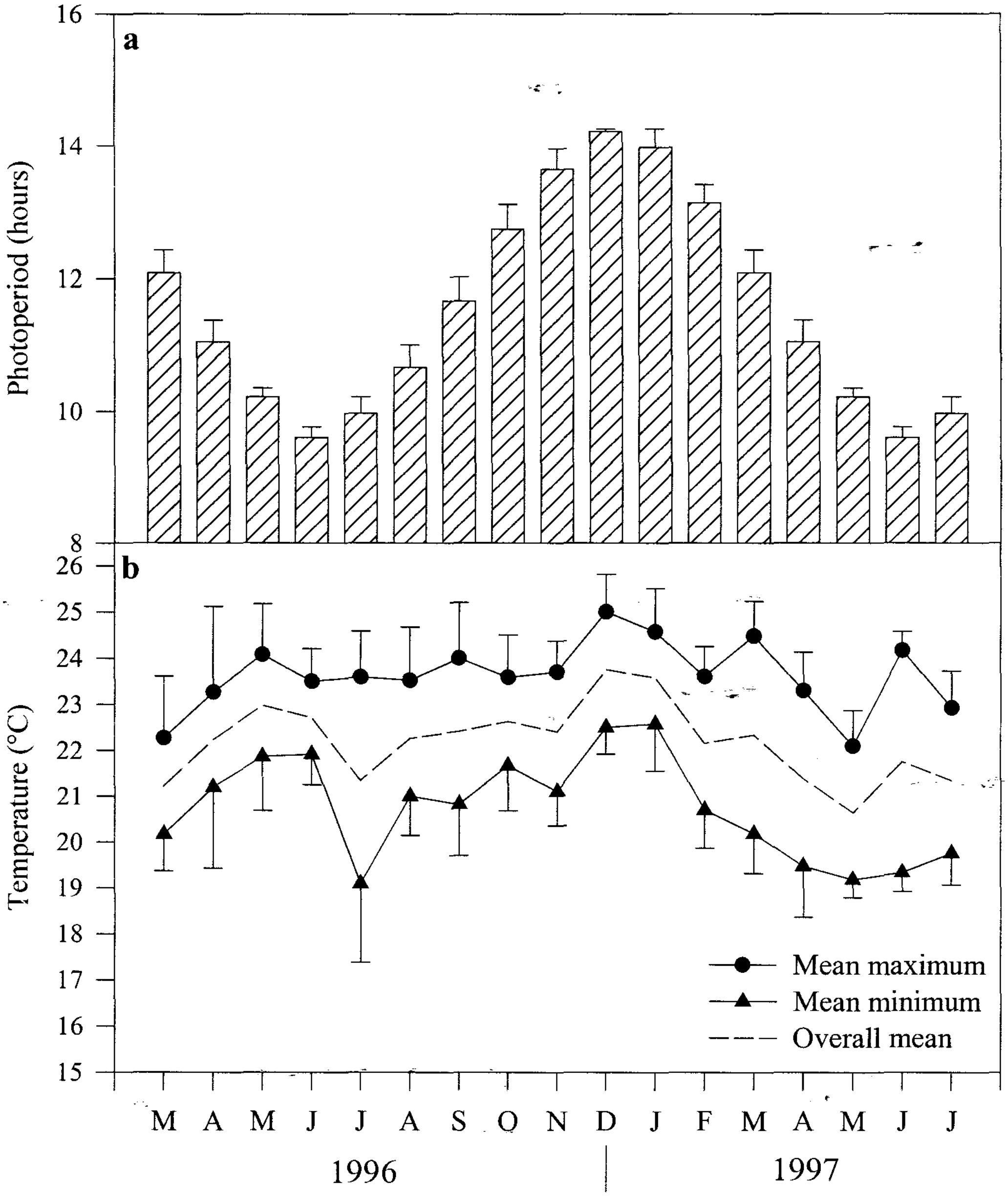

**Figure 2.2** a) The austral daylight hours from sunrise to sunset (S.A. Navy tide tables, 1996 – 1997). b) The monthly mean maximum, mean minimum and overall mean temperatures recorded in the natural photoperiod room throughout the experimental period. Standard deviations are shown for all mean values.

The next morning a vaginal smear was checked for the presence of sperm or sperm fragments. If a sperm plug was detected in the vagina and if the male was sleeping in a separate corner from the female or if the female was aggressive towards the male then it was assumed that mating had occurred (Westlin-van Aarde, 1988). If the male and female were sleeping together they had probably not mated, however, a vaginal smear was taken to confirm this (Earl, 1980; Westlin-van Aarde, 1988). Once it had been established that mating had occurred, the male was removed, and the female was monitored further to determine whether she was pregnant.

**Table 2.1:** The oestrous cycle and acyclic stages of the pouched mouse, *Saccostomus campestris*, as observed from vaginal smears with a description of the cell types present (modified from Westlin-van Aarde, 1988).

| **Stages** | **Description** |
|---|---|
| Pro-oestrus | Few leucocytes, rounded epithelial cells and cornified cells present. |
| Oestrus | Leucocytes absent; many epithelial cells and cornified cells present. |
| Metoestrus | Many leucocytes, some rounded epithelial cells and cornified cells present. |
| Dioestrus | Few to many leucocytes, some rounded epithelial cells and few cornified cells present. |
| Anoestrus | Many leucocytes, some rounded epithelial cells and few cornified cells present; mucoid smear. |
| Tight perforate | Few leucocytes, epithelial cells and cornified cells present. |
| Non-perforate | Vagina closed; smear unobtainable. |

Ehlrich's haematoxylin and Shorr's Stain stained the cells as follow:
Leucocytes = clear cytoplasm with dark blue-black irregularly shaped nucleus (haematoxylin stain)
Epithelial cells =nucleus present (cytoplasm stains blue with red nucleus using haematoxylin stain)
Cornified cells =nucleus absent (cytoplasm stains orange using Shorr's stain)

The females were weighed (to 0.01 g) twice weekly using an electronic balance (Sauter RL4 top loading electronic balance) throughout the duration of the study on the same days. An increase in weight by the end of the first week after mating was indicative of pregnancy and was used to separate pregnant animals from pseudo-pregnant mice.

### *2.3.3 Male pouched mice*

In order to assess reproductive activity of the male without sacrificing the animal, scrotal length (to 0.1 mm) was measured from the base of the penis to just above the epididymides using Vernier callipers. Body weight (to 0.1 g) and scrotal length were measured once a week throughout the study period.

## **2.4 Young pouched mice**

The date of birth and the number of young were noted for each litter with birth date being taken as Day 0. The total body weight of the litter was monitored twice weekly and the sex of the young was established at *c.* Day 8. The young were sexed by measuring the gap between the urethra and anus, as males had a larger gap between the openings (*c.* 2mm) and a small protrusion that was the penis.

Young were weaned from their mother at *c.* 28 days of age, and no later than Day 46 as it had been previously established that males can reach sexual maturity by this age (Earl, 1980). Young were either housed individually or in single-sex groups, comprising siblings with no more than three per cage in the CE room in which they were born.

## 2.5 Sacrifice

When required by the experimental design, animals were sacrificed using an overdose of Eutha-nase (sodium pentobarbitone; Centaur, Johannesburg, S.A.) injected intraperitoneally with a dosage of 0.1 to 0.2 ml per animal depending on the animal's body weight.

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# Chapter 3

## The effect of photoperiod on the oestrous cycle and spermatogenesis of adult domesticated and F1-generation pouched mice.

### 3.1 Introduction

Many mammal species, and populations, exhibit seasonal reproduction during periods of favourable conditions, thus females will only produce young during times of the year that will ensure their survival of the mother and her young. Most mammals in temperate latitudes use predictable cues, such as photoperiod, to time seasonal breeding, while others do not react to photoperiod and may use factors such as food availability and ambient temperature to time their reproduction. In this way, these other mammals employ a more opportunistic strategy enabling them to reproduce at any time of the year if conditions are favourable (Sadlier, 1969; Bronson, 1989; Bronson and Heideman, 1994a).

Most studies of the control of reproduction have been carried out on animals bred for many years in the controlled environments of laboratories and, therefore, most of the information about the physiological mechanisms controlling reproduction is derived from highly domesticated animals (Bronson, 1989). In two studies comparing reproduction in both wild and laboratory-bred field voles (*Microtus agrestis*) and prairie voles (*Microtus* *ochrogaster*) it was found that, although, both stocks of animals responded to photoperiod, under short-day conditions the wild animals matured slower and had a lower growth rate than the laboratory-bred animals (Nelson, 1985a; Spears and Clarke, 1987). The adult male prairie voles showed a reduction in size of the testes and seminal vesicles, however, they remained spermatogenically active and able to impregnate females (Nelson, 1985b).

Prolonged periods of domestication have the effect of intentional or unintentional selection. King (1939), when trying to recreate the domestication process, discovered that three-quarters of wild-caught Norway rats never produced young in the laboratory and thus the colony was founded only on rats that produced young. In this way, only those rats that would reproduce under laboratory conditions, *i.e.* only a quarter of the wild-caught animals were selected for. By the eighth generation, though, there was a decrease in infertility from 75% to only 6 %. Most populations of animals in the wild show varied reproductive responses to the different environmental influences, especially photoperiod (Heideman and Bronson, 1991; Kerbeshian *et al.*, 1994; Bronson and Kerbeshian, 1995; Stanfield and Horton, 1996). Under laboratory conditions, however, wild-caught animals produce a tight genetic bottleneck as unintentional selection culls out the individuals that react adversely to these laboratory conditions (Berry, 1969; Bronson, 1989). Part of domestication is, therefore, the way in which wild animals adapt to the captive environment, which involves both man and the environment he provides. Domestication can be achieved by a combination of genetic change and the effect of environmental influences on development that recur during each generation (Price, 1984; Bronson, 1988; Setchell, 1992). Genetic changes will occur in the population as a result of chance and any shift in selection pressures accompanying the transition from nature to captivity (Price, 1984; Setchell, 1992). Importantly, a reduction in the response to environmental influences, such as photoperiod, has been observed during the domestication process (Price, 1967; Drickamer, 1981; Bronson, 1989). One typical effect of domestication of female small mammals is a decrease in the age of sexual maturity, which is generally associated with an increase in the body weight. A further consequence of domestication is a decrease in the variability of the physiological characteristics (Price, 1967; Drickamer, 1981; Bronson, 1988; 1989; Setchell, 1992).

In nature, photoperiods change daily from a constant daylength at the equator to a difference of greater than 6 hours between summer and winter daylengths in temperate latitudes. However, most studies have used a fixed photoperiod with an abrupt change from one photoperiod to another. A few studies have assessed the effect of gradually changing photoperiods on different aspects of reproduction on small mammals (Heideman and Bronson, 1993; Gorman, 1995; Gorman and Zucker, 1995). Both Siberian (*Phodopus sungorus)* and Syrian (*Mesocricetus auratus*) hamsters become reproductively active in long daylength while under short daylength they undergo gonadal regression. Natural changes in photoperiod influenced reproduction by enhancing the effects of fixed photoperiods in Siberian hamsters (Gorman, 1995), while in Syrian hamsters the only effect of gradually changing photoperiod of 30° and 20° latitude was to reduce within-group synchrony during short daylength when the testes regressed (Heideman and Bronson, 1993).

In the wild, the pouched mouse breeds seasonally during the wet, warm summer months (Smithers and Wilson, 1979; De Graaff, 1981; Skinner and Smithers, 1990). The oestrous cycle and spermatogenesis in domesticated pouched mice are not affected by photoperiod alone, as females exhibit a regular four-day oestrous cycle and remain fertile, while males remain spermatogenically active under short-day conditions (Bernard and Hall, 1995).

The animals used by Bernard and Hall (1995) had been bred in the laboratory since 1958 and, therefore, it is possible that these domesticated animals were no longer responsive to photoperiod. In order to test this, wild-caught pouched mice were brought into the laboratory and subjected to winter and summer daylengths. A direct comparison could not, however, be made between the results for wild-caught and domesticated animals because the experiments were done in different years and the two stocks of pouched mice originated from different geographical distributions (Bronson, 1989).

The original domesticated mice had been trapped in the Mpumalanga (Transvaal) (~25° latitude) while the newly caught wild mice were from the Eastern Cape (~33° latitude). The major aim of the experiments using wild-caught mice was to establish whether the lack of photoresponsiveness reported by Bernard and Hall (1995) was an artefact of domestication. To determine whether the lack of reproductive photoresponse in the adult pouched mice (Bernard and Hall, 1995) was due to fixed photoperiods used in that study, a study was carried out exposing adult laboratory-bred pouched mice to gradual changes in photoperiod at the daily rate of change experienced in the sub-tropical latitudes. The following experiments were, therefore, designed to determine the effect of domesticated and a gradual changing photoperiod on reproductive photoresponsiveness.

## 3.2 The effect of photoperiod on the oestrous cycle adult female pouched mice

### 3.2.1 Materials and Methods

#### *3.2.1.1 The effect of fixed photoperiod on the oestrous cycle of F1-generation female pouched mice*

*Animals:*

- 22 nulliparous F1-generation females (between 147 and 206 days of age) of which:
    - 16 conceived, born and reared under long day conditions (14L : 10D)
    - 6 conceived, born and reared under short day conditions (10L : 14D)

*Experimental design:*

Because of the possibility that an endogenous rhythm might affect the influence of photoperiod on the oestrous cycle, two experiments were run, one in the austral Autumn-Winter and a second in the austral Summer-Autumn.

***<u>Experiment 1:</u>***

***Autumn-Winter (15/4/96 to 30/6/96)***

This experiment used 10 F1-generation females that had been conceived, born and reared under long day conditions. At the start of the experiment the animals were divided into two groups of 5 females, one of which (Group 1) remained in long day conditions to serve as a control for endogenously driven inhibition of the oestrous cycle, while the other (Group 2) was moved to short day conditions and allowed 28 days to acclimatise (Figure 3.1). At the start of the experiment, vaginal smears were taken daily for 16 days to establish whether the females had a regular four-day oestrous cycle. The animals' body mass were recorded twice weekly.

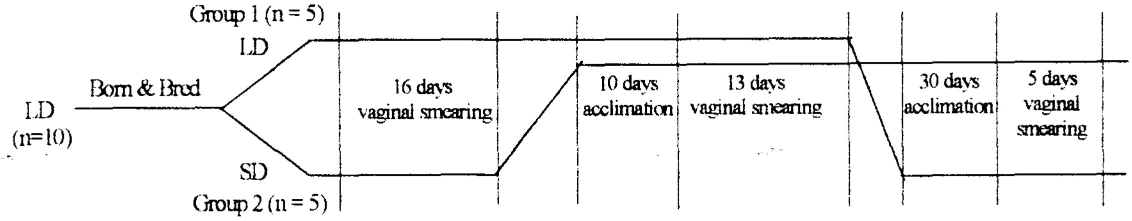

**Figure 3.1** Experimental design showing the periods of acclimation and vaginal smearing of F1-generation female pouched mice in austral Autumn-Winter (15/4/96 – 30/6/96).

After 16 days, Group 2 was moved from short daylength to long day conditions, while Group 1 remained under long day conditions (Figure 3.1). After 10 days acclimation, all mice were smeared for the following 13 days. By the end of this period the mice from Group 1 had spent the first 39 days of the experiment under long day conditions. This group was then moved to short day conditions while the mice from Group 2 remained under long day conditions and served as a control.

After 30 days acclimation, all mice were smeared for five days to establish whether they had a regular four-day oestrous cycle. At the end of the experiment, mice from Group 1 had spent 39 days under long day conditions followed by 30 days under short day conditions, while mice from Group 2 had spent 16 days under short day conditions followed by 53 days under long day conditions.

## *Experiment 2:*

### *Spring-Summer (26/1/98 – 25/4/98)*

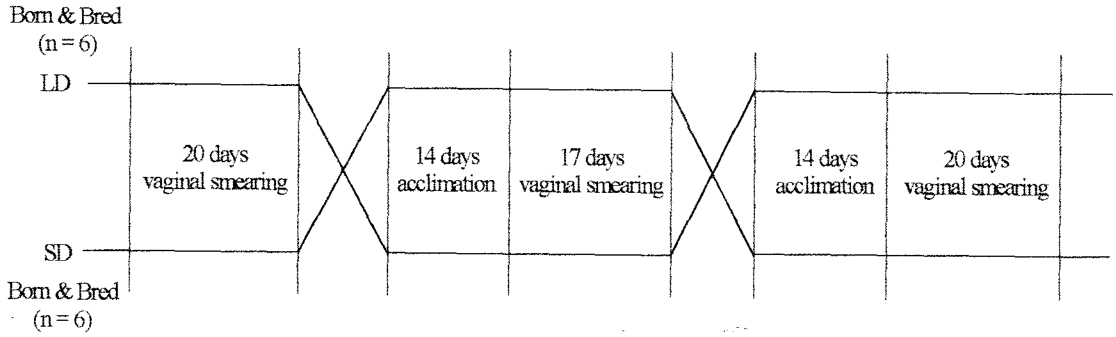

**Figure 3.2** Experimental design showing the periods of acclimation and vaginal smearing of F1-generation female pouched mice during austral Summer-Autumn (26/1/98 – 25/4/98).

Two groups of 6 nulliparous F1-generation females, one born and reared under long day conditions (Group 3) and the other born and reared under short day conditions (Group 4), were monitored by taking vaginal smears daily for 20 days to determine whether they had a distinct four-day oestrous cycle (Figure 3.2). The Group 3 females were then moved to short day conditions while the Group 4 females were moved to long day conditions.

Both groups of animals were allowed to acclimatise to their new photoperiod for 14 days, after which time vaginal smears were taken for a further 17 days. The two groups were returned to the photoperiods in which they were conceived, born and reared. They were allowed 14 days to acclimatise and then vaginal smears were again taken for 20 days. By the end of the experimental period Group 3 females had spend 54 days in long day and 34 days in short day conditions, while Group 4 females had spend 54 days in short day and 34 days in long day conditions. Throughout the experimental period the females' body weights were measured and recorded at twice weekly intervals.

*Data Analyses*

Chi-squared tests were used to compare the occurrence of the four stages of the oestrous cycle with those which would be expected if all the females were in a standard four-day oestrous cycle. Since the oestrous cycle is typically four days in length with each stage lasting for approximately one day, 25 % of all smears should be in each of the stages. However, as it was difficult to separate metoestrus and dioestrus in the acyclic females, these stages were combined and should represent 50 % of all smears. The non-parametric version of the t-test, the Mann-Whitney U test, was used to determine whether photoperiod had an effect on the length of the oestrous cycle of those individuals that exhibited a regular oestrous cycle. All statistical analyses were carried out using the statistical software package, Statgraphics 5.0.

#### *3.2.1.2 The effect of gradually changing photoperiods on the oestrous cycle of adult domesticated female pouched mice*

*Animal Husbandry*

Adult female and male pouched mice of known age were housed individually and placed in a natural photoperiod room. Daylight entered the room through windows, ensuring the animals experienced gradually decreasing and increasing daylengths as they occurred in the external environment (Figure 2.2). Animals were maintained as previously described in sections 2.2.

*Animals*:

- 8 nulliparous & 1 parous domesticated females (between 153 and 422 days of age) of which:
    - 3 females conceived, born and reared under long day conditions (14L : 10D)
    - 6 females conceived, born and reared under short day conditions (10L : 14D)

*Experimental design:*

An initial group of 7 domesticated female pouched mice were placed in the natural photoperiod room on the 2/3/96 (austral autumn). The effect of gradually changing photoperiod on the oestrous cycle was observed by taking vaginal smears for approximately 20 days to establish whether the females exhibited a regular four-day oestrous cycle. Vaginal smears were taken during each of the following seasons: winter (6/5/96 - 23/5/96; 18 days), spring (26/9/96 - 9/10/96; 20 days), summer (6/1/97 - 25/1/97; 20 days), autumn (4/4/97 - 28/4/97; 24 days) and winter (16/6/97 - 5/7/97; 20 days). The smears were taken daily at approximately the same time each day using standard techniques (see section 2.3.1), viewed microscopically and classified depending on the different cells types present (Table 2.1).

At the end of the initial winter sampling period one of the females died and two additional females were added to the group on the 31/7/96. The new females were allowed the eight weeks prior to the next smearing period to acclimatise to the new environment. During the rest of the experimental period two further females died, however, the sample size remained greater than five and although small it was felt that no additional mice were required for the results to remain statistically viable. Body weights (to 0.01 g) were recorded once weekly throughout the experimental period (2/3/96 to 5/7/97) using an electronic balance.

*Data Analyses*

Chi-squared tests were again used to compare the occurrence of the four stages of the oestrous cycle with what would be expected if all the females were in a regular four-day oestrous cycle (*i.e.* 25% each in pro-oestrus and oestrus, 50% combined metoestrus and dioestrus).

The non-parametric test for the one-way Analysis of Variance, the Kruskal-Wallis test, was used to determine whether season had an effect on the length of the oestrous cycle in those individuals that exhibited a regular four-day oestrous cycle. All statistical analyses were carried out using Statgraphics 5.0.

### 3.2.2 Results

#### *3.2.2.1 The effect of fixed photoperiod on the oestrous cycle of adult F1-generation female pouched mice*

**Table 3.1**

***Experiment 1:***

*Autumn-Winter (15/4/96 to 30/6/96)*

All the female pouched mice from Groups 1 and 2 were acyclic at the start of the experiment and remained acyclic, exhibiting either a tight perforate or anoestrus type vaginal smear throughout the experimental period. This is seen in the percentage occurrence of the different stages of the oestrous cycle where the percentage of oestrous smears was very low (Table 3.1). Two females in Group 2 exhibited a regular four-day oestrous cycle under short day conditions, however, under long day conditions these females became inter-cycling and by the end of the experiment they were anoestrus (Table 3.1) (see Table 2.1 for descriptions of the different stages in the oestrous cycle).

Throughout the experimental period, vaginal smears of females from both Groups 1 and 2 were significantly different from those expected if all females were in a standard oestrous cycle, *i.e.* in winter, irrespective of experimental photoperiod, none of the females had a regular four-day oestrous cycle ($\chi^2$; $p < 0.05$; Table 3.1). The last period of vaginal smears in both groups, although short, was long enough to establish that the females remained acyclic with either a tight perforate or anoestrus-type vaginal smear (see Table 2.1).

### ***Experiment 2:***

*Spring-Summer (26/1/98 to 25/4/98)*

All females in Group 3 that had been born and bred under long day conditions ($n = 6$) were acyclic exhibiting tight perforate, anoestrus or inter-cycling vaginal smears under both long and short daylengths. The females in Group 4, which had been born and bred under short day conditions had a mean (± SD) oestrous cycle length of 4.28 ± 0.16 days under the first period of short daylength and 4.13 ± 0.14 days under long daylength (Table 3.1). Of the 6 females in Group 4, only 2 exhibited inter-cycling vaginal smears, under the long daylength they all had a regular four-day oestrous cycle, however under the second period of short daylength all of the females became acyclic, 2 inter-cycling and 4 in anoestrus (see Table 2.1). It appears, therefore, that the second period of short daylength did affect the oestrous cycle of the Group 4 mice (Table 3.1).

**Table 3.1** The effect of photoperiod on the oestrous cycle of F1-generation pouched mice. The table shows percentage occurrence of the different stages of the oestrous cycle and numbers of cyclic and acyclic females with the length of the oestrous cycle for those females exhibiting a regular oestrous cycle.

| | PP | % P | % O | % M/D | Number Tight Perforate | Number Anoestrus | Number Inter-cycling | Number Cycling | Length of Oestrous Cycle | Probability |
|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | LD | 56 | 3 | 41 | 3 | 2 | - | - | - | ** |
| | LD | 66 | 0 | 34 | 2 | 3 | - | - | - | ** |
| | SD | 84 | 4 | 12 | 4 | 1 | - | - | - | ** |
| Group 2 | SD | 59 | 11 | 30 | 3 | - | - | 2 | 4.0 ± 0.0 | ** |
| | LD | 59 | 5 | 36 | 1 | 2 | 2 | - | - | ** |
| | LD | 32 | 0 | 68 | 1 | 4 | - | - | - | * |
| Group 3 | LD | 30 | 5 | 65 | 2 | 2 | 2 | - | - | ** |
| | SD | 41 | 0 | 59 | 2 | 4 | - | - | - | ** |
| | LD | 29 | 0 | 71 | 1 | 5 | - | - | - | ** |
| Group 4 | SD | 24 | 20 | 56 | - | - | 2 | 4 | 4.28 ± 0.16 | NS |
| | LD | 24 | 26 | 51 | - | - | - | 6 | 4.13 ± 0.14 | NS |
| | SD | 25 | 4 | 71 | - | 4 | 2 | - | - | ** |

PP = Photoperiod; P = Proestrus; O = Oestrus; M/D = Metoestrus and Dioestrus combined
NS = non-significant; * P <0.05; ** P <0.01

The results of the Chi-squared analyses showed that the oestrous cycles of Group 3 females were significantly different from those expected, *i.e.* there were no regular 4-day oestrous cycles ($\chi^2$; $p < 0.05$; Table 3.1). The vaginal smears from Group 4 females were not significantly different from those expected in the first session of short day conditions, and in the following session of long day conditions. However, when exposed to the second period of short daylength, the occurrences of the stages of the oestrous cycle were significantly different from those expected ($\chi^2$; $p > 0.05$; Table 3.1).

### *3.2.2.2 The effect of gradually changing photoperiods on the oestrous cycle of domesticated female pouched mice*

**Table 3.2**

There was no significant change in the body masses of the females throughout the experimental period ($p > 0.05$; $H = 14.6$) (Figure 3.5).

In winter 1996, 5 of the 7 females were acyclic exhibiting an anoestrous type smear, *i.e.* although they remained perforate no regular cycle was observed. A regular oestrous cycle was observed in 2 of the females with the length of the oestrous cycle being 4.38 ± 0.52 days. The chi-squared test indicated that the vaginal smears of the females were significantly different from those expected if all the females were in a standard oestrous cycle ($\chi^2$: $p < 0.001$; $DF = 12$; Table 3.2).

During spring 1996 all the females had oestrus-type smears, however, 2 of the 8 females were "inter-cycling", *i.e.* although more oestrus- type smears were observed they did not have a regular four-day oestrous cycle. The other 6 females exhibited regular oestrous cycles of 4.18 ± 0.37 days in length. No significant difference was found between the observed and the expected vaginal smears of females with a regular four-day oestrous cycle ($\chi^2$; $p > 0.05$; DF = 14; Table 3.2).

In summer 1996/97 all the females exhibited a regular oestrous cycle and no significant difference was found between the observed vaginal smears and those expected of females with a regular four-day oestrous cycle ($\chi^2$; $p > 0.05$; DF = 12). The mean length of the oestrous cycle was 4.19 ± 0.47 days.

Of the 6 females smeared in autumn 1997, one female was anoestrous, 4 were "inter-cycling" and one had a regular oestrous cycle of 5.26 ± 1.38 days in length. A significant difference was found between the observed and the expected vaginal smears indicating that the females did not have a regular four-day oestrous cycle ($\chi^2$; $p < 0.01$; DF = 10; Table 3.2).

The vaginal smears carried out during winter 1997 demonstrated that all the females exhibited a regular oestrous cycle of 4.24 ± 0.51 days in length. The chi-squared test indicated that the vaginal smears observed were not significantly different from those expected by females with a regular four-day oestrous cycle ($\chi^2$; $p > 0.5$; DF = 10; Table 3.2).

**Table 3.2** The effect of gradually changing photoperiod on the oestrous cycle of laboratory-bred female pouched mice. The table shows the percentage occurrence of the different stages of the oestrous cycle and the numbers of cyclic and acyclic females with the length of the oestrous cycle for those females exhibiting a recognisable oestrous cycle.

| | **% P** | **% O** | **% M/D** | **Number Tight Perforate** | **Number Anoestrus** | **Number Inter-cycling** | **Number Cycling** | **Length of Oestrous cycle** | **Probability** |
|---|---|---|---|---|---|---|---|---|---|
| Winter '96<br>n = 7 | 27 | 7 | 66 | - | 5 | - | 2 | 4.4 ± 0.535 | ** |
| Spring '96<br>n = 8 | 26 | 22 | 52 | - | - | 2 | 6 | 4.2 ± 0.402 | NS |
| Summer '97<br>n = 7 | 23 | 26 | 51 | - | - | - | 7 | 4.2 ± 0.485 | NS |
| Autumn '97<br>n = 6 | 39 | 11 | 60 | - | 1 | 4 | 1 | 5.3 ± 1.38 | NS |
| Winter '97<br>n = 6 | 23 | 23 | 54 | - | - | - | 6 | 4.2 ± 0.514 | NS |

P = Proestrus; O = Oestrus; M/D = a combination of Metoestrus and Dioestrus
NS = non-significant; ** $P<0.01$.

## 3.3 The effect of photoperiod on spermatogenesis of domesticated and F1-generation adult male pouched mice

### 3.3.1 Materials and Methods

#### *3.3.1.1 The effect of fixed photoperiod on spermatogenesis in the F1-generation adult male pouched mice*

*Animals:*

- 9 adult F1-generation male pouched mice (between 123 and 195 days of age) of which:
    - All were conceived, born and reared under long day conditions (14L : 10D)
    - All were scrotal at start of experiment

*Experimental design:*

The group of 9 F1-generation adult males were monitored under long daylength for 8 weeks starting in late summer (5/2/96 to 1/4/96). Five were moved to short day conditions (LD-SD), 4 males were retained under long day conditions (LD-LD) for a further 9 weeks through to mid-winter (1/4/96 to 4/6/96). It has been suggested that 6 weeks exposure to short daylength is the minimum time for spermatogenic regression to occur in Djungarian hamsters (Hoffmann, 1982), while in the laboratory rat regression occurred between 6 and 9 weeks (Wallen *et al.*, 1987) and golden hamsters showed regression of the testes between 6 and 12 weeks (Wallen *et* al., 1987). The males in this study were retained in the second photoperiod for 9 weeks so the experiment could be terminated at mid-winter to determine whether season influenced spermatogenic activity. Scrotal length (measured using Vernier callipers to the nearest 0.01 mm) and body weight (to the nearest 0.01 g) were monitored weekly throughout the experimental period for both treatments.

The ideal experimental design for the F1-generation males would have been to use two groups of males during autumn-winter, one group born and bred in long daylength and the other born and bred in short daylength. They would have been monitored in the photoperiod in which they were born and bred for a period of time after which half the animals in each group would then be retained in the original photoperiod whilst the other half would be moved to the alternate photoperiod. Both groups would be further monitored for a length of time not less than 6 weeks. The experiment should then be repeated again during the spring-summer period, however, only if the males were non-scrotal. Due to a shortage of F1-generation males this experimental design was not possible and so the above experimental design was used to yield the best results with the number of animals available.

The experiment was only carried out from late summer to winter as the males were scrotal at the start and, since the pouched mouse is reported to breed seasonally in the wild, were expected to become non-scrotal as winter progressed. The experimental design, therefore, allowed a study of the interaction of photoperiod and season on spermatogenesis.

*Histological Examination:*

At the end of the experiment, the males were sacrificed and testes, epididymides and accessory gland complexes were removed, weighed (to 0.01 g), and fixed in Bouin's fluid for histological examination. Tissue from the testis, cauda epididymis and seminal vesicle were embedded in Paraplax, sectioned (at 5μm) on a rotary microtome and stained using Mallory's trichrome one-step method (Humason, 1967). The diameter of 20 seminiferous tubules and thickness of the seminiferous epithelium in these tubules were measured per testis using a Nikon filar optical micrometer and a Nikon binocular microscope. Spermatogenic activity was assessed by examining 100 randomly selected seminiferous tubules and classifying them either as spermatogenically inactive (only spermatogonia and Sertoli cells present in the tubules), in early spermatogenesis (spermatogonia and spermatocytes present in the seminiferous epithelium, but no spermatids present), or in late spermatogenesis (spermiogenesis occurring and spermatozoa present in the lumen of the tubules). Each animal was placed in a specific category if more than 50 % of the tubules were in one of the conditions stated above. For each male, 20 sections of the cauda epididymides were examined and the abundance of spermatozoa was quantified on a scale of 0 (no spermatozoa) to ++++ (all sections packed with spermatozoa).

### *Data Analyses*

To determine whether a relationship existed between the mass of the separate reproductive organs (testes, epididymides and seminal vesicles) and body mass a linear regression was carried out on the data obtained.

A one-way Analysis of Variance (ANOVA) was carried out on the individual groups to check whether body mass and scrotal length changed over time throughout the experimental period. The repeated measures ANOVA was not used as the animals were subjected to changing conditions throughout the experimental period (Radloff, Pers. comm.). A Multifactorial Analysis of Variance (MANOVA) was used to determine whether the two groups (LD-LD and LD-SD) differed significantly during the first part of the experiment, while a one-way ANOVA was carried out on the second part of each experiment to determine whether photoperiod had an effect on body mass and scrotal length.

A t-test was used to determine if there was a significant difference in body mass and reproductive organs between the two groups (LD-LD and LD-SD) at the time the animals were sacrificed. Reproductive activity was also assessed using the t-test to determine whether seminiferous tubule diameter, epithelial thickness and spermatogenic activity were affected by photoperiod.

#### *3.3.1.2 The effect of gradually changing photoperiods on spermatogenesis in adult domesticated male pouched mice.*

*Animals:*

- 31 adult domesticated male pouched mice (between 113 and 492 day of age) of which:
  - 9 conceived, born and reared under long day conditions (14L :10D)
  - 22 conceived, born and reared under short day conditions (10L : 14D)
  - All males were scrotal at the start of the experiment

*Experimental design:*

An original group of 25 adult domesticated male pouched mice were placed in the natural photoperiod room on the 2/3/96, however, due to fatalities, a further 6 animals were added on the 20/9/96. All animals were housed individually.

The mice were monitored weekly by measuring body weight (to 0.01 g) and scrotal length (to 0.01 mm). Scrotal length was used to give an indication of reproductive activity, as a reduction in scrotal length would suggest the animal was becoming non-reproductive.

Five males were sacrificed during each of the following seasons: winter (4/6/96), spring (20/9/96), summer (18/1/97), autumn (1/4/97) and winter (25/7/97). The groups of 5 males consisted of animals of approximately the same ages and with similar photoperiodic histories. Testes, epididymides, and accessory gland complexes were removed, weighed (to 0.01 g) and fixed in Bouin's fluid for histological examination. The amount of body fat in the animals was visually graded from 0 (no fat) to ++++ (fat surrounding all organs) in order to determine whether body fat deposits changed with the season in this way quantifying the condition of the animals.

Tissue from the reproductive organs (testis, cauda epididymides and seminal vesicle) were embedded, sectioned and analysed following the procedure detailed previously.

### *Data Analyses*

A linear regression was used to determine whether a relationship existed between body mass and the masses of the separate reproductive organs (paired testes, paired epididymides and seminal vesicles).

In order to determine whether body mass and scrotal length changed over time throughout the experimental period, a one-way Analysis of Variance (ANOVA) was carried out on the body weights and scrotal lengths of the animals. One-way ANOVAs were used to determine whether there was a seasonal change in body mass, the masses of the reproductive organs and reproductive activity of the sacrificed animals. Where the normal assumptions for ANOVA's failed (Normality and Equal Variance) the non-parametric test, the Kruskal-Wallis Analysis of Variance on Ranks, was carried out.

### 3.3.2 Results

#### *3.3.2.1 The effect of photoperiod on spermatogenesis in adult F1-generation male pouched mice*

**Table 3.3, and Figures 3.3, 3.4**

Body mass of the LD-SD males did not change significantly through the experimental period (p > 0.05, H = 6.45) and although the body mass of the LD-LD males decreased by 9 grams from the 15/4/96 until the termination of the experiment (Table 3.3 and Figure 3.3), these changes were not significantly different (p > 0.05, H = 22.6) it gradually decreased by 9 grams. At termination of the experiment, *i.e.* at autopsy, the LD-LD animals were significantly lighter than the LD-SD specimens (p < 0.05, t = -2.53) (Table 3.3).

**Table 3.3** Body mass, reproductive status and spermatogenic activity (mean ± standard deviation) of F1-generation male pouched mice under different photoperiodic treatments.

| | Day length | | Probability |
|---|---|---|---|
| | LD-LD<br>(n = 9) | LD-SD<br>(n = 10) | |
| Scrotal length (mm) | 20.25 ± 0.65 | 20.70 ± 1.04 | NS |
| Body mass (g) | 68.01 ± 3.68 | 75.95 ± 5.32 | * |
| Paired testes mass (g) | 1.12 ± 0.23 | 1.17 ± 0.18 | NS |
| Paired epididymides mass (g) | 0.28 ± 0.034 | 0.30 ± 0.06 | NS |
| Seminal vesicle mass (g) | 0.41 ± 0.19 | 0.52 ± 0.24 | NS |
| Diameter of seminiferous tubules (µm) | 198.9 ± 19.4 | 210.8 ± 21.2 | NS |
| Height of seminiferous epithelium (µm) | 62.9 ± 7.33 | 67.4 ± 7.17 | NS |
| % Seminiferous tubules in late spermatogenesis | 99.8 ± 0.50 | 96.4 ± 1.52 | NS |
| Number animals in late spermatogenesis | 9 | 10 | |
| Number animals with spermatozoa in cauda epididymides | 9 | 10 | |

NS = non-significant; * = p <0.05

The scrotal length of both groups did not change significantly throughout the experiment (LD-LD: $p > 0.05$, $H = 14.6$; LD-SD: $p > 0.05$, $H = 12.4$) (Table 3.3; Figure 3.3) and at autopsy there was no significant difference in the scrotal length of the two groups ($p > 0.05$, $t = -0.75$).

The masses of the reproductive organs at autopsy were reported as actual masses and not relative masses as no relationship between body mass and that of the paired testes ($r^2 = 1.46$ %), paired epididymides ($r^2 = 3.95$ %) and seminal vesicle ($r^2 = 6.65$ %). The masses of the paired testes, paired epididymides and seminal vesicle of the LD-LD and LD-SD males were not significantly different at autopsy of the experimental period ($p > 0.05$, $t = -0.34$, $p > 0.05$, $t = -0.61$, $p > 0.05$, $t = -0.72$, respectively) (Table 3.3).

Histological examination indicated that the testes of both the LD-LD and LD-SD males remained spermatogenically active with more than 90% of the seminiferous tubules being in late spermatogenesis (99.8 ± 0.50%; 96.4 ± 1.52% respectively; Table 3.3). The seminiferous tubule diameter and the thickness of the seminiferous epithelium were not significantly different for either of the groups. The cauda epididymides of the males in both groups showed an abundance of stored spermatozoa (Rating = LD-LD: 3.65 ± 0.78; LD-SD: 3.62 ± 0.74).

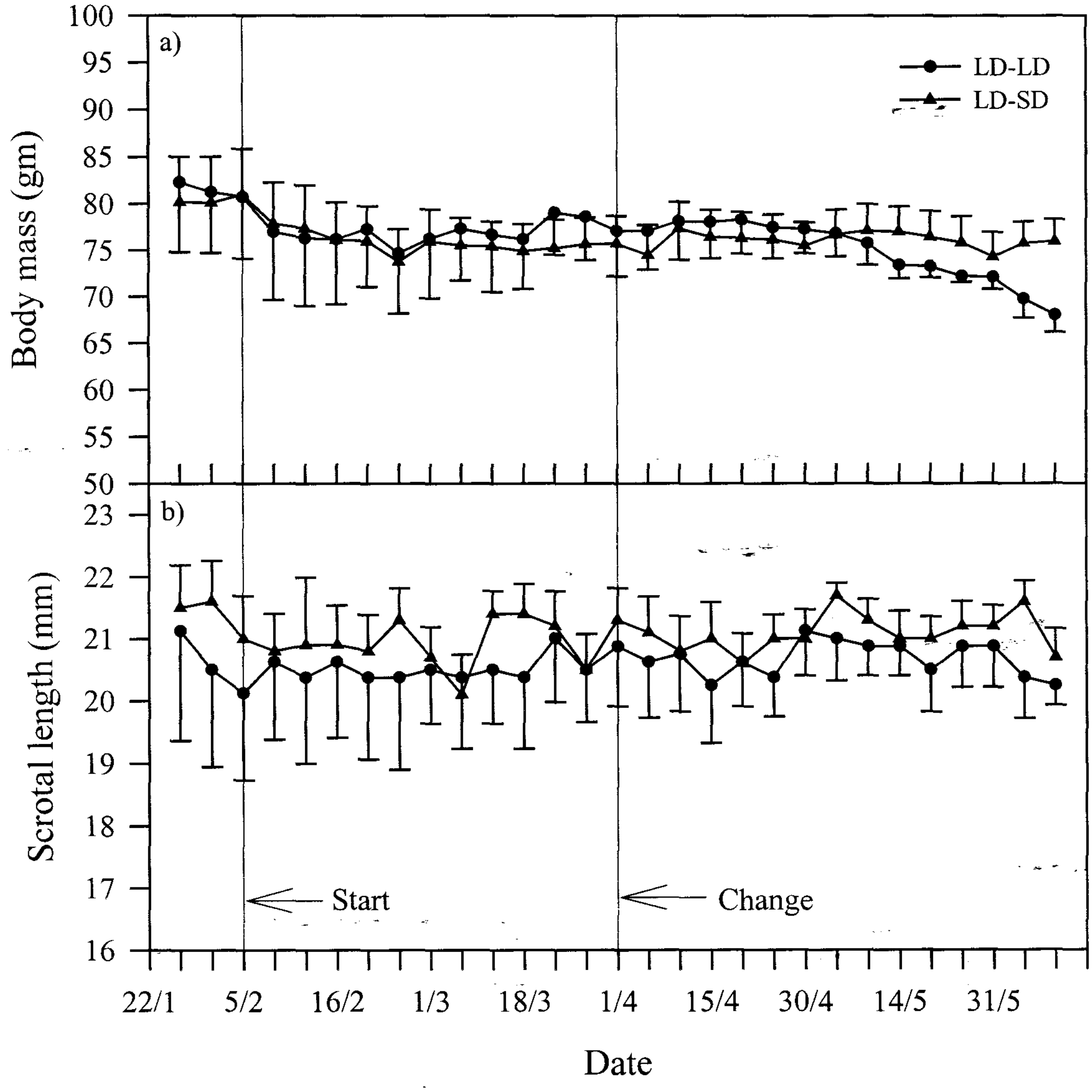

**Figure 3.3** The means ± standard deviations of a) body mass and b) scrotal lengths of F1-generation male pouched mice taken throughout the study period under different photoperiodic treatments. Start = start of the experiment after the acclimation period. Period prior to the start line was the acclimation period. Change = 5 males were moved to short daylength.

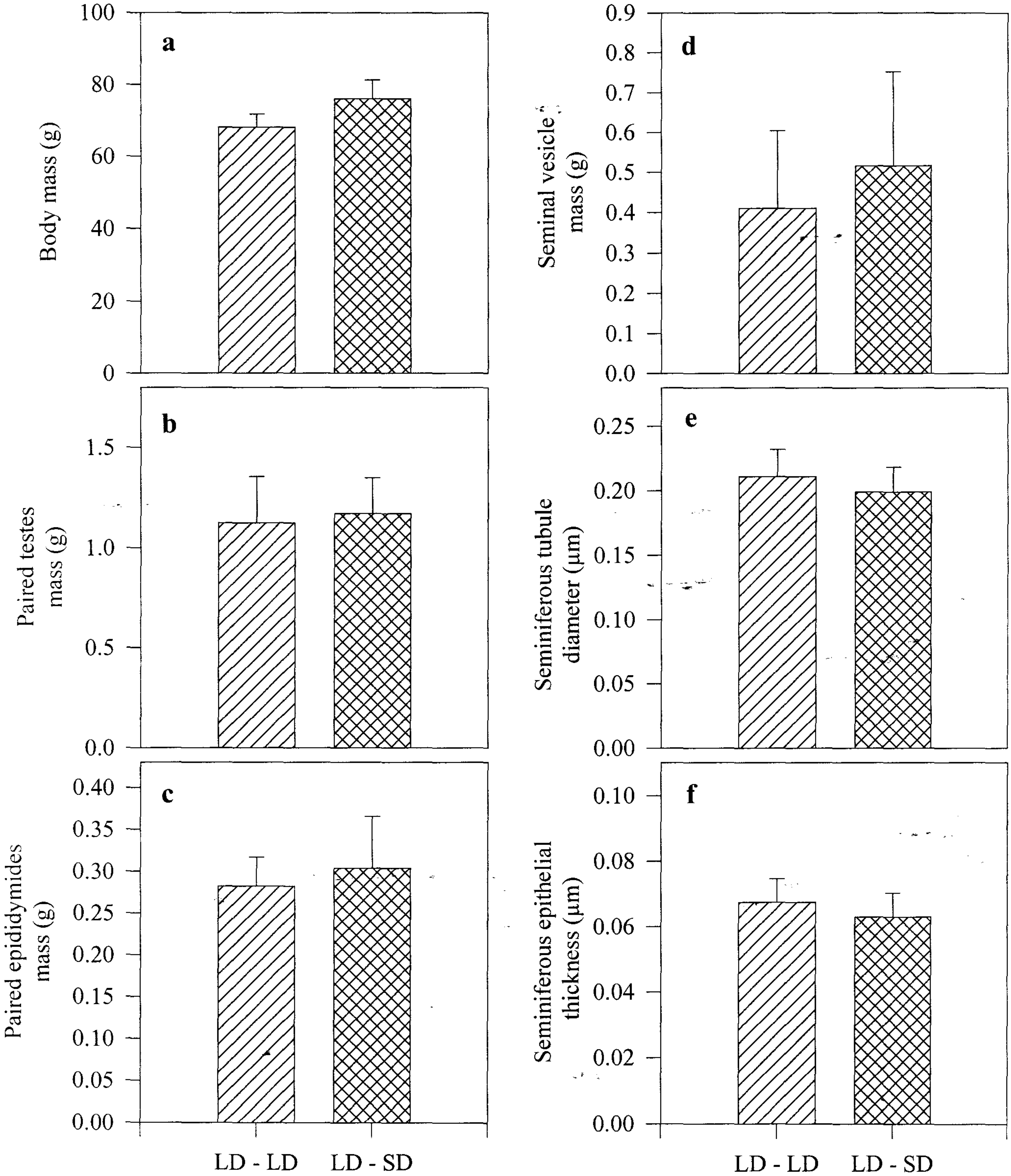

**Figure 3.4** Mean ± standard deviation of a) body mass, b) mass of the paired testes, c) mass of paired epididymides, d) mass of the seminal vesicles, e) seminiferous tubule diameter and f) seminiferous epithelial thickness for F1-generation male pouched mice at autopsy under different photoperiodic treatments. * = $p < 0.05$.

### *3.3.2.2 The effect of gradually changing photoperiods on spermatogenesis in adult domesticated male pouched mice*

**Table 3.4, and Figures 3.5, 3.6, 3.7**

The body masses of the males changed significantly throughout the experimental period with a decrease in body weight observed during both winter 1996 and winter 1997 ($p < 0.01$, H = 32.6) (Figure 3.5). At autopsy, *i.e.* at the end of each seasonal sampling period, no significant differences were observed between the body masses of the experimental groups of animals ($p > 0.05$, F= 0.84) (Table 3.4; Figure 3.6).

A significant change in scrotal length was observed ($p < 0.001$, H = 98.5) (Figure 3.5) as scrotal length decreased during winter 1996 and winter 1997, although the mice did not become non-scrotal or even abdominal. At autopsy no significant difference was found between the mean scrotal length of the groups of males sacrificed during each season ($p > 0.05$, F = 0.51) (Figure 3.6).

The mass of the reproductive organs (paired testes, paired epididymides and seminal vesicle) are reported as actual masses rather than relative masses as there was no relationship between body mass and that of the paired testes ($r^2 = 34.6$ %), paired epididymides ($r^2 = 44.1$ %) and seminal vesicle ($r^2 = 6.75$ %). The masses of the paired testes and paired epididymides were not significantly different at the seasonal sacrifice of the animals ($p > 0.05$, F = 2.24; $p > 0.05$, F = 2.19 respectively) (Table 3.4; Figure 3.6). A significant difference was, however, observed in the masses of the seminal vesicle masses ($p < 0.05$, F = 3.18), specifically between summer 1996/97 and autumn 1997 with the summer 1996/97 seminal vesicles being significantly heavier than the autumn 1997 ones (1.26 ± 0.14 and 0.59 ± 0.068 respectively) (Figure 3.6).

**Table 3.4** Body mass, reproductive status and spermatogenic activity of male pouched mice under gradually changing photoperiods (data are given as mean ± standard deviation).

| | Winter 1996 (4/6/96) (n = 5) | Spring 1996 (20/9/96) (n = 5) | Summer 1996/97 (18/1/97) (n = 5) | Autumn 1997 (1/4/97) (n = 5) | Winter 1997 (25/7/97) (n = 5) | Probability |
|---|---|---|---|---|---|---|
| Scrotal length (mm) | 20.4 ± 2.33 | 21.4 ± 1.16 | 20.4 ± 1.19 | 21.0 ± 1.27 | 21.0 ± 1.09 | NS |
| Body mass (g) | 103.9 ± 14.3 | 116.1 ± 8.38 | 111.4 ± 22.2 | 113.2 ± 15.0 | 102.3 ± 8.60 | NS |
| Paired testes mass (g) | 2.13 ± 0.17 | 2.37 ± 0.20 | 2.10 ± 0.25 | 1.88 ± 0.26 | 2.05 ± 0.37 | NS |
| Paired epididymides mass (g) | 0.543 ± 0.055 | 0.650 ± 0.059 | 0.629 ± 0.094 | 0.566 ± 0.061 | 0.571 ± 0.066 | NS |
| Seminal vesicle mass (g) | 1.01 ± 0.11 | 0.992 ± 0.55 | 1.26 ± 0.32 | 0.588 ± 0.15 | 1.04 ± 0.16 | * |
| Diameter of seminiferous tubules (μm) | 215.1 ± 3.14 | 220.2 ± 4.61 | 226.8 ± 13.8 | 210.6 ± 6.41 | 229.5 ± 16.54 | * |
| Height of seminiferous epithelium (μm) | 66.2 ± 1.49 | 66.2 ± 1.75 | 62.4 ± 3.72 | 59.1 ± 1.25 | 69.7 ± 8.29 | * |
| % Seminiferous tubules in late spermatogenesis | 95.8 ± 2.39 | 97.2 ± 1.30 | 98.4 ± 1.14 | 98.4 ± 1.14 | 88.5 ± 10.66 | * |
| Number animals in late spermatogenesis | 5 | 5 | 5 | 5 | 5 | |
| Number animals with spermatozoa in cauda epididymides | 5 | 5 | 5 | 5 | 4 | |
| Fat index | 3.40 ± 0.55 | 3.20 ±0.45 | 4.40 ± 0.89 | 3.60 ± 0.55 | 2.60 ± 1.14 | NS |

NS = non-significant; * P <0.05

Histological examination of the testes showed that spermatogenesis continued throughout the experimental period, irrespective of season, with more than 90 % of all seminiferous tubules of animals from winter 1996 to autumn 1997 in late spermatogenesis (Table 3.4). The lowest value was recorded in winter 1997 when it dropped to 88.5 ± 10.7 %. Significant differences were found in the seminiferous tubule diameters and the thickness of the seminiferous epithelium between seasons ($p < 0.05$, $F = 2.96$; $p < 0.05$, $H = 11.2$ respectively) (Figure 3.6). However, there were no significant differences in seminiferous tubule diameter between any two seasons and the only significant difference observed in the thickness of the seminiferous epithelium was between autumn 1997 and winter 1997 (Table 3.4). The cauda epididymides of the males, from all the seasons, were packed with spermatozoa, except for one male in winter 1997, which had no spermatozoa in the cauda epididymides.

The body fat index did not change significantly during the study ($p > 0.05$, $H = 8.96$). The males from summer 1996/97 were, however, observed to have the highest fat index (4.40 ± 0.40) while the lowest fat index was in the winter 1997 males (3.00 ± 0.41) (Table 3.4; Figure 3.7).

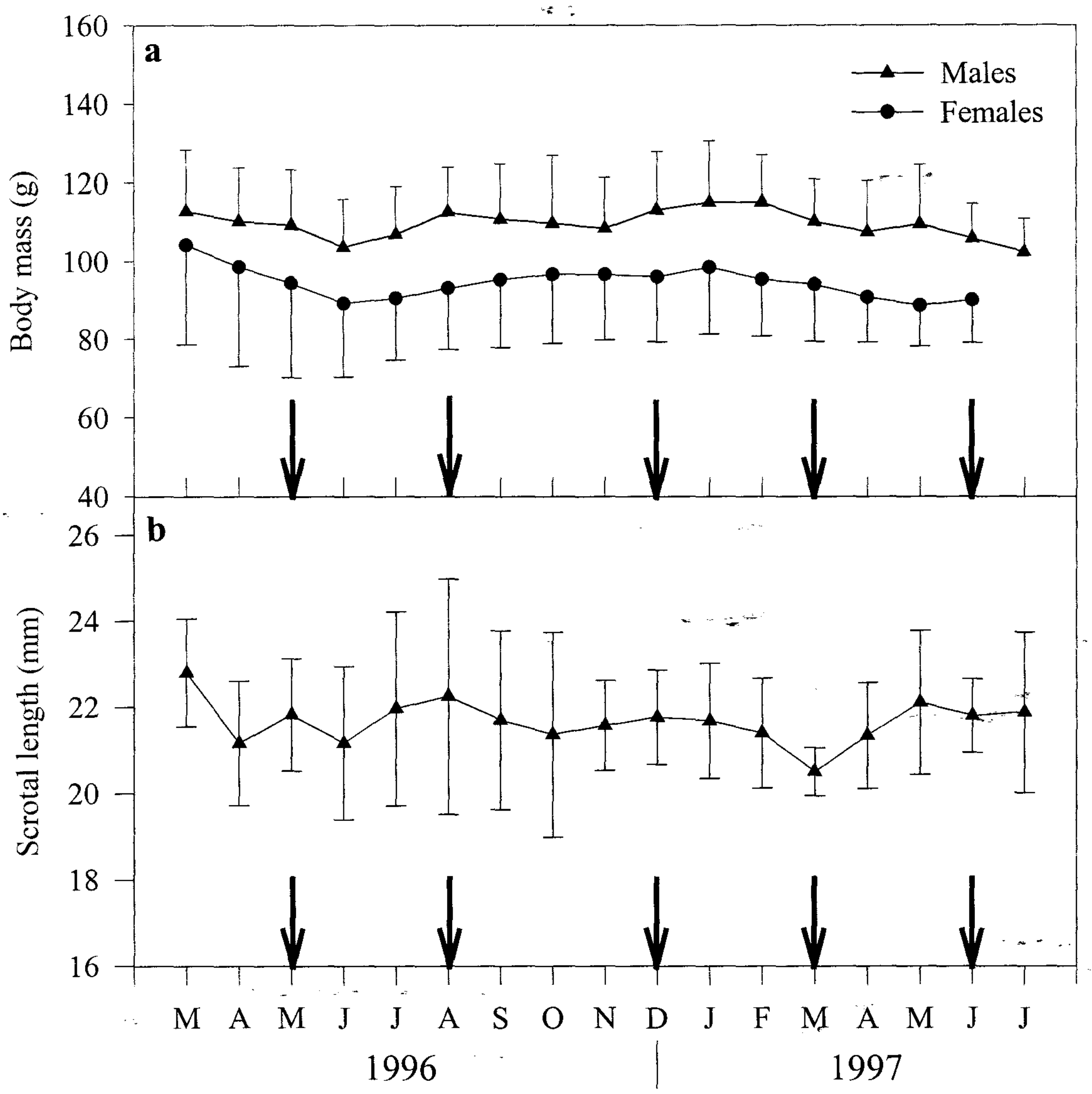

**Figure 3.5** The means ± standard deviations of monthly a) body masses of male and female domesticated pouched mice and b) scrotal lengths of the male domesticated mice throughout the experimental period in natural photoperiod. The arrows indicate periods when a group of 5 males in natural photoperiod were sacrificed in order to obtain the seasonal reproductive sample.

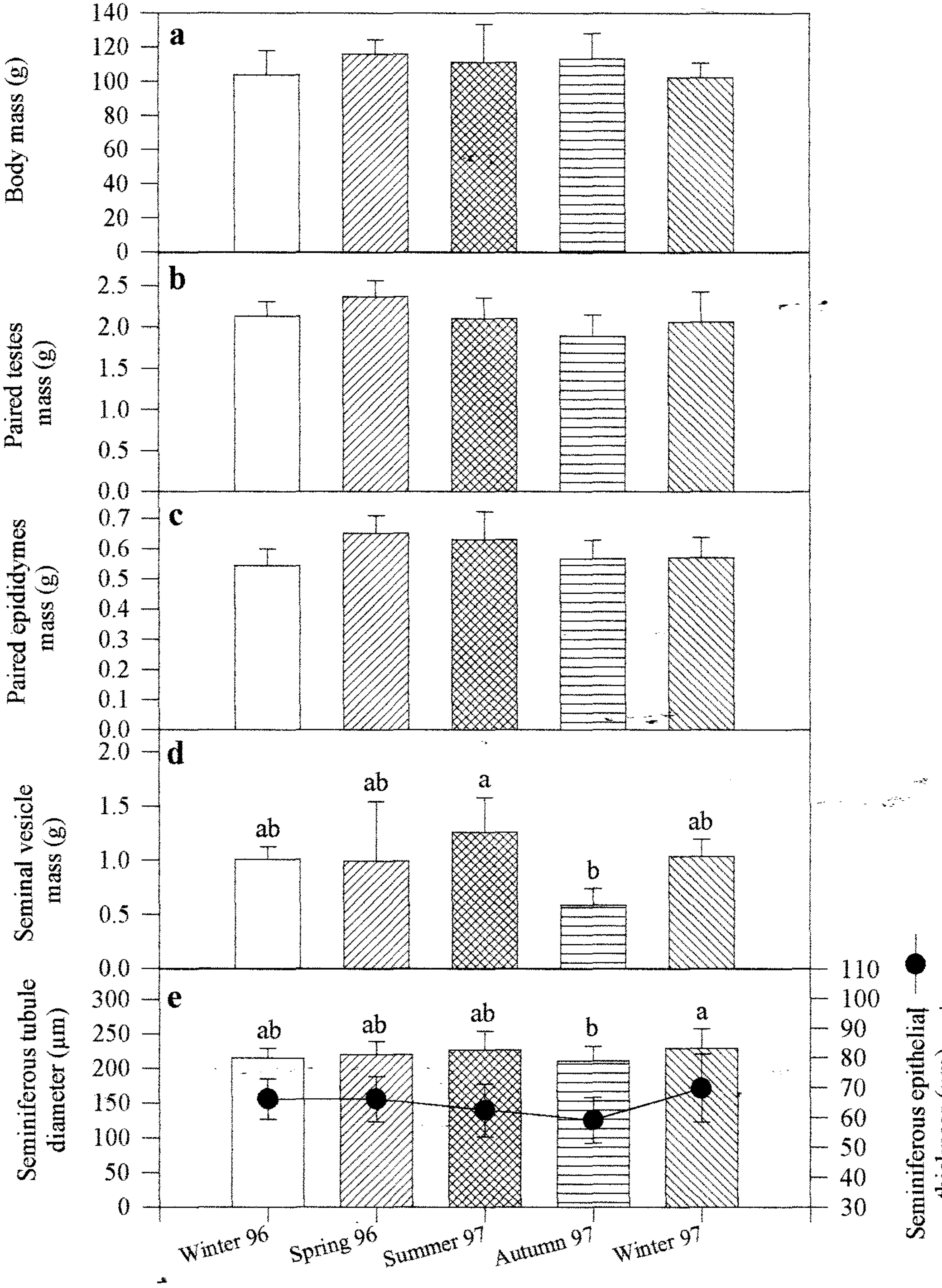

**Figure 3.6** Seasonal comparisons of a) body mass, b) paired testes mass, c) paired epididymides mass, d) seminal vesicles mass, and e) seminiferous tubule diameter and seminiferous epithelium of domesticated male pouched mice at autopsy from winter 1996 to winter 1997 under natural photoperiod. All reproductive indices are shown as mean ± standard deviation. The letters (a, b) denote statistical significances.

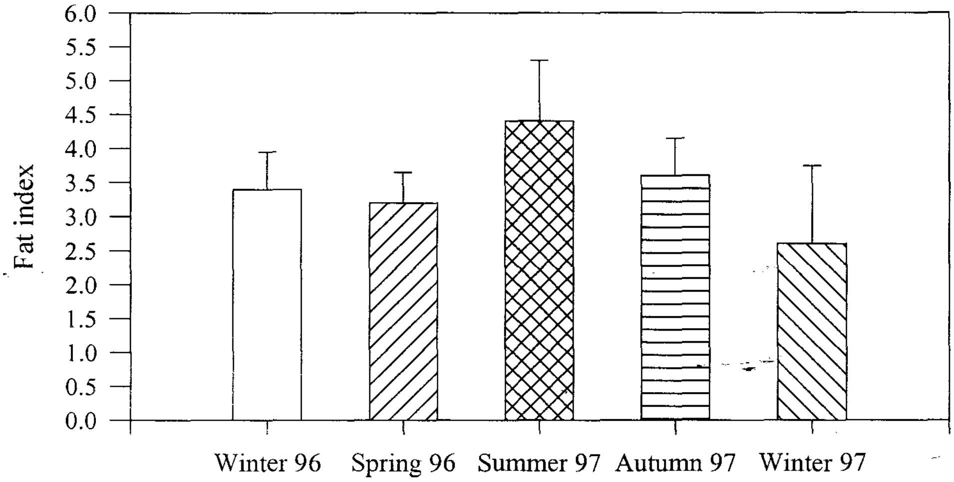

**Figure 3.7** The amount of fat, expressed as the fat index, observed in the groups of domesticated males at autopsy from each season under natural photoperiod. Data are given as mean ± standard deviation.

### 3.3.3 Summary

1. The four groups of mice exposed to abrupt changes in photoperiod from long to short daylength responded differently. Mice in Groups 1, 2 and 3 were not in a regular oestrous cycle at the start of the experiment and remained acyclic irrespective of photoperiod. However, mice in Group 4 were in a regular oestrous cycle at the start of the experiment and became acyclic under short daylength.
2. Gradual changes in photoperiod appeared to influence the oestrous cycle with an increase in the occurrence of normal oestrous cycles in summer and a decrease in autumn.
3. Short daylength did not inhibit spermatogenesis, nor did it have a significant effect on the mass of any of the reproductive organs of adult F1-generation male pouched mice exposed to abrupt changes of photoperiod from long to short day conditions.
4. Although gradual decreases in photoperiod did not inhibit spermatogenic activity, they were associated with a significant reduction in the mass of the seminal vesicles.

## 3.4 Discussion

Although there have been few published studies on reproduction in the pouched mouse, it appears that reproduction in the wild is seasonal (Smithers, 1971; De Graaff, 1981; Skinner and Smithers, 1990). However, spermatogenesis and the oestrous cycle of adult domesticated pouched mice are not inhibited by photoperiod alone (Bernard and Hall, 1995) but may be affected by a reduction in food quality and, or quantity (Tinney *et al.,* 2001). The study by Bernard and Hall (1995) used long periods of fixed daylengths with abrupt changes from one photoperiod to the other. Gradually changing photoperiod has been found to accentuate the effects of photoperiod in Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*) hamsters, which are also photoresponsive when subjected to abrupt changes in photoperiods (Heideman and Bronson, 1993; Gorman, 1995; Gorman and Zucker, 1995). Short daylength inhibited reproduction in adult Syrian hamsters when daylength decreased below 12.5 hours of light (Karp *et al.*, 1990) and 13 hours of light in adult Siberian hamsters, *i.e.* critical daylength. When exposed to gradual changes in photoperiod, reproductive development of male Siberian hamsters was found to continue to maturity at daylengths below the critical daylengths of 13 hours of light (Gorman, 1995). Gradual increases in daylength from 10 to 12 hours of light resulted in accelerated somatic and gonadal development compared to the hamsters born and reared in 12 hours of light (Gorman, 1995). Adult Siberian hamsters exhibited gonadal recrudescence even when gradual changes in daylength did not exceed the critical daylength while decreasing photoperiod greater than the critical daylength inhibited reproductive activity (Gorman and Zucker, 1995).

In the present study, gradually decreasing photoperiods did not inhibit spermatogenic activity of domesticated adult male pouched mice, even with the drop in temperature to 17°C in winter 1996. However, there was a significant seasonal change in the mass of seminal vesicles, which decreased significantly in autumn 1997. Steroidogenesis is more sensitive to adverse conditions, such as food deprivation, than spermatogenesis and a reduction in steroidogenesis will cause a reduction in the size of the accessory glands (Pang *et al.*, 1979; Bronson, 1989; Carballada and Esponda, 1992; Bronson and Heideman, 1994a). The secretions produced by the accessory glands are important in maintaining spermatozoa and could therefore affect their activity or motility (Pang *et al.*, 1979). On the other hand, removal of the accessory glands, especially the seminal vesicles, did not affect the mating behaviour of male rats, although it did decrease the fertility of mice (Pang *et al.*, 1979), rats (Carballada and Esponda, 1992) and hamsters (Chow *et al.*, 1986). The spermatozoa of the golden hamster were still able to fertilise the females' ova, but development of the embryos was affected (Ying *et al.*, 1998). It is thus difficult to conclude whether a change in the mass or activity of the accessory glands will affect reproduction in the pouched mouse. The results from this experiment suggest that gradually changing daylength may have a stronger impact on reproduction of male pouched mice than sudden changes in daylength.

The experiment with female pouched mice generated similar results. In winter 1996, after a period of gradually decreasing photoperiods, 71% of the females were non-cyclic or had extremely lengthened oestrous cycles, *i.e.* inter-cycling, while a few had a regular four-day oestrous cycle. In autumn 1997, a large proportion of domesticated adult female mice (83%) were also anoestrus, tight perforate or inter-cycling. One of the females though, retained a regular four-day oestrous cycle and by winter 1997 all females had begun cycling once again. Thus, although the results are far from clear cut, there is evidence to suggest that the control of reproduction in the female is complex and they use photoperiod as a cue but it is not rigidly enforced and in the presence of other more favourable environmental cues, such as increased food availability and higher ambient temperatures, females will become fertile. The pouched mice in the study were fed *ad libitum* and always had access to food. Consequently, the decreasing daylength that would have been associated with the onset of winter in the field, was not associated with a decline in food quality or quantity in the experiment.

Interactions of cues, such as low ambient temperatures, high humidity and poor quality diets, inhibit reproductive activity in adult female house mice without affecting adult males (Pryor and Bronson, 1981). The low ambient temperatures experienced in the laboratory were not low enough to cause thermoregulatory stress as torpor is only found to occur in some pouched mice below 10°C and even then not all animals entered torpor if there was sufficient food to cover the energetic costs of thermoregulation (Ellison and Skinner, 1992).

The two experiments designed to examine the effects of daylength on reproductive activity of adult F1-generation male and female pouched mice produced ambiguous results. Adult F1-generation females were acyclic in austral winter and this was not affected by photoperiod. In the austral summer, Group 3 females, which had been born and bred under long day conditions, remained non-cyclic throughout the experimental period and did not react to changes in photoperiod. Of Group 4 females, which had been born and bred under short day conditions, 67% had a regular four-day oestrous cycle at the start of the experiment (in short daylength). All females cycled regularly when daylength was changed to long daylength, however, when exposed to short daylengths once again all females became non-cyclic. It is worth emphasising that these females were moved to the second period of short daylength on 23/3/98 and were monitored until 25/4/98, when daylengths in the wild were gradually decreasing. Thus it is possible that the inhibition of the oestrous cycle in the Group 4 females represents an interaction between an endogenous cycle (the mice were F1-generation) and a change in photoperiod.

Opportunistic observations, made while breeding the F1-generation pouched mice indicated that the wild-caught females became reproductively active seasonally as do the pouched mice in the wild, even under constant daylength conditions. This condition persisted throughout their lives in the laboratory, which was approximately 2.5 years. It thus seems likely that the wild-caught females possibly possess a circannual endogenous rhythm, which may be entrained by photoperiod. The zeitgeber is not necessarily photoperiod but it could be another environmental, climatic or dietary factor (Heideman and Bronson, 1994). Endogenous rhythms can, however, override the environmental factor which entrains them (Heideman and Bronson, 1994; Masson-Pévet *et al.*, 1994; Turek and van Cauter, 1994) and then they will free-run at intervals of 8 to 11 months. In the wild, changes in photoperiod, or other environmental factors, would entrain the circannual endogenous rhythm to a 12 month cycle (Woodfill *et al.*, 1991; Masson-Pévet *et al.*, 1994; Concannon *et al.*, 1997).

The extent of photoresponsiveness can vary between populations at different latitudes but also between individuals of the same population with some individuals of the same population being reproductively photoresponsive while others are non-photoresponsive (Nelson, 1987; Heideman and Bronson, 1991; Kliman and Lynch, 1992; Kerbeshian *et al.*, 1994; Stanfield and Horton, 1996; Nelson *et al.*, 1994). This variation has been found to have a genetic basis (Desjardins *et al.*, 1986; Lynch and Lynch, 1986; Heideman and Bronson, 1991; Kliman and Lynch, 1992). Years of domestication can result in a group of animals becoming nonphotoresponsive as unintentional selection for animals that will breed easily and quickly will result in the loss of those in which reproduction was inhibited by short daylength (Price, 1967; Clark and Galef, 1980; Bronson, 1984; Nelson, 1985; Spears and Clarke, 1987; Setchell, 1992). The lack of photoresponsiveness exhibited by the domesticated females may, therefore, be due to either geographical distribution or unintentional selection of a mixed wild population. Similarly the seasonality displayed by the F1-generation was probably inherited from the wild-caught parents. Domestication not only affects sexual maturation of female mammals but may remove responsiveness to environmental factors, such as photoperiod (Sadlier, 1969; Setchell, 1992; Bronson and Heideman, 1994b). An outbred colony of male prairie voles were not affected by photoperiod while the F1-generation were photoresponsive (Nelson, 1985a).

A more opportunistic strategy to use in unpredictable environmental regions is for one of the sexes to reproduce seasonally while the other remains sexually active throughout the year (Bronson, 1988). The two sexes of pouched mice exhibit different amounts of photosensitivity in some but not all aspects of reproduction. *Saccostomus mearnsi,* the second species of the pouched mouse, found in central Kenya, displays a similar pattern of reproduction to *S. campestris* (Keesing, 1998). The reproductive cycle is highly seasonal occurring during the wet season, although 10% population bred during the dry season. Most of the males (85%) remained sexually active throughout the year, while it was the female that dictates seasonality (Keesing, 1998).

Adult F1-generation male pouched mice were not affected by photoperiod remaining spermatogenically active. Differences in reproductive response between the sexes have been found in other animals (Bronson, 1989; Bronson and Heideman, 1994a). The spermatogenic cycle takes 45 to 60 days in male rodents (Clermont, 1972; Bronson, 1989; Bronson and Heideman, 1994a), while only a few days (approximately 3 days) are required for a female to achieve fertility (Dominic, 1969; Kenagy and Bartholomew, 1981; Bronson and MacMillan, 1983; Hamilton and Bronson, 1985; Bronson and Heideman, 1994a). Thus a more opportunistic strategy would be for the male to remain in reproductive readiness if the acyclic female is stimulated to cycle if conditions become favourable.

## 3.5 Summary

1. There is some evidence to support a role of gradually changing photoperiod in the control of the oestrous cycle. In spite of the fact that food was supplied *ad libitum* throughout the experiment, more females were in anoestrus in winter 1996 than in summer 1996/97, and more females were in a regular oestrous cycle in summer 1996/97 than in winter 1997.
2. In only one of four experimental groups, did a change in daylength affect the occurrence of the oestrous cycle of adult F1-generation female pouched mice. In this case, the decrease in daylength mirrored that which the animal would have experienced in the wild and the oestrous cycles were inhibited.
3. Gradual changes in daylength did not affect spermatogenesis in domesticated adult pouched mice, nor did abrupt changes in daylength affect spermatogenesis in adult F1-generation male pouched mice. However, gradually changing daylength had a significant effect on the mass of the reproductive accessory glands suggesting that steroidogenesis may be more sensitive to photoperiod than spermatogenesis.
4. Together, these results suggest a greater role for photoperiod in the control of reproduction than suggested by Bernard and Hall (1995). There is evidence for an endogenous rhythm that may be lost during domestication. There is also evidence that gradually changing daylength may have a stronger effect than abrupt changes in daylength.

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# Chapter 4

## The effect of photoperiod and domestication on the attainment of sexual maturity of domesticated and F1-generation pouched mice.

### 4.1 Introduction

A mammal is considered to have attained sexual maturity when it is able to produce gametes capable of producing viable young (Sadlier, 1969). The rate of attainment of sexual maturity and the onset of fertility are very important to an individual, exerting a strong influence on the reproductive success and affecting the number of young produced by a female in her life time (Reasner and Johnston, 1988). This is particularly important in short-lived mammals that may only survive one or two reproductive seasons, and in which a change in the age of sexual maturity may have a major effect on the dynamics of the population (Hasler *et al.*, 1976). Although genotype will limit the earliest onset of fertility, environmental influences may either delay reproductive maturation or may accelerate it. In this way the genes of the individual interact with environmental factors to regulate the onset of fertility to coincide with a period of favorable conditions thus allowing successful production of young (Bronson, 1985; 1989). Reproduction in adult small mammals is known to be affected by environmental factors, such as nutrition (both quantity and quality), ambient temperature, social factors and photoperiod, with daylength being the most frequently used regulator of reproduction of mammals (Bronson, 1985; 1988; 1989; Bronson and Heideman, 1994). Photoperiod (Hoffman, 1978; Johnston and Zucker, 1980; Yellon and Goldman, 1984; Lee *et al.*, 1987; Ryan and Volk, 1995), social cues (Drickamer, 1981; Reasner and Johnston, 1988), temperature (Bronson, 1989) and food deprivation (Bronson and Marsteller, 1985; Hamilton and Bronson, 1985; Nelson *et al.*, 1985; Willan and Meester, 1987; Bronson, 1989; Krackow, 1989; Nelson *et al.*, 1992; Ruf *et al.*, 1993; Bronson and Heideman, 1994; Bazhan *et al.*, 1996) are also known to affect the reproductive development of peripubertal small mammals (Darrow *et al.*, 1980; Hamilton and Bronson, 1985; Bronson, 1989).

Growth and sexual maturation of some seasonally reproducing small mammals in temperate latitudes are known to be affected by photoperiod (Djungarian hamsters, *Phodopus sungorus*, (Hoffman, 1978; Yellon and Goldman, 1984); white-footed mice, *Peromyscus leucopus (*Johnston and Zucker, 1980); Montane voles, *Microtus montanus* (Horton, 1984a, 1984b); Meadow voles, *Microtus pennsylvanicus* (Lee *et al.*, 1987); Prairie voles, *Microtus ochrogaster* (Nelson, 1985); female ferret, *Mustela putorius* (Ryan and Volk, 1995)). Under laboratory conditions, long day photoperiod causes more rapid growth and earlier onset of sexual maturity than short day photoperiod (Sadlier, 1969; Setchell, 1992; Bronson and Heideman, 1994). In addition to photoperiod and other environmental factors, an internal circadian rhythm that is sensitive to light, together with photoperiod, control the reproductive response (Turek and van Cauter, 1994). Circadian rhythms that measure photoperiod length have been discovered in a number of mammals such as the field vole (*Microtus agrestis*) (Grocock and Clarke, 1974), Djungarian hamster (*Phodopus sungorus*) (Stetson *et al.*, 1989; Horton and Stetson, 1992) and white-footed mouse (*Peromyscus leucopus*) (Whittsett *et al.*, 1983). A critical or threshold daylength of 12.5 hours or more is thought to stimulate and maintain reproduction in many small mammals (Bronson and Heideman, 1994).

The reproductive development and growth of the golden hamster (*Mesocricetus auratus*) on the other hand is not affected by photoperiod and this species will attain sexual maturity at the same age regardless of photoperiod. Male and female golden hamsters are capable of reproducing in short daylength by seven weeks of age. Although the testes of the male exposed to short daylength will regress by 14 weeks of age, between six and 14 weeks of age they are still able to inseminate a female successfully (Darrow *et al.*, 1980; Cherry, 1987; Sisk and Turek, 1987). A similar lack of photoperiodic involvement is found in the onset of sexual maturity in collared lemmings (*Dicrostonyx groenlandicus*) (Hasler, 1975; Hasler *et al.*, 1976).

Wild juvenile Djungarian hamsters (Hoffman, 1978; Stetson *et al.*, 1986) and Montane voles (Horton, 1984b; 1985), born after the summer solstice show inhibited growth and reproductive development, whilst young born during spring and early summer show the most rapid growth and sexual maturation. At both of these times, photoperiod is of a similar length (between 12 and 14 hours of light per day). However, in early spring, daylength is gradually increasing compared to the period after the summer solstice when it gradually decreases (Sadlier, 1969; Johnston and Zucker, 1980). Therefore, it is not necessarily short day conditions that inhibit sexual maturity but rather the time of the year in which the young are born. In other words, the direction of change in daylength towards shorter day lengths is the causal factor when it is above the threshold daylength (Horton, 1984b; Forger and Zucker, 1985; Foster *et al.*, 1988; Horton and Stetson, 1992; Gorman, 1995). The delay in the onset of fertility in young born after the summer solstice probably evolved to prevent breeding during the unfavorable times of the year and thus a waste of an energetically expensive effort, which could result in the death of the young and the female (Pinter, 1968; Horton, 1984a; Forger and Zucker, 1985).

In order to interpret decreasing or increasing daylength the young must have previous photoperiodic experience prior to weaning to use as a comparison. Thus the absolute length of daylength may not be important but whether the daylength being experienced is longer or shorter than that experienced a few weeks prior to weaning (Horton, 1984a). In this way the photoperiod experienced by the mother may affect the rate of reproductive development of her young (Horton, 1985; Lee and Zucker, 1988; Stetson *et al.*, 1986; 1989). The response of juvenile Montane voles (Horton, 1984a, 1984b) and Djungarian hamsters (Lee and Zucker, 1988) to photoperiod depends on the photoperiodic conditions experienced by the mother prior to weaning. Furthermore, the photoperiod experienced by the mother during gestation is enough to influence the postnatal development of the young (Horton, 1984a, 1984b; 1985; Lee *et al.*, 1987; Lee and Zucker, 1988; Stetson *et al.*, 1986; 1989). Photoperiodic information is transferred from the mother to foetuses prior to birth by the transferal of melatonin produced in the maternal pineal gland (Weaver and Reppert, 1986; Horton *et al.*, 1989; Stetson *et al.*, 1989). This continues until weaning as melatonin and gonadotropin-releasing hormone are able to cross the mammary glands in milk and thus to the suckling young (Elliott and Goldman, 1989; Weaver and Reppert, 1986; Horton and Stetson, 1992). In this way the young are able to compare the duration of the prenatal melatonin peak with those that occur postnatally (Weaver *et al.*, 1987).

Seasonal reproduction is found not only in small mammals from temperate latitudes but also in the tropics where both seasonal (*Arvicanthus* spp; *Proamys natalensis*; *Tatera* *nigricauda*; *Rhabdomys pumilio*; *Peromyscus nudipes*) (Neal, 1986; Bronson, 1989; Heideman and Bronson, 1992, 1993; Bronson and Heideman, 1994) and non-seasonal (*Zygodontomys brevicauda*; *Anoura geoffroyi*; *Peromyscus aztecus*; *Elephantulus rufescens*) breeders are found (Neal 1986; Heideman and Bronson, 1990; 1992; 1994; Bronson and Heideman, 1992; Heideman *et al.*, 1992; Demas and Nelson, 1998). Some mammals in the tropics and subtropics, including those that breed seasonally and those that breed all year round, are reproductively photoresponsive to a certain degree. However, whether the regulation of reproduction of the seasonal breeders is under photoperiodic control is not known (Rissman *et al.*, 1987; Heideman and Bronson, 1990). The cane mouse (*Zygodontomys brevicauda*) undergoes sexual maturation under both long and short day photoperiods at the same rate (Heideman and Bronson, 1990).

The aims of this chapter were, therefore, to determine whether short daylength would retard or inhibit the onset of sexual maturation in both F1 and F2-generation wild-caught, and domesticated juvenile male and female pouched mice. Furthermore, to determine if domestication had an effect on the age of sexual maturity.

## 4.2 The effect of photoperiod on the attainment of sexual maturity of female pouched mice.

### 4.2.1 Materials and Methods

#### *4.2.1.1 The effect of photoperiod on sexual maturity of domesticated female pouched mice*

*Animals:*

- 10 nulliparous domesticated females (between 166 and 525 days of age)
  - 5 females conceived, born and reared in long day conditions (166 to 435 days of age)
  - 5 females conceived, born and reared in short day conditions (169 to 525 days of age)
    - 32 juvenile females conceived, born and reared under long day conditions (LD females)
    - 23 juvenile females conceived, born and reared under short day conditions (SD females)

*Experimental design:*

A group of 5 adult female pouched mice, conceived, born and reared under long day photoperiod, were mated under long day photoperiod by placing a male in the cage on the morning of pro-oestrus. A further group of 5 adult females, conceived, born and reared under short day photoperiod, were mated in short day photoperiod. The resultant litters were raised under the respective photoperiods. Once the litters were weaned (*c.* 28 days), the adult female and male mice in long day photoperiod were moved to short day photoperiod and the adult female and male mice in short day photoperiod were moved to long day photoperiod. Both groups of animals were acclimated for four weeks to the new photoperiod and mated with the same males again. Once more, the resultant litters were raised under the photoperiods in which they had been conceived.

*Puberty studies:*

Juvenile female pouched mice (LD: n = 32; SD: n = 23) were separated from the mother and male siblings at 28 days of age and housed in single-sex sibling groups depending on the number of juvenile females per litter. It has been established that the age of vaginal opening and first oestrus (*i.e.* the first oestrous-type smear) does not differ significantly between single females or single-sex groups of 5 young females in the absence of an adult female (Westlin-van Aarde, 1989). The young females were checked every morning for vaginal perforation, *i.e.* opening of the vagina. Once vaginal perforation was observed, vaginal smears were then taken until the first oestrous-type smear. The young females were weighed twice weekly on the same day. The juvenile females in long day photoperiod are referred to as LD females (n = 32) and those in short day photoperiod as SD females (n = 23).

### *4.2.1.2 The effect of photoperiod on sexual maturity of F1/ F2-generation female pouched mice*

*Animals:*

- 4 wild-caught females of unknown parity or age
- 6 nulliparous F1-generation females (between 172 and 437 days of age) of which:
  - 2 F1-generation females were conceived, born and reared under long day conditions
  - 4 F1-generation females were conceived, born and reared under short day conditions
    - 27 juvenile females (F1 = 18; F2 = 9) were conceived, born and reared under long day conditions (LD females)
    - 23 juvenile females (F1 = 22; F2 = 1) were conceived, born and reared under short day conditions (SD females)

*Experimental design:*

A group of 4 wild-caught female pouched mice were placed in long day photoperiod and acclimated for four weeks before experimentation began. Vaginal smears were taken during this acclimation period to establish whether the females exhibited regular four-day oestrous cycles. Due to the shortage of wild-caught females a further 6 F1-generation adult females of known age were also used for the experiment, thus the F2-generation of these animals were observed. Four of these females had been born and reared under short day conditions and were moved to long day and acclimated for four weeks.

After the period of acclimation vaginal smears were taken and a wild-caught male was introduced into the cage of the female on the morning of pro-oestrus. The resultant litters were raised under the photoperiod in which they had been conceived. Once the litters were weaned (*c.* 28 days) the adult males and females were moved to short day photoperiod and acclimatised for four weeks. The females were mated with the same males they had been mated with previously in long day photoperiod and the resultant litters were raised under the photoperiod in which they had been conceived.

*Puberty studies:*

The method of analysis was the same as previously described for juvenile domesticated female pouched mice (see section 4.2.1.1). The juvenile females from long day photoperiod are referred to as LD females and those from short day photoperiod as SD females.

#### *4.2.1.3 Data Analyses*

The analyses of both the domesticated, and the F1/F2-generation and wild-caught females were carried out using the same method. T-tests were used to examine the effect of photoperiod on the age of sexual maturity of the domesticated, and F1/F2-generation and wild-caught females independently. On the failure of normality or homogeneity the non-parametric test equivalent, the Mann-Whitney U-test, was carried out.

A two-way ANOVA was carried out to determine whether domestication had an effect on the age of sexual maturity, taking into account photoperiod. If the data was not normally distributed, it was log-transformed and the two-way ANOVA repeated (Underwood, 1997).

### 4.2.2 Results:

#### *4.2.2.1 The effect of photoperiod on the attainment of sexual maturity of domesticated female pouched mice*

**Table 4.1 and Figure 4.1**

The ages of vaginal perforation and first oestrus of the LD ($n = 32$) and SD ($n = 23$) juvenile females differed significantly ($p < 0.0001$, $T = 888.5$; $p < 0.0001$, $T = 910.0$ respectively), with LD females becoming perforate 4.4 days earlier and reaching first oestrus 7.8 days earlier than SD females (Figure 4.1). If oestrus is taken as being an indicator of sexual maturity, then the LD females attained sexual maturity *c.* 7.8 days earlier than the SD females. Photoperiod also had a significant affect on the interval between vaginal perforation and first oestrus ($p < 0.05$, $T = 777.5$) with LD females having a shorter period between vaginal perforation and first oestrus than SD females (Table 4.1; Figure 4.1).

**Table 4.1** The effect of photoperiod on age and body mass at vaginal perforation and first oestrus of juvenile domesticated female pouched mice. Standard deviations are given for all mean values.

| | Photoperiod | | Significance |
|---|---|---|---|
| | LD | SD | |
| Sample size (n) | 32 | 23 | |
| Age at perforation (days) | 36.66 ± 2.49 | 41.09 ± 3.98 | ** |
| Body mass (g) at perforation | 41.45 ± 5.56 | 48.45 ± 7.70 | ** |
| Age at first oestrus (days) | 45.69 ± 4.81 | 53.48 ± 7.84 | ** |
| Body mass (g) at first oestrus | 53.97 ± 6.35 | 61.26 ±10.41 | ** |
| P-O Interval (days) | 9.03 ± 4.22 | 12.22 ± 6.48 | * |

LD = long day, SD = short day; P-O Interval = the number of days between perforation and the first oestrous-type vaginal smear.
* = $p < 0.05$; ** = $p < 0.01$

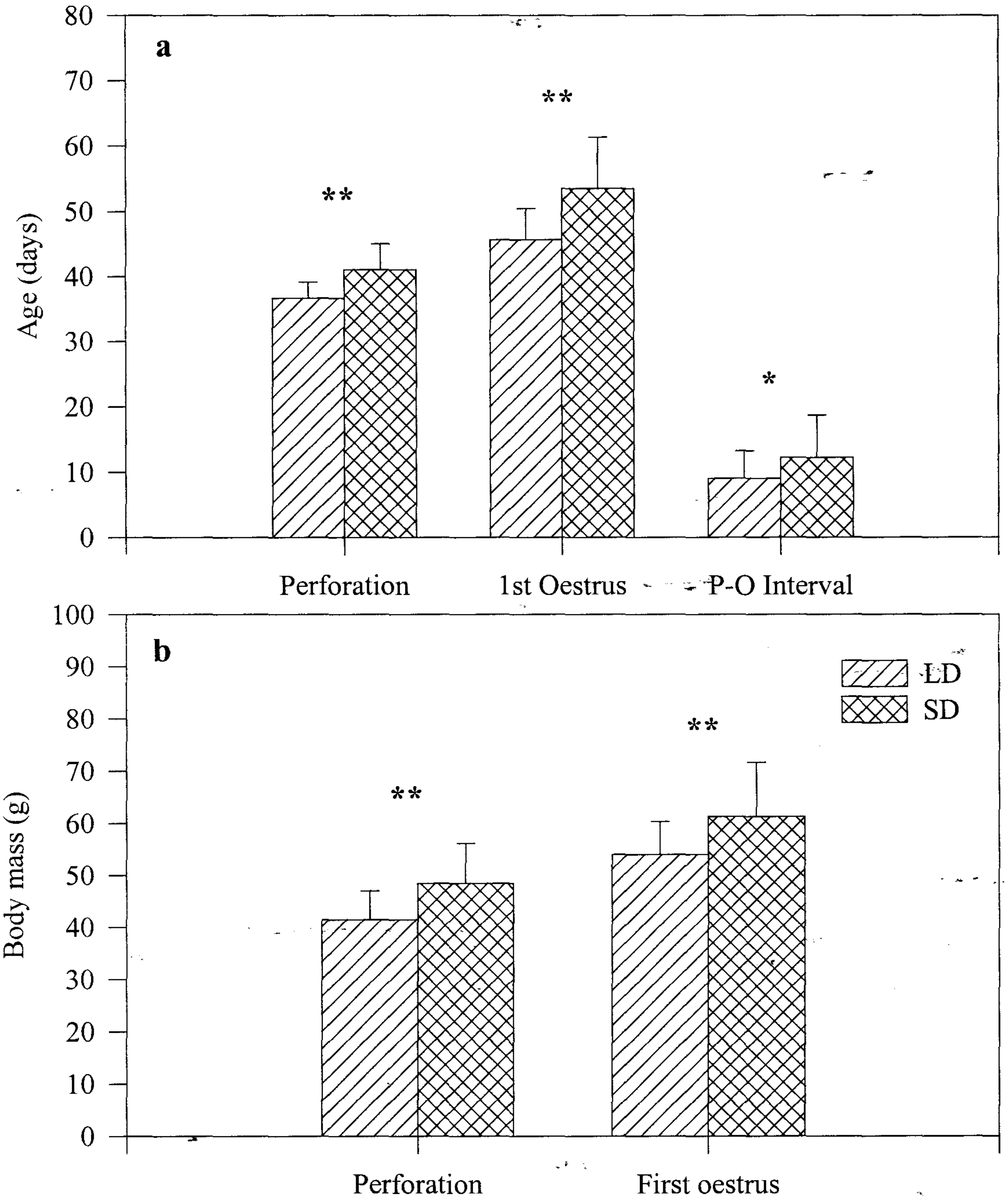

**Figure 4.1** Mean age in days (± standard deviation) at perforation and first oestrus (a) and mean body mass at vaginal perforation and first oestrus (b) of domesticated pouched mice in both long and short daylength. * = $p < 0.05$; ** = $p < 0.01$

Mean body mass of LD females at the age of vaginal perforation was significantly lighter than that of SD females ($p < 0.001$, $t = -3.91$). This same trend was seen in the mean body mass at first oestrus, which was significantly lighter in the LD females than the SD females ($p < 0.01$, $T = 821.5$) (Figure 4.1). All domesticated females in both photoperiods became perforate and reached first oestrus.

### *4.2.2.2 The effect of photoperiod on the attainment of sexual maturity of F1/ F2-generation female pouched mice*

**Table 4.2 and Figure 4.2**

Significant differences were observed in the ages of vaginal perforation and first oestrus between the LD and SD juvenile females ($p < 0.01$, $t = -2.77$ and $p = 0.05$, $T = 337.0$ respectively). The LD females became perforate 6.4 days earlier and had their first oestrous-type vaginal smear 8.6 days earlier than SD females. Photoperiod also significantly affected the interval between vaginal perforation and first oestrus ($p < 0.05$, $t = -2.48$), as LD females had a shorter period between vaginal perforation and first oestrus than SD females (Table 4.2; Figure 4.2).

The mean body masses of LD and SD F1/F2-generation females were not significantly different at the time of vaginal perforation ($p > 0.05$, $t = -0.79$), although LD females were generally slightly lighter than SD females. Similarly, at first oestrus, body masses of the LD females did not differ significantly from those of SD females ($p > 0.05$, $t = -0.97$), however, LD females tended to be slightly lighter than SD females (Table 4.2; Figure 4.2).

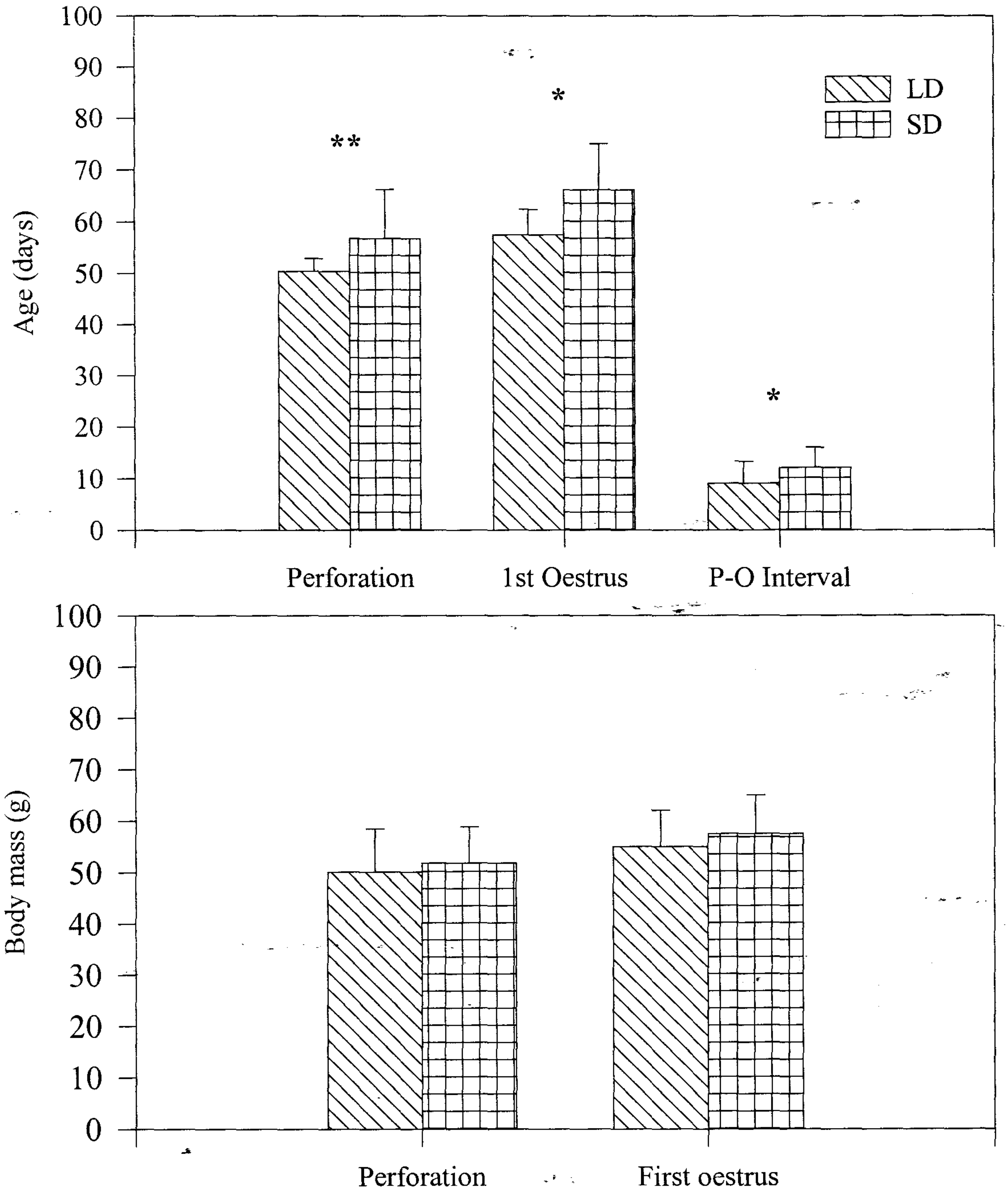

**Figure 4.2** Mean age in days (± standard deviation) at vaginal perforation and first oestrus (a), and mean body mass at vaginal perforation and first oestrus (b) of F1/F2-generation pouched mice in both long and short daylength. * = $p < 0.05$; ** = $p < 0.01$

**Table 4.2** The effect of photoperiod on age and body mass at vaginal perforation and first oestrus of F1/F2-generation female pouched mice. Standard deviations are shown for all mean values.

| | Photoperiod<br>LD | <br>SD | Significance |
|---|---|---|---|
| Sample size (n) | 27 | 23 | |
| Age at perforation (days) | 50.41 ± 6.85 | 56.78 ± 9.39 | ** |
| Body mass (g) at perforation | 50.05 ± 8.38 | 51.80 ± 7.06 | NS |
| Age at first oestrus (days) | 55.91 ± 7.43 | 66.13 ± 8.95 | * |
| Body mass (g) at first Oestrus | 54.92 ± 7.08 | 57.45 ± 7.51 | NS |
| Age at P-O Interval (days) | 8.47 ± 4.54 | 12.13 ± 3.89 | * |

LD = long day, SD = short day; P-O Interval = the number of days between perforation and the first oestrous-type vaginal smear.
NS = non-significant; * = $p < 0.05$; ** = $p < 0.01$

Although all the F1/F2-generation females became perforate, not all reached first oestrus. In long daylength only 19 of 27 females (70%) and, under short day conditions, 15 of 23 females (65%) reached first oestrus (Figure 4.2). The 8 LD females that did not have a first oestrus were born and reared during January and June (austral late summer and autumn), thus were conceived and born during decreasing photoperiods. Of the 8 SD females, that did not have a first oestrus, 4 were born and reared between January and June, and 4 between July and December (*i.e.* austral late winter and early spring). Thus during the decreasing photoperiods 40% of LD females and 80% of SD females did not become sexually mature. During the period of increasing photoperiod the percentage of females that never reached sexual maturity decreased dramatically to 0% in long daylength and 22% in short daylength.

### *4.2.2.3 The effect of domestication on the age of sexual maturity in juvenile female pouched mice*

A two-way ANOVA carried out to determine the effect of photoperiod and domestication on the age of sexual maturity showed that both photoperiod and domestication significantly affected the age of perforation ($p < 0.0001$, $F = 20.55$ and $p < 0.0001$, $F = 152.57$ respectively). All interactions between the factors were found to be non-significant ($p > 0.05$, $F = 0.67$). Domesticated females in long daylength became perforate *c.* 4.43 days earlier than domesticated females in short daylength, 13.75 days earlier than F1/F2-generation females in long daylength and 20.12 days earlier than F1/F2-generation in short daylength. Domesticated females in short daylength became perforate significantly earlier than F1/F2-generation females in both long and short daylength (9.32 and 15.69 days respectively).

The age of first oestrus was significantly affected by domestication and photoperiod ($p < 0.0001$, $F = 61.35$ and $p < 0.0001$, $F = 27.64$ respectively) as domesticated females in long daylength reached first oestrus significantly earlier than domesticated females in short daylength (7.79 days earlier), and F1/F2-generation females in both long and short daylength (11.81 and 20.44 days earlier respectively). All interactions between the factors were found to be non-significant ($p > 0.05$, $F = 0.073$). Domesticated females in short daylength reached first oestrus significantly earlier than F1/F2-generation females in short daylength (12.65 days earlier) but only 4.02 days earlier than F1/F2-generation females in long daylength.

Domestication did not significantly affect the interval between perforation and first oestrus ($p > 0.05$, $F = 0.045$). Photoperiod did significantly lengthen the interval between perforation and first oestrus ($p < 0.01$, $F = 9.19$) as domesticated females in short daylength had significantly longer interval between perforation and first oestrus than domesticated females in long daylength. All interactions between factors were not found to be significant ($p > 0.05$, $F = 1.21$).

Body mass at perforation was significantly affected by domestication and photoperiod ($p < 0.0001$, $F = 17.91$ and $p < 0.01$, $F = 9.59$ respectively) as domesticated females in long daylength were significantly lighter than domesticated females in short daylength and F1/F2-generation females in both long and short daylengths. Domestication did not affect body mass at first oestrus ($p > 0.05$, $F = 0.0015$). Photoperiod did have a significant effect on the body mass at first oestrus ($p < 0.05$, $F = 4.24$) as domesticated females in long daylength were significantly lighter than domesticated females in short daylength. All interactions were found to be non-significant (perforation: $p > 0.05$, $F = 3.44$; first oestrus: $p > 0.05$, $F = 0.29$).

### *4.2.3 Summary:*

1. Photoperiod significantly affected the attainment of sexual maturity. Both vaginal perforation and first oestrus occurred at younger ages under long day conditions than under short day conditions in both the domesticated and F1/F2-generation wild-caught mice.
2. Domesticated females, irrespective of photoperiod, reached sexual maturity at a significantly earlier age than F1/F2-generation females.
3. The F1/F2-generation of wild-caught females experienced the first oestrus at the same body mass but at different ages depending on the photoperiod under which they were reared. By contrast, in domesticated, daylength affected both the age and body mass at which vaginal perforation and first oestrus occurred.
4. All domesticated female pouched mice experienced a first oestrous-type vaginal

smear while only *c.* 68% of F1/F2-generation female pouched mice experienced first oestrus.

## 4.3 The effect of photoperiod on the attainment of sexual maturity of domesticated and F1/F2-generation male pouched mice.

### 4.3.1 Materials and Methods

#### *4.3.1.1 The effect of photoperiod on the attainment of sexual maturity of domesticated male pouched mice*

*Animals:*

- 19 domesticated females of unknown parity, age or original photoperiod
- 21 nulliparous domesticated females (between 102 and 435 days of age)
  - 13 females were conceived, born and reared under long day conditions
  - 8 females were conceived, born and reared under short day conditions
    - 71 juvenile males were conceived, born and reared under long day conditions (LD males)
    - 70 juvenile males were conceived, born and reared under long day conditions (SD males)

*Experimental design:*

A group of 27 adult domesticated female pouched mice (14 females of unknown parity and age, and 13 nulliparous females of known age) were mated under long day photoperiod and a second group of 13 (5 females of unknown parity and age, and 8 nulliparous females of known age) were mated under short day photoperiod. The resultant litters were raised under the photoperiods in which they had been conceived. Once the litters were weaned (juvenile males at *c.* 30 days) the same female and male mice were moved to the alternate photoperiod, acclimatized for four weeks and mated again. Once more, the resultant litters were raised under the photoperiod in which they had been conceived.

Sample of between 5 and 11 juvenile males were sacrificed on 8, 11, 15, 17, 19, 26, 32, 39, 46 and 50 days of age in both photoperiods. The day of birth was called Day 0. Each age group consisted of animals from at least 3 different litters. Where possible, juvenile males from specific females and males were sacrificed at the same ages in both photoperiods. At sacrifice, body masses were measured and testes recorded as abdominal, inguinal or scrotal depending on the position in relation to the scrotal sac.

### *Histological examination:*

The testes and epididymides were removed, weighed (to 0.01g) and prepared for histological examination as described previously (see Section 3.3.1).

The diameter of 20 round seminiferous tubules and the thickness of the seminiferous epithelial layer were measured for each testis. 100 randomly selected seminiferous tubules were examined for the presence of elongated spermatozoa (ES). If ES were present then 20 sections of the cauda epididymides for each animal were examined and the abundance of spermatozoa given a score ranging from 0 (no spermatozoa) to ++++ (many spermatozoa). In the absence of ES the most advanced cell types in the seminiferous epithelium were counted in 100 randomly selected seminiferous tubules per animal. The age at which the lumen of the seminiferous tubules appeared was also noted.

#### *4.3.1.2 The effect of photoperiod on the attainment of sexual maturity of F1/F2-generation male pouched mice*

*Animals:*

- 4 wild-caught females of unknown parity or age
- 6 nulliparous F1-generation females (between 172 and 630 days of age)
    - 2 F1-generation females were conceived, born and reared under long day conditions
    - 4 F1-generation females were conceived, born and reared under short day conditions
        - 21 juvenile males (F1 = 8; F2 = 13) were conceived, were born and reared under long day conditions (LD males)
        - 13 juvenile males (F1 = 4; F2 = 10) were conceived, born and reared under short day conditions (SD males)

*Experimental design:*

Four wild-caught female and male mice were placed in long day photoperiod and acclimated for four weeks. The females were mated once it was established they had a regular four-day oestrous cycle using vaginal smears taken daily. The resultant litters were raised under the same photoperiod in which they were conceived. Once the litters were weaned (*c.* 28 days) the adult female and male mice were moved to short day photoperiod, acclimatised for four weeks and mated again. The resultant litters were raised under the photoperiod in which they were conceived.

Due to the small number of F1-generation animals and as the primary aim of the study was to determine the age of sexual maturity, it was decided to sample from those age groups that had been found to possess spermatozoa in the domesticated mice. Thus, 4 to 8 juvenile males were sacrificed at 46, 50 and 53 days of age in both long and short day photoperiods. Each age group consisted of animals from at least three different litters. Where possible, juvenile males from specific adult females and males were sacrificed at the same ages in both photoperiods. At sacrifice, body masses were measured and testes recorded as abdominal, inguinal or scrotal depending on their position in relation to the scrotal sac.

*Histological examination:*

The testes and epididymides were removed, weighed (to 0.01g) and histologically examined following the procedure outlined in section 4.3.1.1.

#### *4.3.1.3 Data Analyses*

The analyses of data of both the domesticated and F1/F2-generation males were carried out using the same method. A linear regression was carried out to determine whether there was a relationship between body mass and the mass of the paired testes. Both sets of data were log-transformed as the original data was not linearly distributed.

To determine the effect photoperiod had on the age groups a two-way ANOVA was carried out on the domesticated and F1/F2-generation males separately. As there was no non-parametric equivalent for the two-way ANOVA the data were log-transformed and the two-way ANOVA was repeated (Underwood, 1997).

## 4.3.2 Results:

### *4.3.2.1 The effect of photoperiod on the attainment of sexual maturity of domesticated male pouched mice*

**Table 4.3, 4.4, 4.5, 4.6 and Figure 4.3, 4.4, 4.5, 4.6, 4.7**

*Body and Testis masses:*

A two-way ANOVA showed that photoperiod had a significant affect on body mass ($p < 0.05$; $F = 6.05$) however a significant interaction was not found between photoperiod and age on body mass ($p > 0.05$, $F = 1.92$). The mean body mass of mice under long and short day conditions were very similar from day 8 to day 19. Thereafter, the mice under short day conditions were consistently lighter (Table 4.3 and Figure 4.3). These differences were only significant on days 39 and 46. Body masses of the 50 day old males in short day conditions approached those of the males in long day conditions, who had reached adult body mass at an earlier age than the SD males (Figure 4.3).

**Table 4.3** Comparisons of body masses and the masses of paired testis for long and short day juvenile domesticated pouched mice at different ages. Standard deviations are given for all mean values

| Ages (days) | Body mass (g) | | | Paired testis mass (g) | | |
|---|---|---|---|---|---|---|
| | LD | SD | Significance | LD | SD | Significance |
| 8 | 6.69 ± 0.83 | 6.24 ± 0.81 | NS | 0.01 ± 0.002 | 0.02 ± 0.002 | NS |
| 11 | 8.99 ± 1.97 | 9.52 ± 1.32 | NS | 0.03 ± 0.01 | 0.03 ± 0.009 | NS |
| 15 | 10.90 ± 2.37 | 11.19 ± 2.40 | NS | 0.05 ± 0.007 | 0.06 ± 0.007 | NS |
| 17 | 12.30 ± 2.71 | 15.43 ± 2.22 | NS | 0.06 ± 0.01 | 0.08 ± 0.009 | * |
| 19 | 15.79 ± 3.78 | 15.95 ± 3.74 | NS | 0.07 ± 0.02 | 0.09 ± 0.03 | NS |
| 26 | 36.77 ± 8.45 | 33.96 ± 6.82 | NS | 0.26 ± 0.08 | 0.22 ± 0.04 | NS |
| 32 | 48.90 ± 7.82 | 44.35 ± 5.07 | NS | 0.43 ± 0.16 | 0.37 ± 0.06 | NS |
| 39 | 56.20 ± 6.53 | 47.63 ± 6.36 | ** | 0.78 ± 0.15 | 0.65 ± 0.17 | NS |
| 46 | 68.81 ± 15.37 | 54.30 ± 10.27 | * | 1.40 ± 0.41 | 0.95 ± 0.29 | ** |
| 50 | 71.09 ± 9.58 | 63.63 ± 9.98 | NS | 1.62 ± 0.21 | 1.35 ± 0.30 | * |

NS = non- significant; * = $p < 0.05$; ** = $p < 0.01$

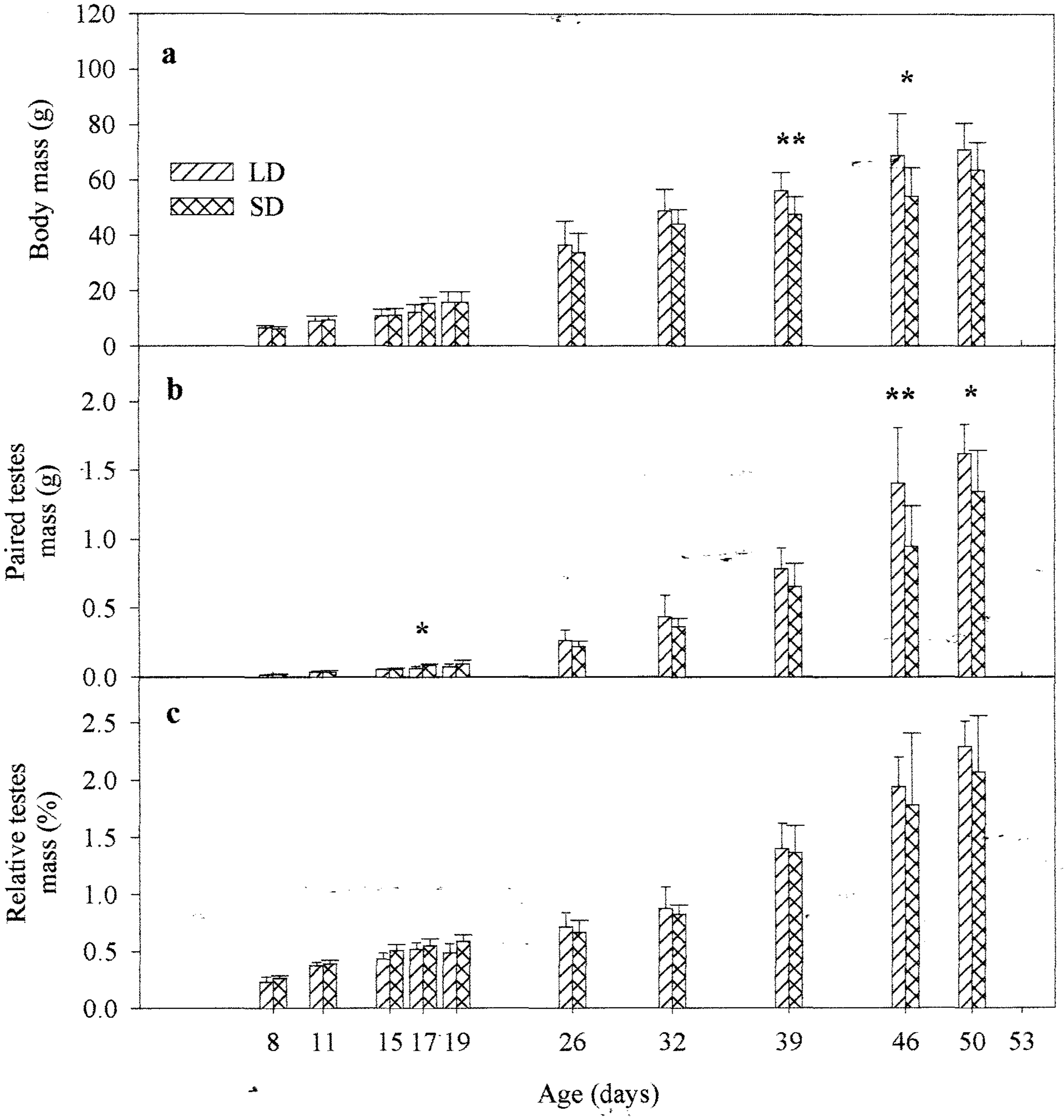

**Figure 4.3** The mean ± standard deviation for a) body mass, b) mass of paired testis, and c) relative paired testis mass of domesticated male pouched mice in both long and short daylength. * = $p < 0.05$; ** = $p < 0.01$

Photoperiod had a significant effect on the mass of paired testis (two-way ANOVA; $p < 0.01$; $F = 6.98$) and there was a significant interaction between photoperiod and age ($p < 0.01$, $F = 2.65$). From 26 days of age, the mean mass of paired testis of the SD males was consistently lower than those of the LD males and the mass of paired testis was found to be significantly different at 17, 46 and 50 days of age (Figure 4.3).

A linear regression of the log of the dependent variable, masses of paired testis, versus the log of the independent variable, body mass, indicated that the masses of paired testis were a function of body mass ($r^2 = 84.6$ % and 76.8 % in LD and SD males, respectively) thus it would be more appropriate to report the relative masses of the paired testis. A two-way ANOVA showed no significant differences between the relative mass of the paired testis between LD and SD males ($p > 0.05$, $F= 0.26$) neither was there an interaction between photoperiod and age ($p > 0.05$; $F = 0.42$) (Table 4.4).

**Table 4.4** Comparison of the relative paired testis mass of juvenile domesticated pouched mice in both long and short daylength. Standard deviations are given for all mean values.

| Ages (days) | Relative paired testis mass (g) | | |
|---|---|---|---|
| | LD | SD | Significance |
| 8 | 0.23 ± 0.05 | 0.26 ± 0.02 | NS |
| 11 | 0.37 ± 0.03 | 0.39 ± 0.03 | NS |
| 15 | 0.43 ± 0.05 | 0.51 ± 0.05 | NS |
| 17 | 0.52 ± 0.06 | 0.55 ± 0.06 | NS |
| 19 | 0.49 ± 0.08 | 0.59 ± 0.06 | NS |
| 26 | 0.71 ± 0.13 | 0.67 ± 0.10 | NS |
| 32 | 0.87 ± 0.19 | 0.82 ± 0.08 | NS |
| 39 | 1.39 ± 0.22 | 1.36 ± 0.24 | NS |
| 46 | 1.93 ± 0.26 | 1.77 ± 0.63 | NS |
| 50 | 2.29 ± 0.22 | 2.07 ± 0.49 | NS |

NS = non- significant

*Seminiferous tubules:*

Seminiferous tubule diameter increased from 8 to 46 days of age in both photoperiods and a two-way ANOVA indicated that neither photoperiod nor the interaction between photoperiod and age had a significant effect ($p > 0.05$, $F = 0.97$ and $p > 0.05$; $F = 1.89$ respectively). At 50 days of age, however, the seminiferous tubule diameter of the LD males was significantly greater than that of the SD males (Table 4.5 and Figure 4.4).

**Table 4.5** Comparisons of seminiferous tubule diameter and seminiferous epithelial thickness for long and short day juvenile domesticated pouched mice at different ages using t-tests at each age. Mean ± standard deviations are given for all values.

| Ages (days) | Seminiferous tubule diameter (μm) | | | Seminiferous epithelial thickness (μm) | | |
|---|---|---|---|---|---|---|
| | LD | SD | Significance | LD | SD | Significance |
| 8 | 47.34 ± 5.56 | 44.62 ± 0.96 | NS | 16.75 ± 1.35 | 16.70 ± 0.73 | NS |
| 11 | 45.91 ± 1.39 | 46.84 ± 3.44 | NS | 16.36 ± 0.57 | 17.83 ± 1.33 | * |
| 15 | 59.48 ± 2.49 | 52.29 ± 5.37 | NS | 18.79 ± 1.50 | 20.86 ± 2.25 | NS |
| 17 | 54.93 ± 2.69 | 57.00 ± 3.18 | NS | 20.23 ± 1.12 | 24.09 ± 1.85 | ** |
| 19 | 63.26 ± 4.56 | 64.62 ± 4.49 | NS | 22.61 ± 1.25 | 26.63 ± 1.74 | ** |
| 26 | 85.17 ± 7.00 | 92.44 ± 2.48 | NS | 30.98 ± 2.41 | 35.80 ± 0.74 | ** |
| 32 | 111.62 ± 10.72 | 117.81 ± 5.98 | NS | 39.76 ± 5.70 | 44.08 ± 2.52 | NS |
| 39 | 140.32 ± 8.92 | 134.37 ±11.03 | NS | 52.03 ± 4.92 | 50.43 ± 2.70 | NS |
| 46 | 166.92 ± 14.37 | 164.74 ± 9.62 | NS | 59.93 ± 7.29 | 59.90 ± 3.16 | NS |
| 50 | 196.09 ± 10.61 | 185.04 ± 9.61 | * | 67.99 ± 3.30 | 63.15 ± 3.78 | ** |

NS = non- significant; * = $p < 0.05$; ** = $p < 0.01$

Photoperiod had a significant effect on the thickness of the seminiferous epithelial layer (two-way ANOVA; $p < 0.05$, $F = 5.94$) and there was a significant interaction between photoperiod and age (two-way ANOVA; $p < 0.001$, $F = 3.45$). From 8 days of age until 32 days of age the SD males had thicker seminiferous epithelium than the LD males (Table 4.5). After 32 days of age the epithelium of the LD males was thicker than the SD males (Table 4.5 and Figure 4.4). Significant differences occurred at 11, 17, 19, 26 and 50 days of age.

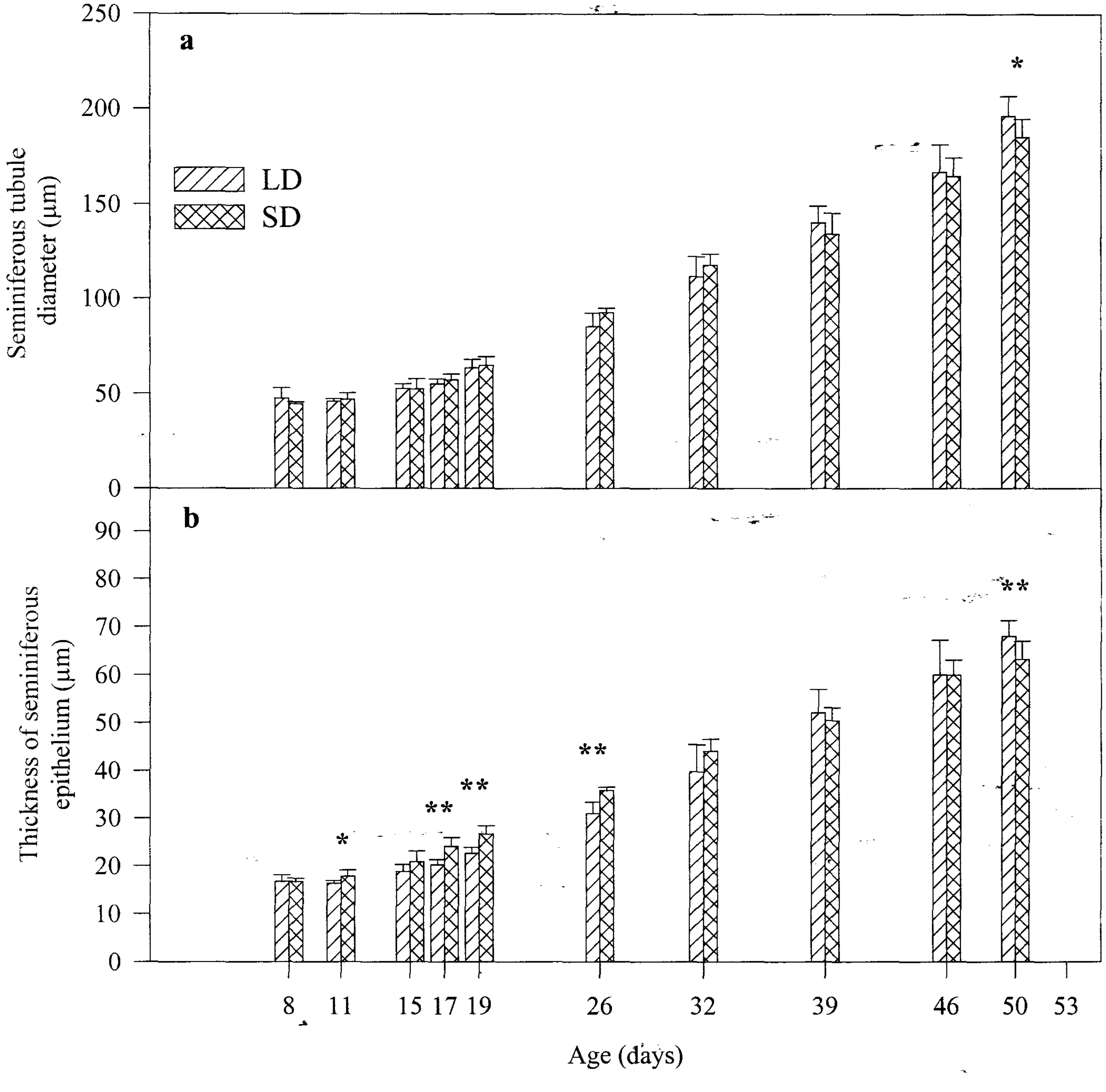

**Figure 4.4** The mean ± standard deviation of a) seminiferous tubule diameter and b) the thickness of the seminiferous epithelial layer of juvenile domesticated male pouched mice in long and short daylength. * = $p < 0.05$; ** = $p < 0.01$

*Spermatogenic activity:*

Gonocytes were found up until the age of 11 days in all LD juvenile males, while 2 of 5 males at 15 days of age from short daylength still had gonocytes present in the seminiferous tubules. The gonocytes were located in the centre of the tubules and were large round cells with a large spherical nucleus and 1 to 3 nucleoli (Kluin and De Rooij, 1981) (Figure 4.5a). In both LD and SD males the first type-A spermatogonia were observed at 8 days of age, the first Intermediate spermatogonia at 17 days of age, type-B spermatogonia at 19 days of age in LD males while early pachytene spermatocytes were observed in 19 day old SD males (Table 4.6).

At 26 and 32 days of age, in both photoperiods, the spermatogenic cells had reached the late pachytene stage (LP) and rounded spermatids ($S_p$) were found at 39 days of age (Figure 4.5b). Elongated spermatids (ES) were first observed at 46 days of age in 9 LD and 10 SD males with ES in 23.4 ± 22.0% and 31.9 ± 21.0% of the seminiferous tubules respectively (Figure 4.6a). Spermatozoa were not present in the cauda epididymides of any LD males, while 1 SD males had spermatozoa present in the cauda epididymis by 46 days of age (Figure 4.6b). The 50 day old specimens had ES in 55.6 ± 18.2% of seminiferous tubules (LD males) and 62.5 ± 10.3% of the seminiferous tubules (SD males) (Figure 4.7a). Of the 10 LD males at 50 days of age, 4 were had spermatozoa present in the cauda epididymides while 7 of the 11 SD males had spermatozoa in the cauda epididymides (Figure 4.7b). Males were considered as being sexually mature when spermatozoa were present in the cauda epididymis (Table 4.6).

Sertoli cells were immature at 8 days of age in both photoperiods and were only found to be mature from 19 days of age. The immature Sertoli cells were easy to distinguish from the type-A spermatogonia and the gonocytes as they were smaller and more variable in shape (Figure 4.5a). Unlike the type-A spermatogonia the elongated longitudinal axis of gonocytes were perpendicular to the basement membrane of the seminiferous tubule (Miething, 1989). Immature Sertoli cells were distinguished from the mature Sertoli cells as they were smaller in size.

**Table 4.6** Stage of spermatogenesis within the seminiferous tubules of juvenile, domesticated, male pouched mice showing the latest cell type and the percentage tubules in that stage.

| | 8 days | | 11days | | 15 days | | 17 days | | 19 days | | 26 days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LD | SD | LD | SD | LD | SD | LD | SD | LD | SD | LD | SD |
| Number of animals | 8 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Latest cell type | A | A | A | A | A | A | I | I | B | EP | LP | LP |
| % Seminiferous tubules with latest cell type | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 32.0 | 64.0 | 63.0 |
| Number animals in late spermatogenesis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number animals with spermatozoa in cauda epididymides | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | 32 days | | 39 days | | 46 days | | 50 days | |
|---|---|---|---|---|---|---|---|---|
| | LD | SD | LD | SD | LD | SD | LD | SD |
| Number of animals | 10 | 10 | 10 | 10 | 10 | 11 | 10 | 11 |
| Latest cell type | LP | LP | $S_p$ | $S_p$ | ES | ES | ES | ES |
| % Seminiferous tubules with latest cell type | 85.3 | 94.4 | 49.0 | 41.0 | 23.4 | 31.9 | 55.6 | 62.5 |
| Number animals in late spermatogenesis | 0 | 0 | 0 | 0 | 9 | 10 | 10 | 11 |
| Number animals with spermatozoa in cauda epididymides | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 7 |

A = type A spermatogonia; I = intermediate spermatogonia; B = type B spermatogonia; EP = early pachytene speratocytes; LP = late pachytene spermatocytes; $S_p$ = rounded spermatids; ES = elongated spermatids.

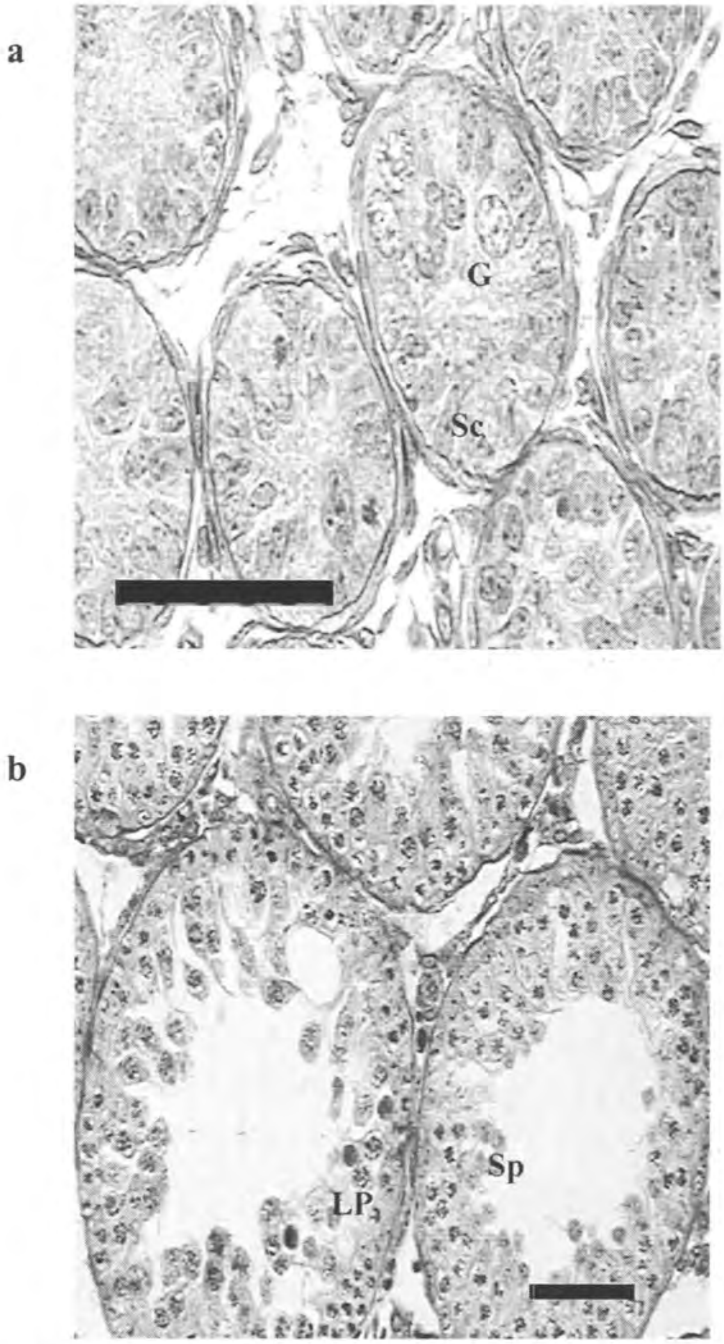

**Figure 4.5** Seminiferous tubules from juvenile domesticated pouched mice at a) 15 days of age in long daylength, and b) 39 days of age in long daylength. G = gonocytes, Sc = Sertoli cells, LP = late leptotene stage, Sp = rounded spermatids. Scale bar = 50 μm.

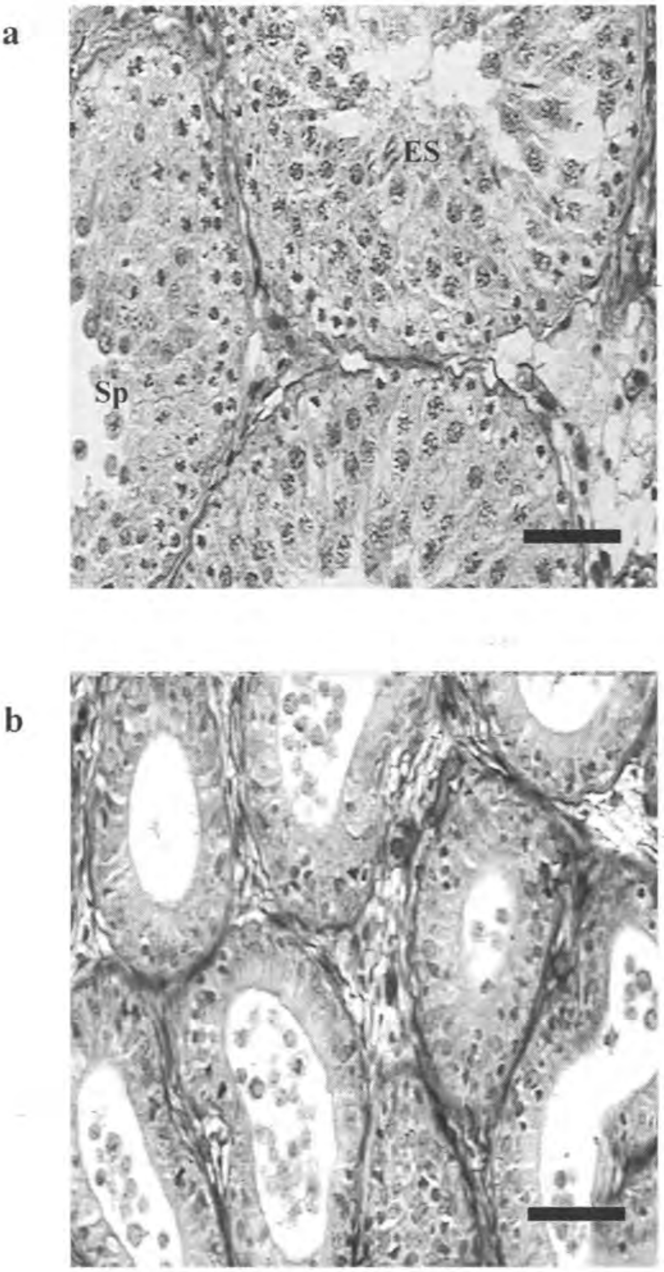

**Figure 4.6** Transverse sections through a) seminiferous tubules, and b) cauda epididymis of juvenile pouched mice at 46 days of age in long daylength. Sp = rounded spermatids, ES = elongated spermatids. Scale bar = 50 μm.

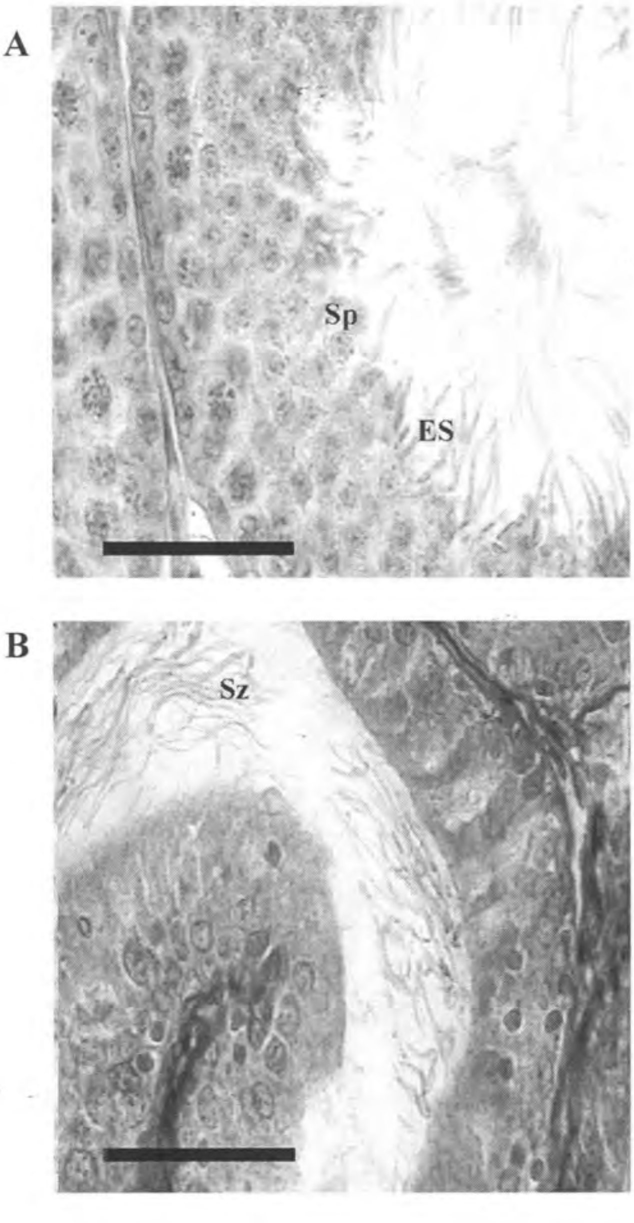

**Figure 4.7** Sections through a) seminiferous tubules and b) cauda epididymis of 50 day old domesticated juvenile pouched mice in short daylength. Sz = spermatozoa. Scale bar = 50 μm.

### *4.3.2.2 The effect of photoperiod on the attainment of sexual maturity of F1/F2-generation male pouched mice*

**Table 4.7, 4.8, 4.9 and Figure 4.8, 4.9, 4.10, 4.11, 4.12**

*Body and testis masses:*

Photoperiod had a significant effect on mean body mass of the F1/F2-generation juvenile males (two-way ANOVA; $p < 0.01$, $F = 13.31$) as LD males were significantly heavier than the males in short daylength at 46 days of age (Table 4.7; Figure 4.8). There was no significant interaction between photoperiod and age ($p > 0.05$, $F = 0.44$).

Photoperiod had a significant effect on the mean masses of paired testis ($p < 0.01$, $F = 12.81$), and at 50 days of age, the mean testis mass of LD males was significantly greater than that of the SD males (Table 4.7; Figure 4.8). There was not a significant interaction between photoperiod and age ($p > 0.05$, $F = 0.21$).

**Table 4.7** Comparisons of body masses and the masses of paired testis for long and short day juvenile F1/F2-generation male pouched mice at different ages. Mean ± standard deviations are given for all values.

| Ages (days) | Body mass (g) | | | Paired testes mass (g) | | |
|---|---|---|---|---|---|---|
| | LD | SD | Significance | LD | SD | Significance |
| 46 | 54.05 ± 3.23 | 46.02 ± 2.78 | ** | 0.62 ± 0.09 | 0.47 ± 0.15 | NS |
| 50 | 55.31 ± 4.69 | 51.31 ± 6.24 | NS | 0.84 ± 0.11 | 0.64 ± 0.17 | * |
| 53 | 63.49 ± 9.12 | 54.56 ± 1.24 | NS | 0.93 ± 0.19 | 0.76 ± 0.23 | NS |

NS = non- significant; * = $p < 0.05$; ** = $p < 0.01$

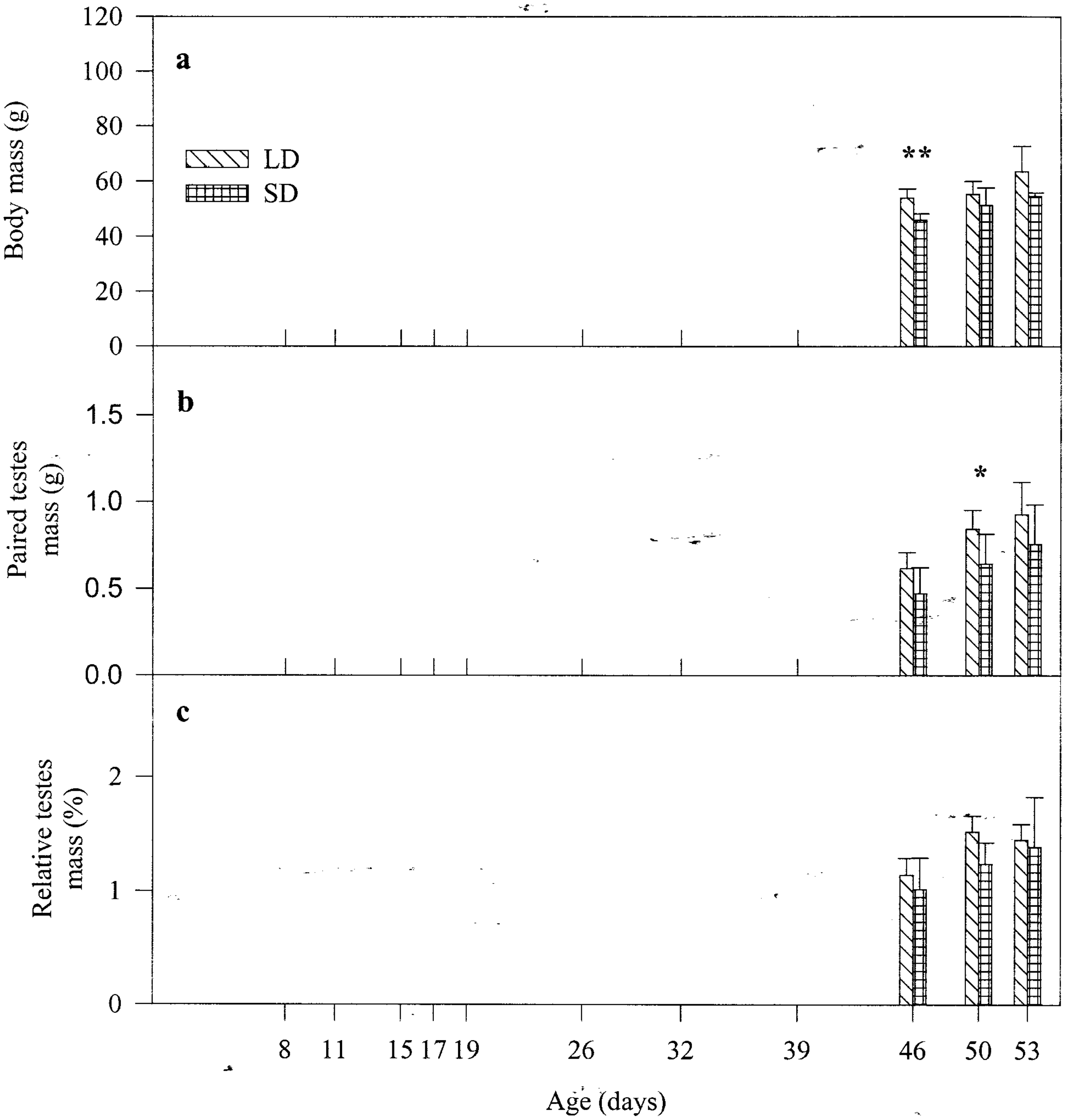

**Figure 4.8** The mean ± standard deviation for a) body mass, b) mass of paired testis and c) relative mass of paired testis of juvenile F1/F2-generation pouched mice in both long and short daylength. * = $p < 0.05$; ** = $p < 0.01$

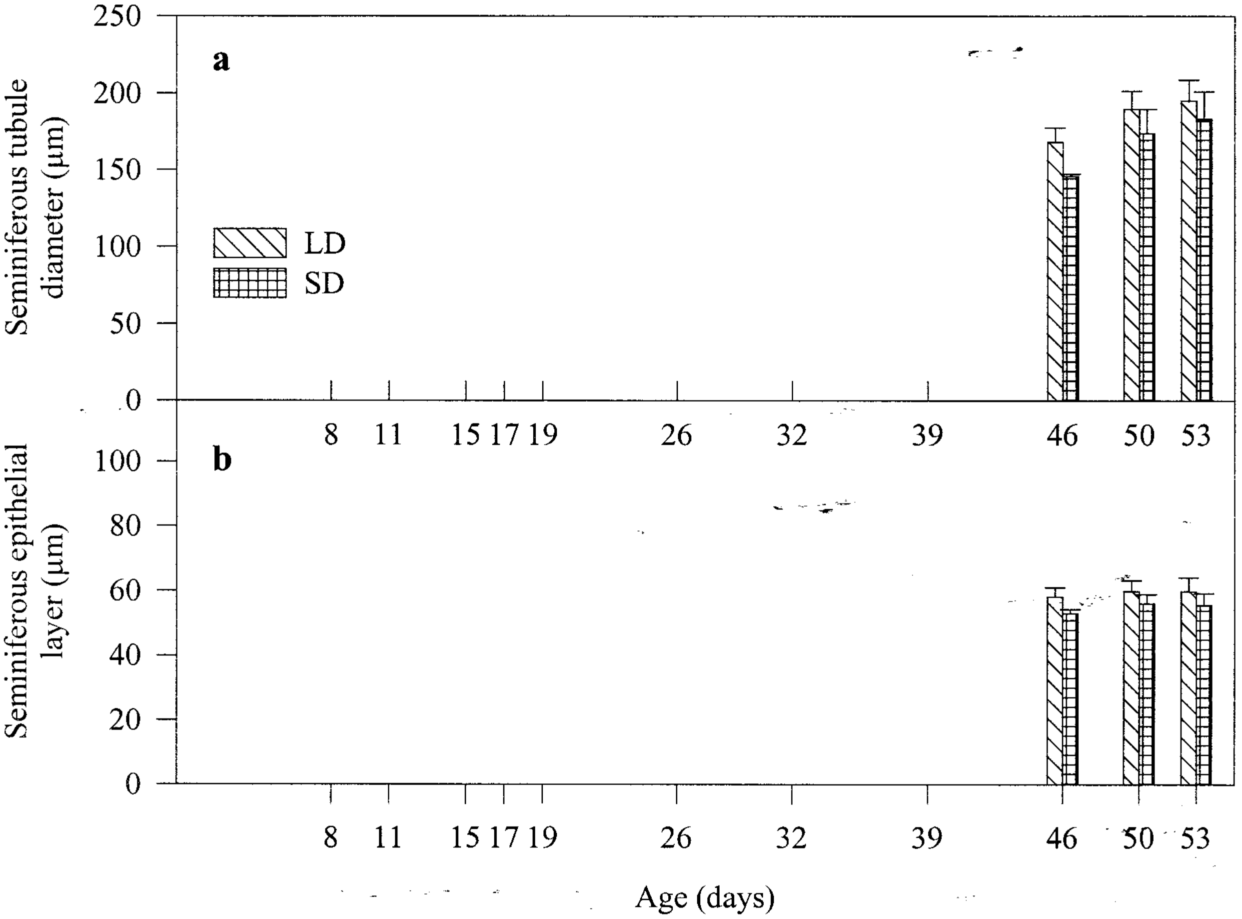

**Figure 4.9** The mean ± standard deviation for the a) seminiferous tubule diameter and b) thickness of the seminiferous epithelium of juvenile F1/F2-generation male pouched mice in different photoperiodic treatments.

A linear regression of the dependent variable, mass of paired testis, and the independent variable, body mass, indicated that, although there was a positive correlation between the masses of paired testis and body mass in LD males ($r^2 = 63.7\%$), the correlation between the two variables was less apparent in the SD males ($r^2 = 51.3\ \%$). It is, therefore, more relevant to report the actual mass of paired testis rather than the relative testis mass.

*Seminiferous tubules:*

Photoperiod did not have a significant effect on the diameter of the seminiferous tubules ($p > 0.05$, $F = 1.62$), or the thickness of the seminiferous epithelium ($p > 0.05$; $F = 1.25$), and there was no statistically significant interaction between photoperiod and age for either the diameter of the seminiferous tubules ($p > 0.05$, $F = 0.26$) or seminiferous epithelial thickness ($p > 0.05$, $F = 0.88$). The diameter of seminiferous tubules were significantly affected by photoperiod only at 46 days of age (Table 4.8; Figure 4.9).

**Table 4.8** Comparisons of the mean ± standard deviations of seminiferous tubule diameter and seminiferous epithelial thickness for long and short day juvenile F1/F2-generation male pouched mice at different ages.

| Ages (days) | Seminiferous tubule diameter (µm) | | | Seminiferous epithelial thickness (µm) | | |
|---|---|---|---|---|---|---|
| | LD | SD | Significance | LD | SD | Significance |
| 46 | 168.05 ± 9.40 | 166.40 ± 24.53 | * | 58.09 ± 2.95 | 58.89 ± 6.87 | NS |
| 50 | 189.54 ± 11.96 | 181.47 ± 21.88 | NS | 59.85 ± 3.29 | 58.21 ± 5.53 | NS |
| 53 | 194.93 ± 13.93 | 183.22 ± 17.85 | NS | 59.77 ± 4.32 | 55.54 ± 3.69 | NS |

NS = non- significant; * = $p < 0.05$

*Spermatogenic activity*

Elongated spermatids (ES) were observed in 26.5 ± 12.3% of the seminiferous tubules of all the LD males at 46 days of age. By contrast, ES were present in 30.5 ± 36.0% of the seminiferous tubules of 2 of the 4 SD males and not present in the other two (Table 4.9 and Figure 4.10a). Spermatozoa were, however, not present in the cauda epididymides of any of the males at 46 days of age (Figure 4.10b).

At 50 days of age all the LD males (100%) and 4 of the 5 SD males (80%) had ES present in 70.3 ± 11.1% and 50.2 ± 38.2% of the seminiferous tubules respectively (Figure 4.11a). At 50 days of age only 2 of the 8 LD males (25%) and 2 of the 5 SD males (40%) had spermatozoa present in the cauda epididymides (Figure 4.11b).

All males in both photoperiods had ES present in the seminiferous tubules at 53 days of age, while the percentage of the seminiferous tubules containing ES had increased to 79.1 ± 19.3% in LD males and 72.6 ± 14.8% in SD males (Figure 4.12a). This is reflected in the increased numbers of animals having spermatozoa present in the cauda epididymides (5 of the 7 LD males (71%) and 4 of the 5 SD males (80%)) (Figure 4.12b).

### *4.3.2.3* ***The effect of domestication on the age of sexual maturity of juvenile male pouched mice***

It is not possible to statistically compare the results from the experiments with domesticated and F1/F2-generation, wild-caught mice, however, it appears that domestication has had little effect on the attainment of sexual maturity in males (presence of spermatozoa in the cauda epididymides). All males under long day conditions were in late spermatogenesis at day 50 and spermatozoa were first seen in the cauda epididymides at 46 days of age (n = 1 for domesticated mice) and 50 days of age (n = 4 for F1/F2-generation mice) (Table 4.6 and 4.9).

### *4.3.3 Summary:*

1. Photoperiod significantly affected the body mass of the juvenile domesticated and F1/F2-generation male pouched mice. Between 39 and 50 days of age, body mass was lighter in the mice exposed to short day conditions.
2. Photoperiod had a significant effect on testis mass of both juvenile domesticated and F1/F2-generation male pouched mice. Between 39 and 50 days of age, testis mass was lighter in the males exposed to short daylength. In the domesticated juvenile males there was a significant, positive correlation between testis size and body mass and photoperiod had no effect on relative testis mass.
3. Domestication appears to have little effect on the attainment of sexual maturity in male pouched mice.

**Table 4.9** Stage of spermatogenesis within the seminiferous tubules of juvenile F1-generation male pouched mice showing latest cell type and the percentage tubules in that stage.

| | 46 days | | 50 days | | 53 days | |
|---|---|---|---|---|---|---|
| | LD | SD | LD | SD | LD | SD |
| Number of animals | 6 | 4 | 8 | 5 | 7 | 5 |
| Latest cell type | ES | ES | ES | ES | ES | ES |
| % Seminiferous tubules with latest cell type | 26.5 ± 12.3 | 30.5 ± 36.0 | 70.3 ± 11.1 | 50.2 ± 38.2 | 79.1 ± 19.3 | 72.6 ± 14.8 |
| Number animals in late spermatogenesis | 6 | 2 | 8 | 4 | 7 | 5 |
| Number animals with spermatozoa in cauda epididymides | 0 | 0 | 2 | 2 | 5 | 4 |

A = type A spermatogonia; I = intermediate spermatogonia; B = type B spermatogonia; $S_1$ = primary spermatocytes; $S_2$ = secondary spermatocytes; $S_p$ = rounded spermatids; ES = elongated spermatids.

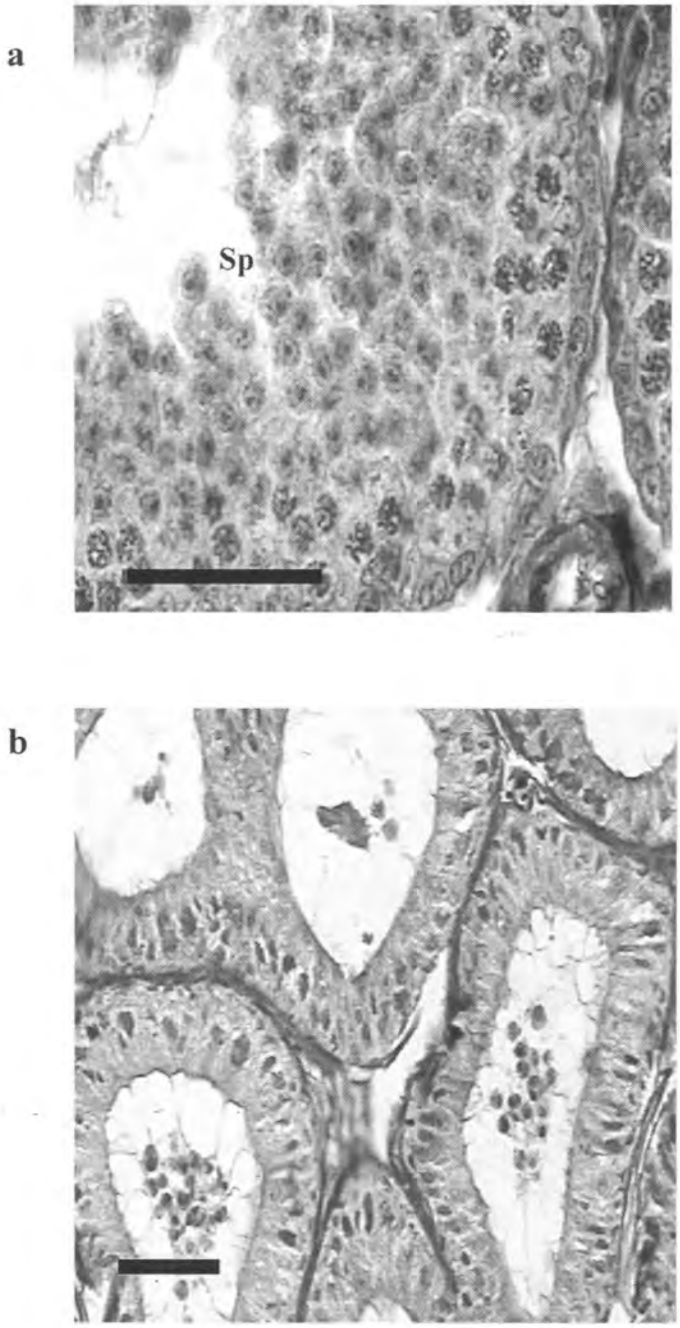

**Figure 4.10** Transverse sections through a) seminiferous tubules and b) cauda epididymides of F1/F2-generation juvenile pouched mice at 46 days of age in long daylength. Sp = rounded spermatids. Scale bar = μm.

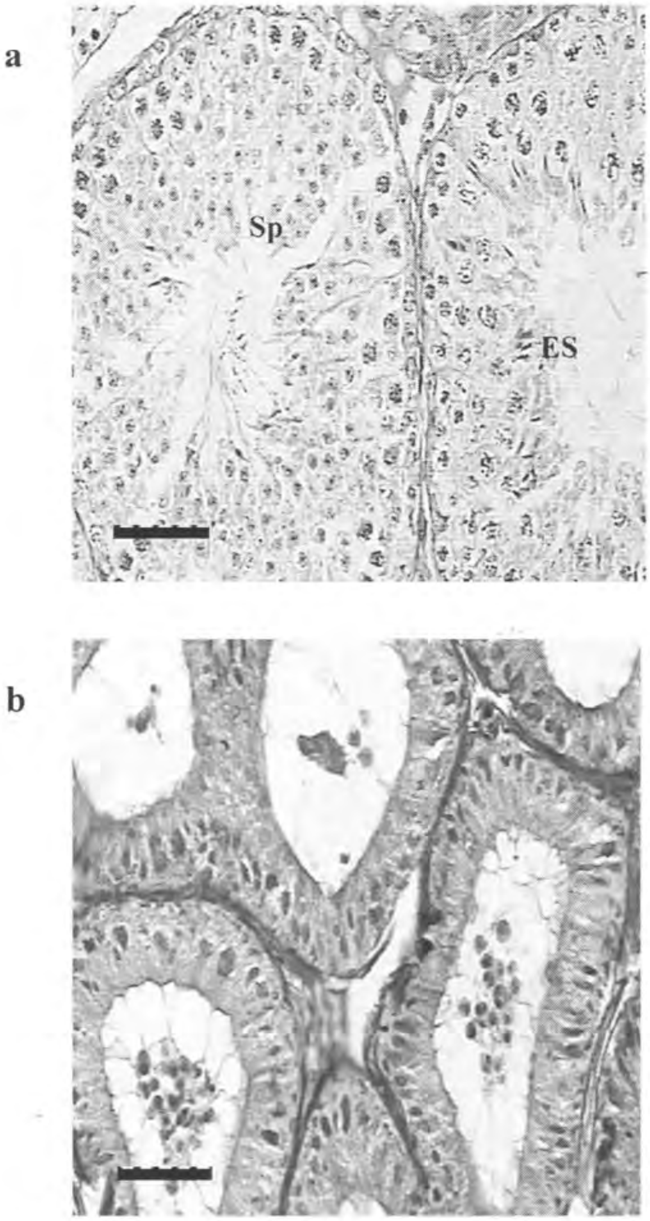

**Figure 4.11** Transverse sections of a) seminiferous tubules and b) cauda epididymides of F1/F2-generation juvenile pouched mice at 50 days of age in short daylength. Sp = rounded spermatids, ES = elongated spermatids. Scale bar = 50 μm.

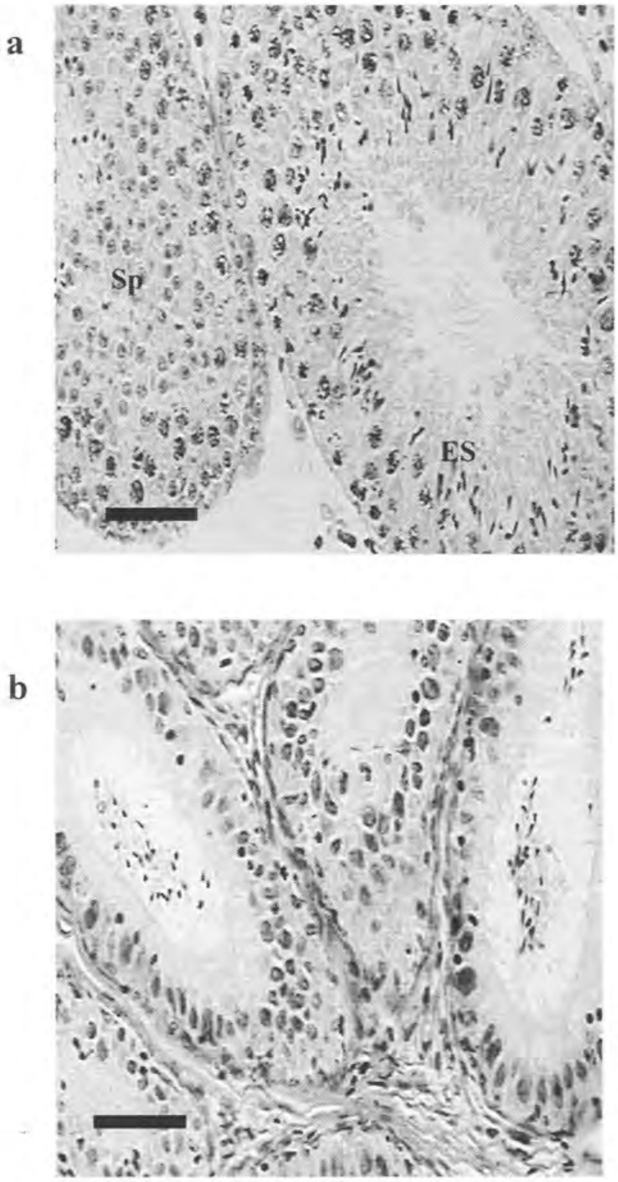

**Figure 4.12** Sections through a) seminiferous tubules and b) cauda epididymides of F1/F2-generation juvenile pouched mice at 53 days in short daylength. Sp = rounded spermatids, ES = elongated spermatids. Scale bar = 50 µm.

## 4.4 Discussion

Male and female pouched mice in this study responded differently to photoperiod, which influenced the attainment of sexual maturity in the females but had little effect in the males. The onset of sexual maturity in female pouched mice was retarded, but not halted in short daylength. Domestication was associated with an increase in body size in both males and females but only in the females, did the domesticated and F1/F2-generation wild-caught mice respond differently to photoperiod. Domestication had the effect of accelerating the age at which female pouched mice attained sexual maturity. Domestication and long daylength separately resulted in increased growth rate of juvenile male pouched mice, however, they had little effect on the attainment of sexual maturity. Both the domesticated and F1/F2-generation males first had spermatozoa in the cauda epididymides at about the same age irrespective of photoperiod.

The actions of domestication and natural selection are similar, with both producing population to population variation and in the process decreasing but not eliminating individual variation (Setchell, 1992). Domestication has the effect of unintentional selection thus causing a genetic bottleneck under laboratory conditions so mice that will reproduce easily and quickly under these conditions are selected (Nelson, 1985; Bronson, 1989). One of the first results of domestication is an increase in body size possibly due to the selection process in the laboratory, *i.e.* by keeping animals that breed earlier one is also selecting for the larger animals. A further result of domestication is to accelerate the age for the onset of fertility (Price, 1967; Berry, 1969; Bronson, 1989; Clark and Galef, 1980; Setchell, 1992). King (1939) showed that in eight generations of laboratory breeding the incidence of infertility in wild-caught Norway rats (*Rattus norveticus*) had decreased from 75% to only 6%. This was due to the fact that only a quarter of the wild-caught rats bred under laboratory conditions and thus the rats that did not breed were culled or died out. The same results of domestication were seen in the present study where the domesticated pouched mice grew faster, were larger and the females reached sexual maturity earlier than the wild mice.

Many evolutionary biologists view males and females of the same species as if they were different species, as pubertal strategies often differ between the sexes even within the same population (Sadlier, 1969; Hasler *et al.*, 1976; Hamilton and Bronson, 1985). In females there are three factors that influence pubertal strategies;

1. Natural selection may accelerate the onset of fertility so that a female can fit the greatest number of pregnancies into her life. This is important in short-lived mammals that may only survive one breeding season but must not result in increased mortality.
2. The female needs to be physiologically and physically developed before reproduction occurs and;
3. Reproduction must occur only if environmental conditions are favourable (Bronson, 1989).

This final point is particularly important in female mammals as lactation is the most energetically expensive process and once a female reaches her first ovulation she risks pregnancy (Millar, 1977; Bronson, 1984; 1985; Gittleman and Thompson, 1988). The premature onset of sexual maturity could result in pregnancy and lactation during unfavourable environmental conditions, possibly wasting energy and risking death of the female. By contrast, males only need to compete with each other for the chance to mate with a female and for territories (Bronson, 1989; Lee *et al.*, 1987) and a different set of factors will affect the onset of spermatogenesis.

All the F1/F2-generation males in this study produced spermatozoa at about the same age, irrespective of daylength, while not all the F1/F2-generation females reached sexual maturity. It is probable that those females that did not reach first ovulation, would have done had the experiment run longer, and it has been suggested that in a population there may be females that attain sexual maturity at an early age while others attain sexual maturity at a later age (Falconer, 1984; Bronson, 1989).

Finally there is the question as to why photoperiod should affect the age of sexual maturity of female pouched mice and not in males? The lengths of the gametogenic cycles of female and male mammals differ considerably. Female mammals have a much shorter gametogenic cycle and, when halted can proceed through the final stages of reproductive development rapidly once environmental conditions become favourable (Hamilton and Bronson, 1985; Bronson and Heideman, 1994). The spermatogenic cycle of male mammals, however, takes several weeks from the development of spermatogonia to the mature spermatozoa and thus it is often more beneficial for the male to reach sexual maturity and then remain sexually ready (Sadlier, 1969). This is important in the unpredictable climates of sub-tropical and tropical latitudes where a more opportunistic and flexible strategy needs to be adopted (Bronson, 1985; 1989; Bronson and Heideman, 1994). In sub-tropical latitudes the highest rainfall normally occurs during a certain period of the year (summer in most of South Africa excluding the Western Cape), however, the rainfall period may be extended in some years or rain may fall during other times of the year (Neal, 1986; Perrin, 1986; Bronson and Heideman, 1994). Food availability and quality peak during high rainfall periods and therefore are also unpredictable and some small mammals extend their breeding periods if the rainfall period is extended (Rautenbach, 1982; Neal, 1986; Perrin and Swanepoel, 1987;

Willan and Meester, 1987; Korn, 1989; Makundi, 1995).

The attainment of puberty within a species may be either age-dependent or weight-dependent as individuals of the same species reach sexual maturity at either a certain age or a certain body weight (Falconer, 1984). In the F1/F2-generation pouched mice, the occurrence of the first oestrus was mass dependent while in the domesticated mice, it was both mass and age dependant. In the F1/F2-generation mice, long daylength accelerated growth and the critical body mass was reached at an earlier age than under short daylength. In the domesticated mice, long daylength accelerated growth and first oestrus occurred at a younger age and at a lower body mass. An obvious benefit of accelerated growth and sexual development under long day conditions is that it may allow a young female to produce a litter before winter sets in. This point is highlighted by the fact that the interval between vaginal perforation and first ovulation was significantly shorter under long day conditions. The fact that the onset of sexual maturity in domesticated pouched mice was not body mass dependant is probably an artefact of selective breeding within a constant and protected environment.

## 4.5 Summary

1. Photoperiod affected the onset of sexual maturity of female pouched mice as short daylength delayed growth and sexual development of both domesticated and F1/F2-generation wild-caught female pouched mice.
2. Domestication affected the onset of sexual maturity in female pouched mice, as years of laboratory breeding increased body size and consequently accelerated the onset of fertility.
3. Neither photoperiod nor domestication significantly affected the onset of sexual

maturity in male pouched mice, age-dependent, who thus exhibited an opportunistic strategy as they became sexually mature and were able to reproduce when females reached sexual maturity.

4. Domestication reduced the variability seen in the wild-caught female pouched mice probably by unintentional selection of females that would reproduce easily and quickly under laboratory conditions.

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# Chapter 5

## The effect of photoperiod on the litter size, ovulation rate and sex ratio of domesticated and wild-caught female pouched mice.

### 5.1 Introduction

Of all aspects of population dynamics litter size, together with the number of young weaned per litter, are the most important to the growth and survival of small mammals (Pelikán, 1981; Loeb and Schwab, 1987; Bronson, 1985; 1989). Litter size is generally defined as the number of live young produced by a female during one reproductive event and her reproductive success is dependent on the number of offspring she has throughout her life span that survive to reproduce (Spencer and Steinhoff, 1968; Bronson, 1989; Sikes, 1995). Physiological, environmental and behavioral factors can influence litter size prior to birth by altering the number of eggs ovulated, fertilized and implanted, plus the loss of fetuses caused by fetal resorption (Smith and McManus, 1975; Clarke, 1985; Krackow, 1992; Westlin *et al.*, 1995). Litter size is therefore a function of ovulation rate (number of corpora lutea in the ovary), implantation rate and absorption rate. Physiological factors include body size, condition and parity of mother plus the size and development of young (Williams, 1979; Loeb and Schwab, 1987; Huck *et al.*, 1988; Bronson, 1989). Environmental factors influencing litter size can be divided into ultimate, which include climate (temperature, rainfall and humidity) and food availability, and proximate factors, which include photoperiod and food availability (Millar, 1977; Bronson, 1985; 1989; Sikes, 1995). Behavioural factors, such as predator avoidance, may not be as important but may still influence litter size to a small degree, while others, such as social influences, can be important. Many of these influences may interact with each other, such as food availability and predation, body size and predation, rainfall and food availability, and condition of mother and food availability. Further adjustments to litter size occur after parturition prior to weaning when maternal infanticide and death of pups result in a decrease in the litter size (Bronson and Marsteller, 1985; Krackow and Hoeck, 1989; Sikes, 1995).

The energy requirements during mammalian reproduction have received much attention by evolutionary biologists, behavioural ecologists and physiologists. Physiologists are mainly interested in measuring the energetic costs of specific reproductive events such as pregnancy and lactation (Smith and McManus, 1975; Millar, 1977; Bronson, 1985; 1989; Gittleman and Thompson, 1988). A pregnant small mammal requires slightly more energy than a non-reproductive female and this is compensated by a modest increase in oxygen consumption and food intake of 18 to 25% (Millar, 1977). Lactation, on the other hand, is one of the most expensive events of reproduction and most small mammals need to increase their food intake considerably, requiring 66 to 188% more food than non-reproductive females (Smith and McManus, 1975; Millar, 1977; Gittleman and Thompson, 1988). By increasing her food intake, the mother provides enough energy for her own needs and enough milk to maintain rapid growth of her young (Lee *et al.*, 1987). Therefore, one would expect litter size to significantly affect the energy required by the mother (Millar, 1977; Bronson, 1985; 1989; Gittleman and Thompson, 1988; Sikes, 1995).

Of the environmental factors, food is the source of energy required by small mammals for reproduction and most small mammals will adjust their litter size according to the amount of food available to them when out foraging (Rivers and Crawford, 1974; McClure, 1981; Sikes, 1995; Bazhan *et al.*, 1996). Food deprivation is known to affect litter size by impacting on ovulation rate (Beer *et al.*, 1957; Bronson and Marsteller, 1985) implantation, fetal resorption, birth weights of pups, mortality of young after parturition and finally growth of young (Smith and McManus, 1975; Krackow and Hoeck, 1989; Krackow, 1992; Westlin *et al.*, 1995). Food availability is critical as the mother needs to find enough food to sustain herself and the pups and as larger litters may remain with the mother for longer, this increases the interval between litters, the demand for food and the possibility of predation on both mother and young (Millar, 1977; Gittleman and Thompson, 1988; Bronson, 1989; Heideman and Bronson, 1992).

The physiological factors that affect litter size are mainly concerned with the mother, her body size and condition, although the size and development (altricial and precocial) of the young is also important. Body size of the mother is important as it limits the number of young she can carry so as not to be hampered by the fetal mass. The size of the young are important as small litters tend to have larger pups that mature more rapidly after parturition than the smaller pups of large litters (Eisenberg, 1981; Bronson, 1989). The juvenile mortality rate of larger litters is higher than in smaller litters and fewer young are weaned from larger litters overall thus removing the advantages of a larger litter, unless the mother can sustain the larger litter (Cameron, 1973; Eisenberg, 1981; Glazier, 1985; Bronson, 1989; Sikes, 1995).

The pouched mouse has a highly variable litter size (Smithers, 1971: mean = 7.4, range = 5 to 10; Smithers and Wilson, 1979: mean = 6.7, range = 1 to 10; Earl, 1980: mean = 4.9, range = 2 to 8 pups; Westlin-van Aarde, 1988: young primiparous females mean = 7.1, range = 3 to 13 and adult multiparous females mean = 7.9, range = 3 to 11). Although the number of young in some small mammals increases with parity (Perrin, 1986; Dobson and

Myers, 1989; Read and Harvey, 1989; Huck *et al.*, 1990), this is not the case in pouched mice as litter size of primiparous females was not significantly different from that of adult multiparous females (Westlin van-Aarde, 1988). Perrin and Clarke (1987) reported that although the respiratory rate (a proportionate measure of energy expenditure) of one pouched mouse increased by 55 % during pregnancy and 110% during lactation, which was significantly greater than those for non-reproducing pouched mice, the mouse did not increase the intake of food above that of a non-reproducing mouse. The pouched mouse probably compensates for the elevated energy requirements of lactation by utilizing fat and protein stores (Perrin and Clarke, 1987; Speakman *et al.*, 1992).

The ovary of the pouched mouse accumulates corpora lutea (CL) and thus luteal bodies in different developmental stages from a number of previous ovulations are present (Westlin-van Aarde, 1989). Westlin-van Aarde (1989) also found that in mid pregnancy (Day 13 - 15) several sets of active CL were found in the ovaries. By Day 19 of pregnancy, two days prior to parturition, the CL from earlier ovulations had regressed leaving only the set that formed after conception and thus these CL would represent those of one ovulation. The CL are dark pink structures, larger than CL from earlier ovulations, and thus at about Day 19 it is possible to count the number of CL, and therefore ovulations (Westlin-van Aarde, 1989).

Due to the energy expense of reproduction, especially during lactation, a female producing more than one litter in a reproductive season will deteriorate physiologically and this could lead to not only a reduction in litter size at birth but also to a manipulation of the sex ratio of these litters. Ideally production of male and female progeny (of an individual and subsequently the population) should conform to a 50:50 sex ratio as the production of a sex ratio biased towards one or the other sex could lead to lowered fitness of the individual (Fisher, 1930; Huck *et al.*, 1990). Clutton-Brock and Iason (1986) reported that few nonhuman mammals showed significant trends of sex ratio, skewed towards one sex, at birth and maternal parity throughout the female's life. Trivers and Willard (1973) predicted that females in poor condition would produce smaller female-biased litters resulting from differential male mortality while litters of females in good condition would be male-biased. Females in poor condition would have a higher probability of producing a weaker son and thus female offspring would have a greater chance of reproducing as an adult than a male. Subsequent studies, however, refute the "condition" hypothesis of Trivers and Willard (1973) (Myers, 1978; Burley, 1982; Clutton-Brock and Iason, 1986). Although deviations of the sex ratio both antenatally and postnatally prior to weaning have been found when females are physiologically stressed these can be explained in other ways (Myers, 1978; Burley, 1982; Clutton-Brock and Iason, 1986; Krackow, 1997).

The reproductive success of male and female offspring may differ as one sex may be energetically more costly to rear and this could influence the fitness of the mother. Parents may therefore invest in the sex that has the greater probability of reproductive success (Trivers and Willard, 1973; Clutton-Brock and Iason, 1986; Armitage, 1987; Wright *et al.*, 1995). Myers (1978) suggests that in vertebrate populations sexual dimorphism is frequently found in the polygynous species where the male is often, although not always, larger than the female of the species. Males are more energetically expensive to rear as they have a higher growth rate and take longer to grow than their female siblings and therefore have greater energy requirements during development (Trivers and Willard, 1973; Myers, 1978; Clutton-Brock and Iason, 1986).

Many factors have been found to adjust the sex ratio of litters such as litter size (McClure, 1981; Myers *et al.*, 1985; Huck *et al.*, 1990), food quality and availability (Trivers and Willard, 1973; Rivers and Crawford, 1974; Myers, 1978; McClure, 1981; Clutton-Brock *et al.*, 1985; Meikle and Drickamer, 1986; Bazhan *et al.*, 1996), social influences (Pratt and Lisk, 1989), hormonal influences (Hahn and Hays, 1963; Pratt and Lisk, 1991; Gorman *et al.*, 1994; James, 1996), age and parity of mothers (Huck *et al.*, 1988; Krackow and Hoeck, 1989; Krackow, 1992), climatic factors (Myers *et al.*, 1985), as well as the time of mating within the oestrous cycle (Huck *et al.*, 1990; Hornig and McClintock, 1994; James, 1996, Havelka and Millar, 1997).

The aims of this chapter were firstly to determine whether short daylength affected ovulation rate and, or litter size, and particularly whether short daylength (which may be a cue for less favorable conditions) would result in a reduced ovulation rate and in litter size. Secondly, whether daylength affected the sex ratio of the litter. And thirdly, whether the selection for larger body size during domestication would affect ovulation rate, litter size or sex ratio.

## 5.2 The effect of photoperiod on the litter size of domesticated and wild-caught female pouched mice

### 5.2.1 Materials and Methods

#### *5.2.1.1 The effect of photoperiod on the litter size of domesticated female pouched mice*

*Group 1:-* *Litter size data collected throughout the entire study period*

*Animals:*

- 37 adult domesticated female pouched mice (between 102 and 459 days of age)
    - 12 nulliparous females conceived, born and reared under long day conditions (14L : 10D)
    - 11 nulliparous females conceived, born and reared under short day conditions (10L : 14D)
    - 14 females of unknown parity or birth photoperiod or age

*Experimental design:*

A group of 25 females (consisting of 12 nulliparous females conceived, born and reared under long day conditions and 13 females of unknown parity or age) were mated first under long day conditions with the resultant litters being born and bred under the same photoperiod. Once the litters were weaned the original male and female parental pairs were then moved to short day conditions, acclimated for four weeks and allowed to raise a second litter.

A further group of 12 females, consisting of 11 nulliparous females conceived, born and reared under short day conditions and 1 female of unknown parity or age, were mated first under short day conditions with the resultant litters being born and raised in the same photoperiod. The male and female pairs were then moved to long day conditions, acclimated for four weeks and allowed to raise a second litter, which was reared under the conditions in which they were conceived.

*Group 2:-* *Experimental data*

*Animals:*

- 12 adult, nulliparous domesticated female pouched mice (between 166 and 232 days of age)
    - 6 females conceived, born and reared under long day conditions (14L : 10D)
    - 6 females conceived, born and reared under short day conditions (10L : 14D)

*Experimental design:*

In Group 2 there was far less experimental variation in the age of the mice than in Group 1 and this experiment was designed to remove the possible effect that age could have. It has been found in some small mammals that the age of females affects the litter size. Both young females (*i.e.* females that have just reached maturity) and old females (> 730 days) may give birth to less young than a female in her prime (Perrin, 1986; Dobson and Myers, 1989; Read and Harvey, 1989; Huck *et al.*, 1990).

A group of 6 nulliparous domesticated female pouched mice were mated under long day conditions and a further 6 females under short day conditions. The size of the resultant litters was recorded at parturition.

Once the litters were weaned the male and female pairs in long daylength were moved to short daylength and the males and females in short daylength were moved to long daylength. After four weeks of acclimation the females were mated with the same male and a record again made of the size of the resultant litters at parturition.

### *5.2.1.2 The effect of photoperiod on litter size of wild-caught and F1-generation female pouched mice*

*Animals:*

- 4 wild-caught females of unknown parity or age
- 3 adult nulliparous F1-generation female pouched mice conceived, born and bred under short day conditions (between 191 and 437 days of age)

*Experimental design:*

The wild-caught mice were allowed to mate in order to provide an F1-generation large enough to provide males and females for all the experiments carried out to establish the effect of domestication. In doing so the 4 wild-caught female pouched mice were mated as shown in Table 5.1 with the resultant litters being raised under the same photoperiods. Once a litter was weaned the females were either mated again or moved to the alternate daylength and after a four-week acclimation period, mated again with the same males.

**Table 5.1** Successful mating of wild-caught females used throughout the entire study.

| Females | Litter Number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | LD | LD | SD | SD | SD | LD |
| 2 | SD | SD | LD | | | |
| 3 | LD | SD | SD | LD | | |
| 4 | LD | LD | SD | SD | SD | |

LD = long daylength (14L : 10D)
SD = short daylength (10L : 14D)

A further group of 3 F1-generation adult nulliparous females were mated under short day conditions and the resultant litters were reared under the same photoperiod. Once the litters were weaned the females and males were moved to long daylength, acclimated for four weeks and mated again. The sizes of all resultant litters at parturition were recorded.

### *5.2.1.3 Data Analyses*

*Domesticated pouched mice:*

A two-way ANCOVA was carried out to determine the effect of photoperiod and parity on litter size of domesticated pouched mice using body mass as the covariate in the analysis to control for any possible effect body mass may have on litter size (Underwood, 1997).

*Wild-caught pouched mice:*

The very small sample size for the wild-caught animals precluded an analysis of the effect of parity and a one-way ANCOVA was carried out to determine whether photoperiod affected litter size. Body mass was again used as the covariate.

*Comparison of domesticated and wild-caught pouched mice:*

The effect of domestication on litter size was determined using a two-way ANOVA, taking into account the effect of photoperiod on litter size as well. Only data from Group 1 domesticated females were compared to the wild-caught and F1-generation females. The data were not normally distributed and as the log-transformation of the data did not change the distribution of the data substantially, the two-way ANOVA was carried out on the raw data (Underwood, 1997). The data in Group 1 included the Group 2 data.

## 5.2.2 Results

### *5.2.2.1 The effect of photoperiod on the litter size of domesticated female pouched mice*

**Table 5.2 and Figure 5.1**

Litter size of domesticated female pouched mice was not significantly affected by either photoperiod (Group1: $p > 0.05$, $F = 3.4$; Group 2: $p > 0.05$, $F = 0.1$) or parity (Group 1: $p > 0.05$, $F = 0.3$; Group 2: $p > 0.05$, $F = 0.8$). All interactions were non-significant (Group 1: $p > 0.05$, $F = 0.4$; Group 2: $p > 0.05$, $F = 0.9$). There was, however, a trend for smaller litters in short daylength in both Group 1 and 2 females (Table 5.2; Figure 5.1a). Figure 5.1b shows the difference in litter size with changing photoperiod where SD litter size was subtracted from the LD litter size, of a particular female. In this way it was found that 23 of the 37 Group 1 females had larger litters and 9 had smaller litters under long day conditions, while 5 females had the same number of young in both photoperiods. Litter sizes ranged from 2 to 11 young per litter under long daylength and 1 to 10 under short daylength.

**Table 5.2** The means ± standard deviations for litter size of adult domesticated and F1-generation female pouched mice at weaning in long and short daylength.

| Treatment | n | Domesticated Group 1 | n | Domesticated Group2 | n | F1-generation |
|---|---|---|---|---|---|---|
| LD | 37 | 7.08 ± 2.3 | 12 | 7.42 ± 1.8 | 11 | 5.73 ± 1.8 |
| SD | 37 | 5.97 ± 2.5 | 12 | 5.42 ± 3.1 | 13 | 4.46 ± 1.8 |
| Significance | | NS | | NS | | NS |

LD = long daylength; SD = short daylength; n = number of litters born and bred.
NS = non-significant; * = $p < 0.05$; ** = $p < 0.01$.

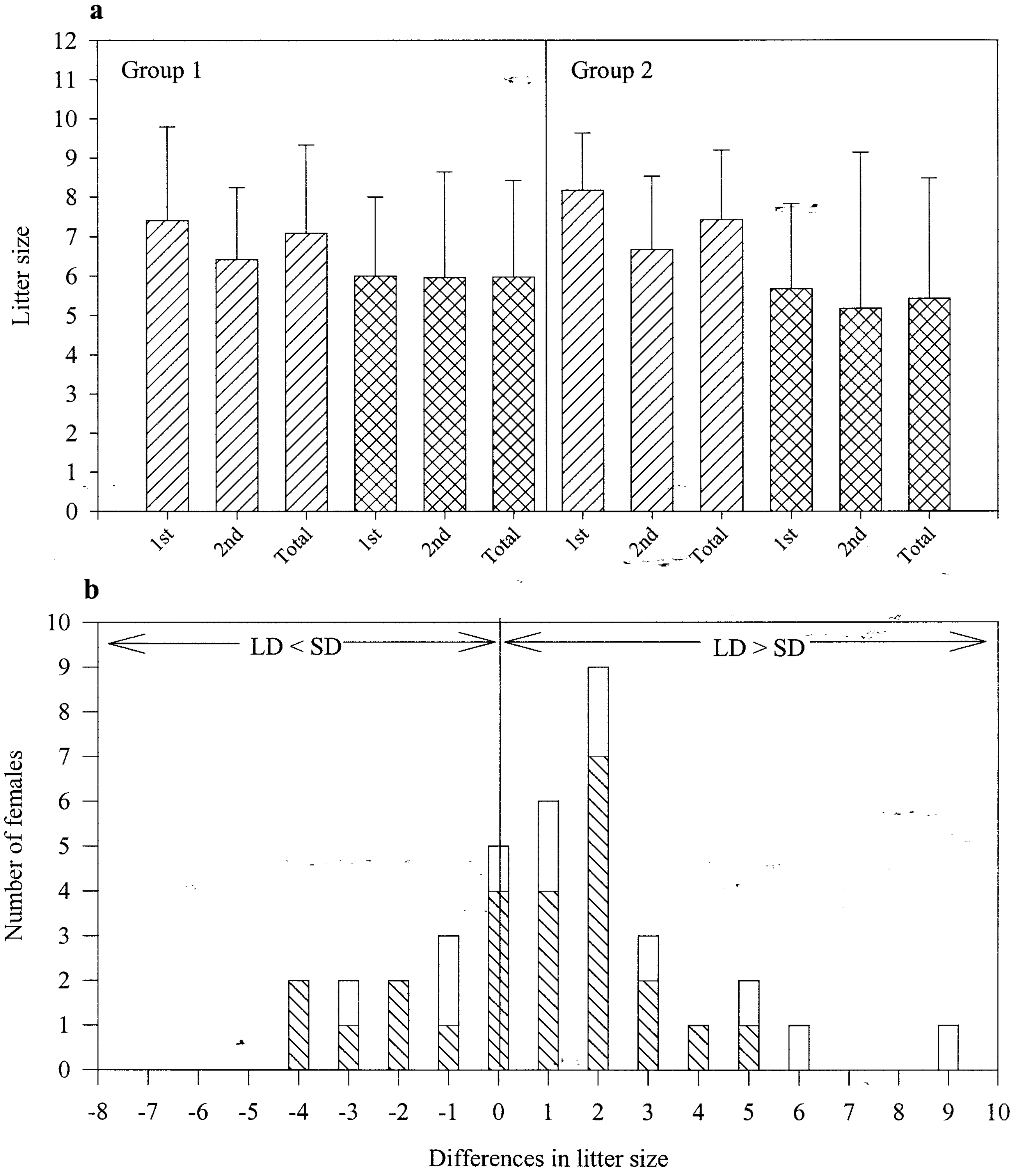

**Figure 5.1** a) Litter order for Group 1 and Group 2 domesticated female pouched mice in both long and short daylength. Standard deviations given for all mean values. b) Differences in litter size in Group 1 and Group 2 domesticated females.

Of the 12 Group 2 females, 8 had larger litters and 3 females had smaller litters in long daylength while 1 female had the same number of young in both photoperiods. Litters ranged from a minimum of 4 young to a maximum litter size of 10 young in long daylength, while in short daylength the range in litter size was from 1 to a maximum of 10 young at parturition.

#### *5.2.2.2 The effect of photoperiod on the litter size of wild-caught and F1-generation female pouched mice*

**Table 5.2 and Figure 5.2**

Eleven litters were successfully raised in long daylength and 13 litters in short daylength. Litter size of the wild-caught and F1-generation female pouched mice at parturition was not affected by photoperiod ($p > 0.05$, $F = 3.2$) (Table 5.2; Figure 5.2a). As the wild-caught females had more than one litter in each photoperiod the litters in a particular photoperiod were averaged before calculating the difference in litter sizes of a female between long and short daylengths. Three of the 7 females had larger litters and 2 had smaller litters in long daylength while 2 had the same size litters in both photoperiods (Figure 5.2b). Litters in long daylength ranged between 4 and 9 young while the litters in short daylength ranged between 1 and 7 young.

An analysis of the effect of parity on litter size could not be carried out as the females had different numbers of litters.

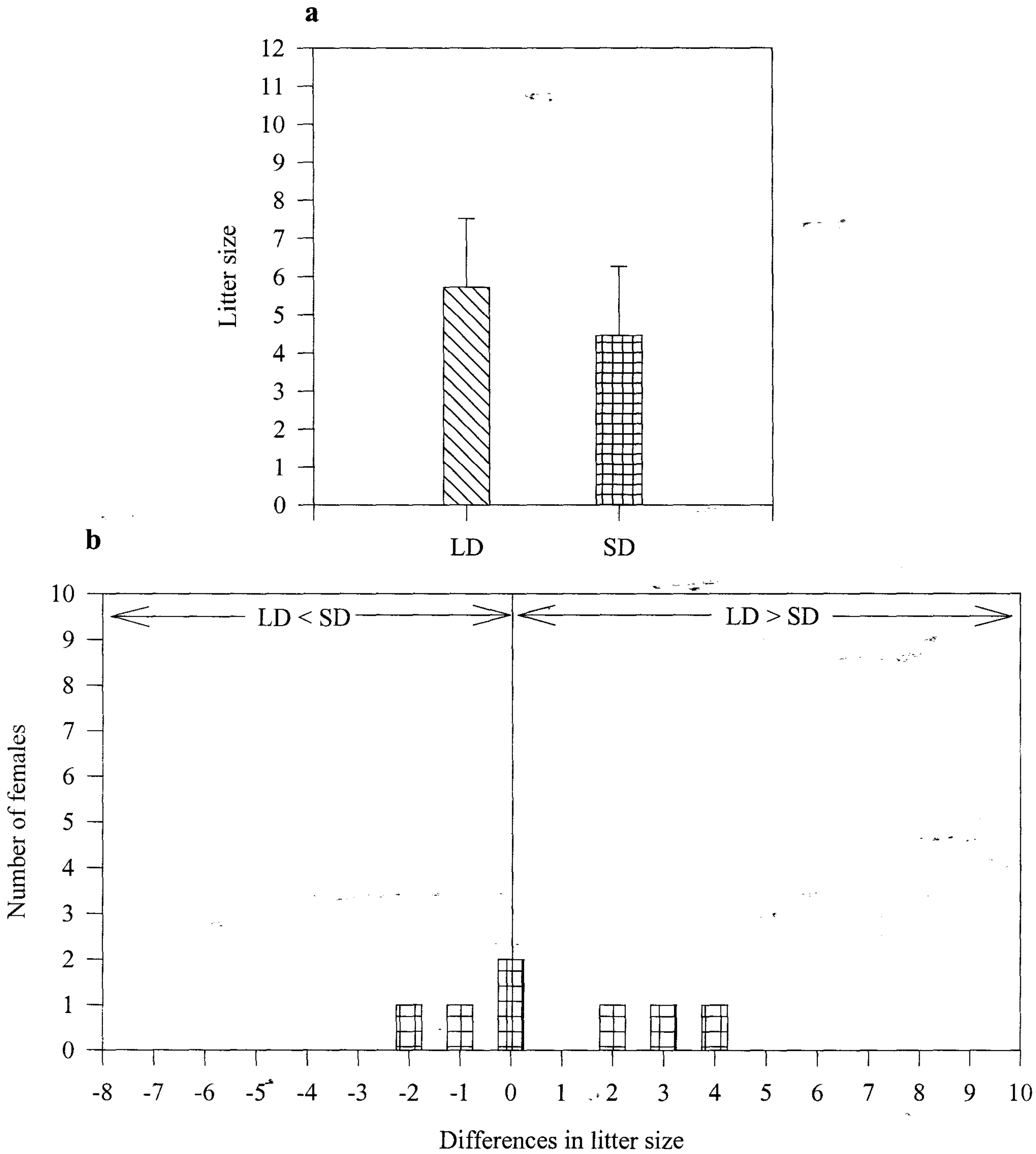

**Figure 5.2** a) Mean ± standard deviation for litter size in wild-caught and F1-generation female pouched mice in both long and short daylengths. b) Differences in litter size in wild-caught and F1-generation females.

#### *5.2.2.3 The effect of domestication and photoperiod on the litter size of female pouched mice*

**Table 5.2**

In this section, only data from Group 1 domesticated female pouched mice were compared to that of the wild-caught and F1-generation female pouched mice. A two-way ANOVA carried out to determine the effect of photoperiod and domestication on litter size indicated that both photoperiod and domestication had a significant effect on litter size (photoperiod: $p < 0.05$, $F = 5.1$; domestication: $p < 0.01$, $F = 7.4$). Domesticated females in long daylength had significantly larger litters than the domesticated and wild-caught females in short daylength (Table 5.2). All interactions were found to be non-significant ($p > 0.05$, $F = 0.02$).

## 5.3 The effect of photoperiod on the ovulation rate of domesticated and wild-caught female pouched mice

### 5.3.1 Materials and Methods

#### *5.3.1.1 The effect of photoperiod on the ovulation rate of domesticated female pouched mice*

*The number of ova within the oviduct*

*Animals:*

- 16 adult nulliparous domesticated female pouched mice (between 266 and 522 days of age)
    - 8 females conceived, born and reared under LD conditions (14L : 10D)
    - 8 females conceived, born and reared under SD conditions (10L : 14D)

*Experimental design:*

A group of 8 adult domesticated female pouched mice were monitored under long day conditions and a second group of 8 females under short day conditions by taking vaginal smears daily at approximately 08H00. Once a typical four-day oestrous cycle was observed the females were sacrificed on the morning of oestrus between 08H30 and 09H30.

The ovaries, oviducts and a small piece of the uterus were removed. The oviduct was placed in Dulbecco's modified Eagle's medium (DMEM) and separated from the ovarian bursa and the mesosalpinx, straightened and placed on a wax-spot slide with a small amount of DMEM under a Weiss dissecting microscope. A coverslip was placed on the wax-spot slide and gently depressed, in this way the oviduct was compressed thus making it easier to distinguish the ova under light microscopy using a Nikon binocular compound microscope (Figure 5.3) (Bedford *et al.*, 1997). The number of ova in both left and right oviducts were noted. The ovaries were examined macroscopically for new corpora lutea (CL), which were distinguishable from the older CL as they were larger and dark pink structures. It was possible, therefore, to count the number of CL in both ovaries and therefore estimate the number of ova ovulated (Westlin-van Aarde, 1988; 1989).

*Number of corpora lutea in late pregnancy*

*Animals:*

- 15 adult nulliparous domesticated female pouched mice (between 232 and 557 days of age)
    - 9 adult females conceived, born and bred under long day conditions (14L : 10D)
    - 6 adult females conceived, born and bred under short day conditions (10L : 14D)

*Experimental design:*

A group of 9 domesticated female pouched mice were mated under long day conditions and a further 6 females under short day conditions. Once pregnancy was confirmed, females were sacrificed on Day 19 or 20 of pregnancy, taking the day after mating as Day 1. Gestation length is normally approximately 21 days (Earl, 1980; Westlin-van Aarde, 1988; Skinner and Smithers, 1990). The ovaries were removed, weighed and examined macroscopically for CL representing the last ovulation. The number of implanted ova was taken as the sum of viable fetuses (fetuses attached firmly to the placenta), and the number of resorbing fetuses. The expected litter size was taken as the number of viable fetuses found within the uterine horns.

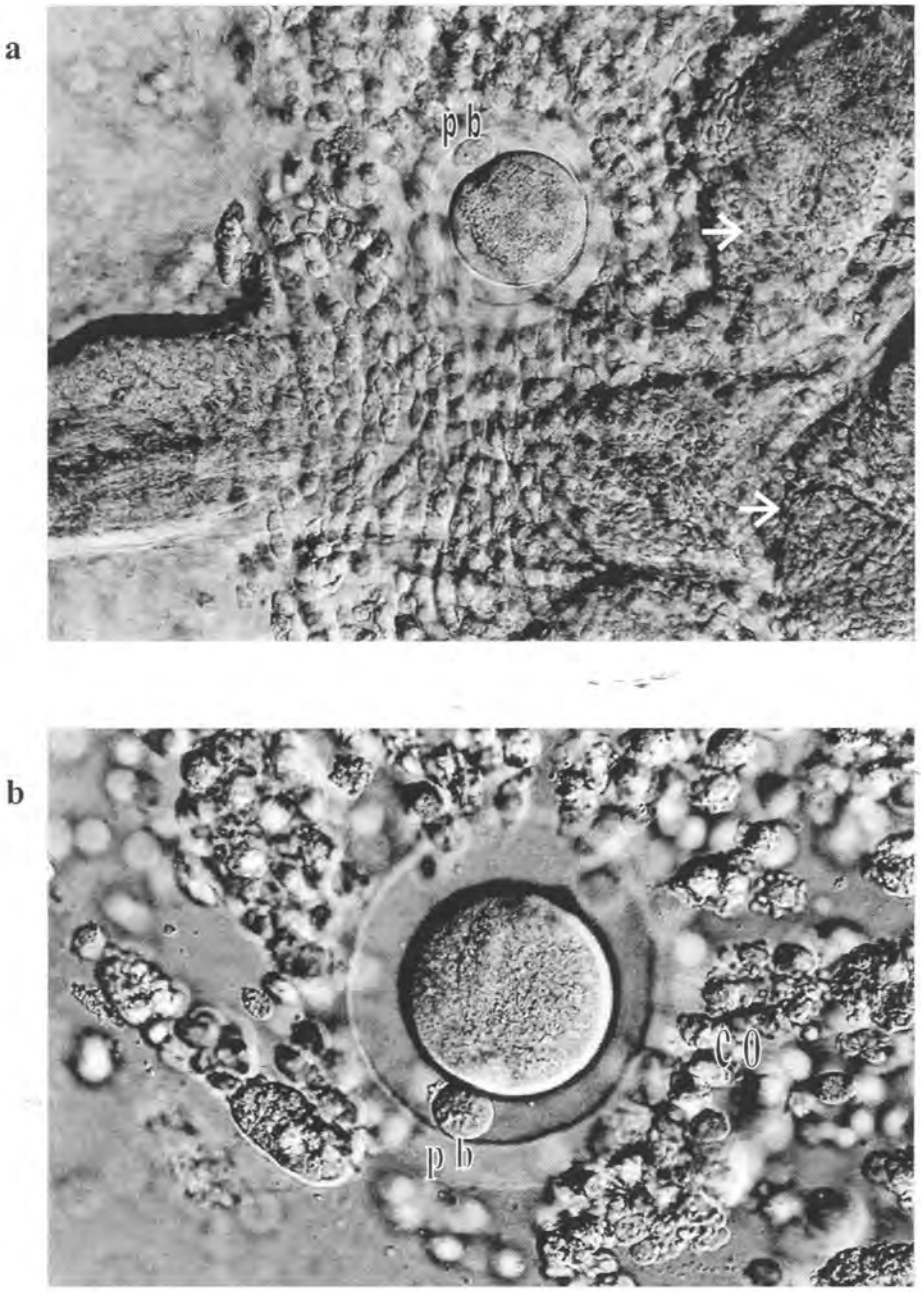

**Figure 5.3** Unfertilised ova in the oviducts of female pouched mice showing a) the ova, polar body (pb) and ciliated wall of the oviduct (→) (x200 magnification), and b) the ova, polar body (pb) and the cells of the cumulus oophorus (co) (x400 magnification).

### *5.3.1.2 The effect of photoperiod on the ovulation rate of F1-generation female pouched mice*

*Animals:*

- 9 adult nulliparous F1 female pouched mice conceived, born and reared under SD conditions (10L : 14D) (between 148 and 450 days of age)

*Experimental design:*

As insufficient F1-generation female pouched mice had been born and reared under long daylength a group of 4 adult F1-generation females were moved from short to long daylength and acclimatised for four weeks. After the acclimation period, the 4 F1-generation females pouched mice under long day conditions as well as a second group of 5 females under short day conditions were all monitored by taking vaginal smears daily at approximately 08H00. As with the domesticated female pouched mice, the F1-generation females were monitored until a typical four-day oestrous cycle was observed and then the females were sacrificed on the morning of oestrus between 09H00 and 09H30. It had been established previously that the ova of F1-generation females took longer to reach the ampulla than in domesticated females (Pers. obs.). Thus to make sure all the ova were harvested the procedure was carried out at a later time in the F1-generation female pouched mice.

The ovaries, oviducts and a piece of the uterus were removed under a dissecting microscope and treated in the same way as for the domesticated females. The number of ova within both left and right oviducts and the number of CL in each ovary were noted, and the ovaries were weighed.

#### *5.3.1.3 Data Analyses*

Possible correlations between the reproductive factors examined (body mass, ovarian mass, number of CL, number of implantation sites, litter size and number of ova) were tested using Pearson's correlation coefficient. T-tests were carried out to determine the effect of photoperiod on the different parameters measured if they were not correlated to body mass otherwise a one-way ANCOVA was carried out (Underwood, 1997). On failure of normality or homogeneity the non-parametric test was used.

In order to determine the effect of domestication on the different reproductive factors examined, a two-way ANOVA was carried out taking into account the effect of photoperiod between the domesticated and F1-generation female pouched mice.

### 5.3.2 Results

#### *5.3.2.1 The effect of photoperiod on the ovulation rate of domesticated female pouched mice*

*Number of ova within the oviduct*

**Table 5.3, 5.4, and Figure 5.4**

The number of CL found in the ovaries was significantly correlated with the number of ova within the oviducts ($r = 0.6$, $n = 16$, $p < 0.05$) (Table 5.4). Photoperiod did not have a significant effect on either the total number of ova found within the oviducts ($p > 0.05$, $t = 1.0$) or the total number of CL in the ovaries ($p > 0.05$, $t = 0.4$) (Table 5.3; Figure 5.4).

**Table 5.3** The mean ± standard deviations of body mass, the number of corpora lutea, the number of ova and the paired ovarian mass of domesticated female pouched mice in both long and short day photoperiods.

| Treatment | LD (n = 8) | SD (n = 8) | Significance |
|---|---|---|---|
| Body mass (g) | 92.17 ± 6.6 | 104.02 ± 14.4 | * |
| Number of corpora lutea | 9.63 ± 0.9 | 9.38 ± 1.6 | NS |
| Number of ova | 8.63 ± 0.9 | 7.75 ± 2.4 | NS |
| Ovarian mass (g) | 0.12 ± 0.04 | 0.16 ± 0.06 | NS |

LD = long daylength (14L : 10D), SD = short daylength (10L : 14D)
n = sample size
NS = non-significant; * = $p < 0.05$

**Table 5.4** Pearson's correlation coefficients of body mass and ovarian mass, number of CL, number of ova within the oviducts of domesticated female pouched mice.

| Reproductive factors | Sample correlations | | |
|---|---|---|---|
| | Number of CL | Number of ova | Ovarian mass |
| Number of ova | r = 0.59<br>(n = 16)<br>* | | |
| Ovarian mass (g) | r = 0.31<br>(n = 13)<br>NS | r = - 0.02<br>(n = 13)<br>NS | |
| Body mass (g) | r = 0.45<br>(n = 16)<br>NS | r = - 0.05<br>(n = 16)<br>NS | r = 0.49<br>(n = 16)<br>NS |

Coefficient = r
n = sample size
NS = non-significant; * = $p < 0.05$

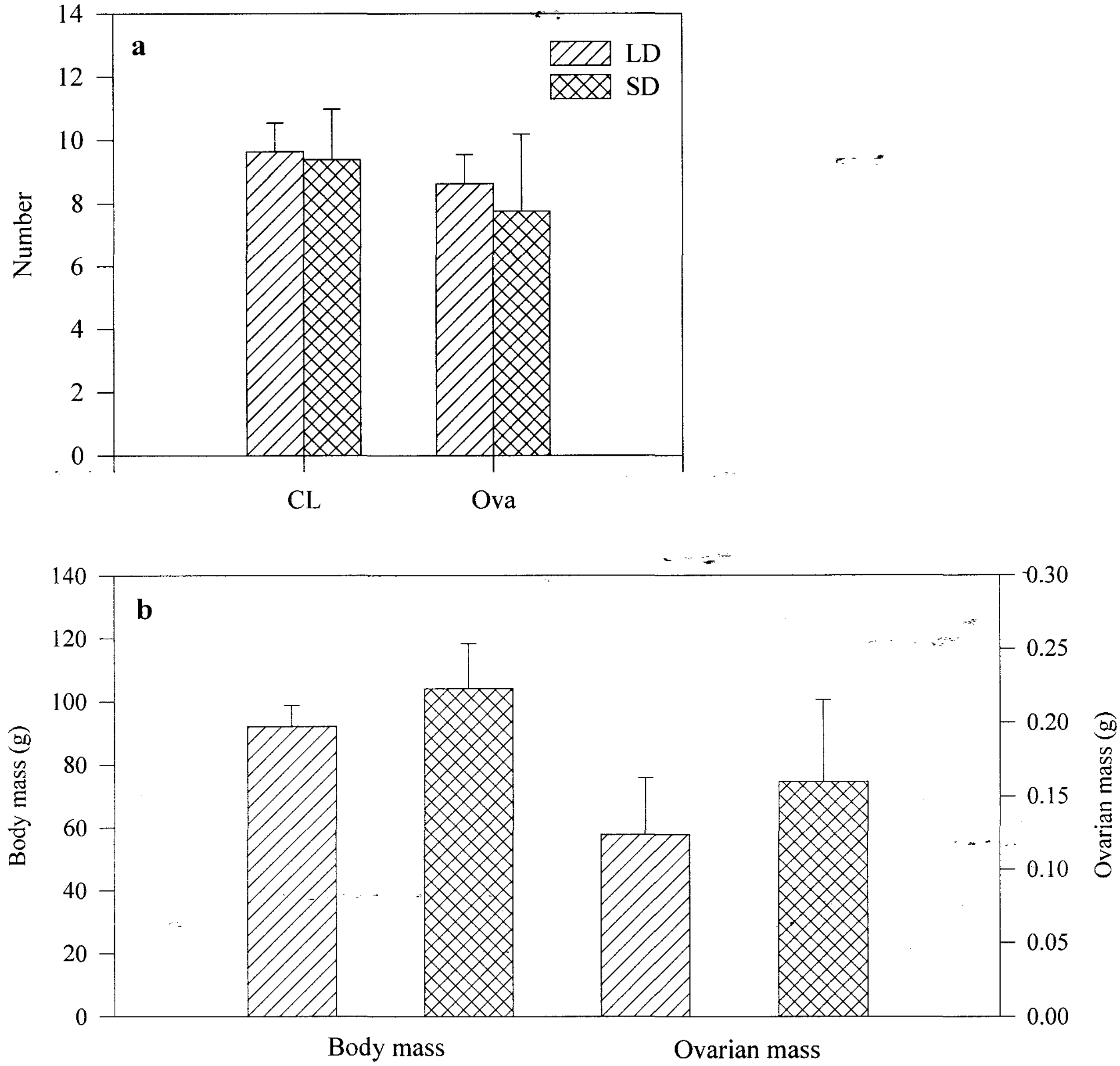

**Figure 5.4** Mean ± standard deviation of a) number of corpora lutea and number of ova, and b) body mass and ovarian mass of domesticated female pouched mice in both long and short daylengths. * = $p < 0.05$

*Number of CL at late pregnancy*

**Table 5.5, 5.6, and Figure 5.5**

Correlations were found between the number of implanted ova and the expected litter size ($r = 0.82$, $p < 0.001$), between the number of CL and the ovarian mass ($r = 0.65$, $p < 0.01$), and between the ovarian mass and body mass ($r = 0.53$, $p < 0.05$) of the domesticated female pouched mice (Table 5.6).

Photoperiod did not significantly affect the total number of CL found in both ovaries ($p > 0.05$; $t = -0.3$); the number of implanted ova ($p > 0.05$; $t = 1.1$) or the expected litter size ($p > 0.05$; $T = 34.0$) (Table 5.5; Figure 5.5).

**Table 5.5** The mean ± standard deviations for body mass, number of corpora lutea found in the ovaries and paired ovarian mass of females sacrificed on Day 19 of gestation in both long and short day photoperiods.

| Treatment | LD (n = 9) | SD (n = 6) | Significance |
|---|---|---|---|
| Body mass (g) | 120.92 ± 18.8 | 127.83 ± 5.0 | NS |
| Number of corpora lutea | 10.67 ± 1.9 | 11.00 ± 2.4 | NS |
| Number of implanted ova | 8.67 ± 1.9 | 7.33 ± 2.9 | NS |
| Expected litter size | 8.00 ± 2.1 | 4.83 ± 3.7 | NS |
| Ovarian mass (g) | 0.2 ± 0.04 | 0.2 ± 0.04 | NS |

LD = long daylength (14L: 10D); SD = short daylength (10L: 14D)
n = sample size
NS = non-significant

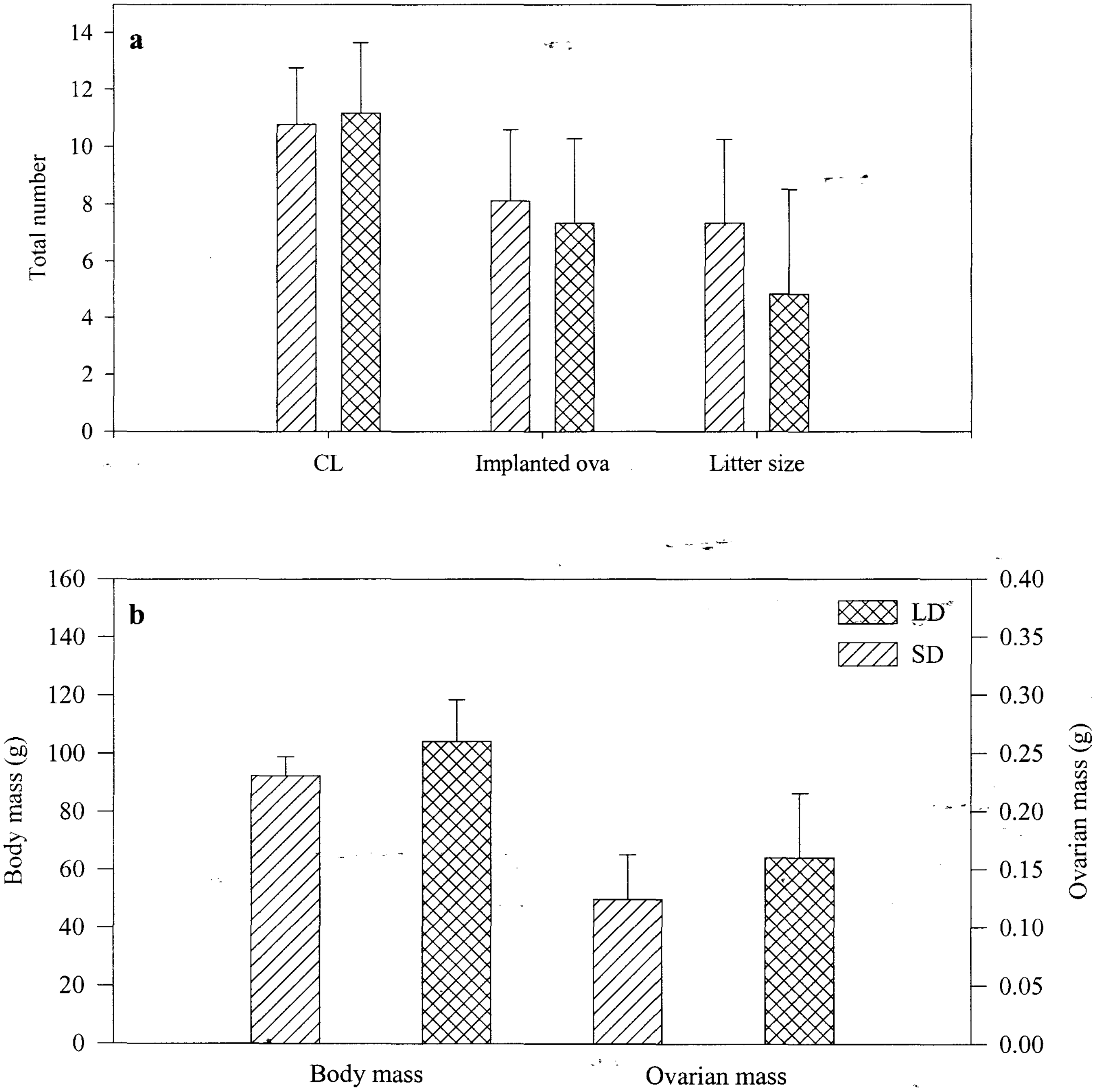

**Figure 5.5** Mean ± standard deviation of a) number of corpora lutea, implanted ova and expected litter size, and b) body mass and ovarian mass of domesticated female pouched mice in both long and short daylengths.

**Table 5.6** Pearson's correlation coefficients of body mass and ovarian mass, number of CL, number of implanted ova and the expected litter size of domesticated female pouched mice at Day 19 of pregnancy.

| Reproductive factors | Sample correlations | | | |
|---|---|---|---|---|
| | No. of CL | No. of implanted ova | Litter size | Ovarian mass |
| No. of implanted ova | r = 0.46<br>(n = 15)<br>NS | | | |
| Expected litter size | r = 0.28<br>(n = 15)<br>NS | r = 0.82<br>(n = 15)<br>** | | |
| Ovarian mass (g) | r = 0.65<br>(n = 15)<br>** | r = 0.49<br>(n = 15)<br>NS | r = 0.31<br>(n = 15)<br>NS | |
| Body mass (g) | r = 0.37<br>(n = 15)<br>NS | r = 0.19<br>(n = 15)<br>NS | r = -0.01<br>(n = 15)<br>NS | r = 53<br>(n = 15)<br>* |

Coefficient = r; n = sample size
NS = non-significant; * = $p < 0.05$; ** = $p < 0.01$

Photoperiod did not significantly affect body mass or ovarian mass of females ($p > 0.05$, T = 53.0; $p > 0.05$, t = 0.3 respectively) (Table 5.5; Figure 5.5).

### ***5.3.2.2 The effect of photoperiod on the ovulation rate of F1-generation female pouched mice.***

**Table 5.7, 5.8, and Figure 5.6**

The number of CL found in the ovaries was significantly correlated with the number of ova within the oviducts of F1-generation female pouched mice (r = 0.95, $p < 0.001$). No other correlations were significant (Table 5.7).

The total number of ova in the oviducts of the LD F1-generation females was not significantly different from those within the oviducts of the SD F1-generation females (p >

0.05, T = 18.5) (Table 5.8; Figure 5.6). Similarly the total number of CL in both ovaries of LD F1-generation females was not significantly different from the F1-generation females in short daylength (p > 0.05, T = 20.0) (Table 5.8; Figure 5.6).

**Table 5.7** Pearson's correlation coefficients of body mass ovarian mass, number of CL, number of ova within the oviducts of F1-generation females of wild-caught pouched mice.

| Reproductive factors | Sample correlations | | |
|---|---|---|---|
| | Number of CL | Number of ova | Ovarian mass |
| Number of ova | r = 0.95<br>(n = 9)<br>** | | |
| Ovarian mass (g) | r = 0.007<br>(n = 9)<br>NS | r = 0.09<br>(n = 9)<br>NS | |
| Body mass (g) | r = -0.55<br>(n = 9)<br>NS | r = -0.54<br>(n = 9)<br>NS | r = 0.65<br>(n = 9)<br>NS |

Coefficient = r
n = sample size
NS = non-significant; * = p< 0.05; ** = p < 0.01

**Table 5.8** The mean ± standard deviations of body mass, the number of corpora lutea, the number of ova and the paired ovarian mass of F1-generation female pouched mice in both long and short day photoperiods at ovulation.

| Treatment | LD (n = 4) | SD (n = 5) | Significance |
|---|---|---|---|
| Body mass (g) | 73.76 ± 8.0 | 70.14 ± 1.4 | NS |
| Number of corpora lutea | 5.75 ± 1.3 | 5.80 ± 1.1 | NS |
| Number of ova | 5.75 ± 1.3 | 6.00 ± 1.02 | NS |
| Ovarian mass (g) | 0.05 ± 0.2 | 0.05 ± 0.02 | NS |

LD = long daylength (14L : 10D), SD = short daylength (10L : 14D)
n = sample size; NS = non-significant

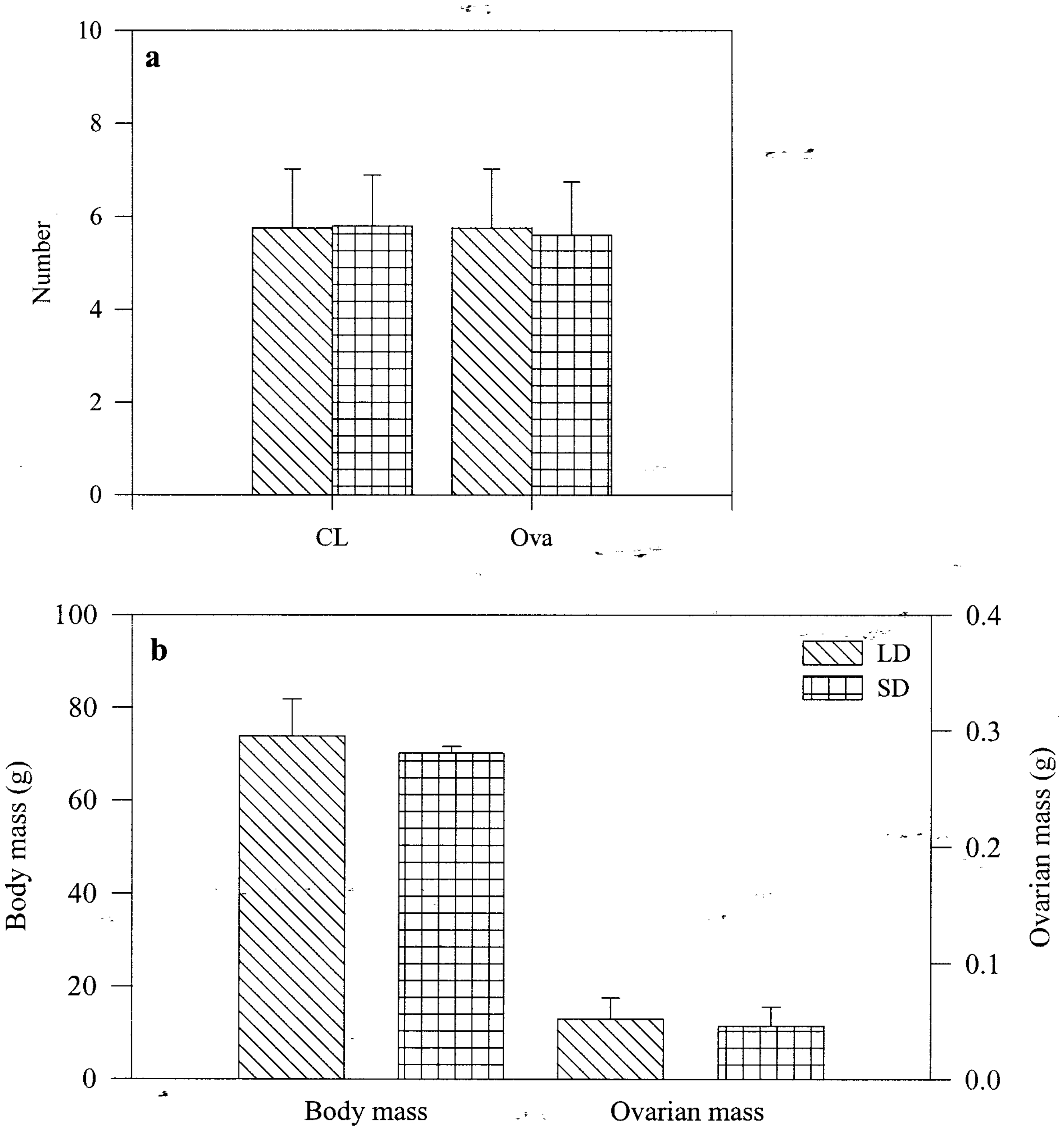

**Figure 5.6** Mean ± standard deviation of a) number of CL and number of ova, and b) body mass and ovarian mass of F1-generation female pouched mice in both long and short daylengths at ovulation.

Body mass and ovarian mass were not significantly affected by photoperiod ($p > 0.05$, $t = 1.0$ and $p > 0.05$, $t = 0.5$ respectively) although LD F1-generation females tended to be slightly heavier than SD F1-generation females (Table 5.8; Figure 5.6).

### *5.3.2.3 The effect of domestication and photoperiod on the ovulation rate of female pouched mice.*

Domestication had a significant effect on the total number of CL found in the ovaries ($p < 0.0001$, $F = 50.0$) as the domesticated females had significantly more CL present in their ovaries than the F1-generation females in both photoperiods at ovulation. The interaction between domestication and photoperiod was not significant ($p > 0.05$, $F = 0.08$).

The number of ova in the oviducts was also significantly higher in the domesticated females than in the oviducts of the F1-generation females ($p < 0.01$, $F = 13.2$) and the domesticated females in long daylength had significantly more ova in their oviducts than the F1-generation females in both long and short daylength. The domesticated females in short daylength did not have significantly more ova in their oviducts than the F1-generation females in either photoperiod. All interactions were found to be non-significant ($p > 0.05$, $F = 0.3$).

Domesticated females were significantly heavier than F1-generation females ($p < 0.0001$, $F = 42.1$) and domesticated females in long daylength were significantly lighter than domesticated females in short daylength. Body mass of F1-generation females was not significantly affected by photoperiod. Ovarian mass was also affected significantly by domestication ($p < 0.0001$, $F = 53.8$) as domesticated females had significantly heavier ovaries than F1-generation females. Photoperiod, however, did not affect ovarian mass of the domesticated and F1-generation females ($p > 0.05$, $F = 0.7$). All interactions were non-significant (Body mass: $p > 0.05$, $F = 3.7$; Ovarian mass: $p > 0.05$, $F = 1.6$).

## 5.4 The effect of photoperiod on the sex ratio of domesticated and wild-caught female pouched mice

### 5.4.1 Materials and Methods

#### *5.4.1.1 The effect of photoperiod on sex ratio of domesticated female pouched mice*

*Animals:*

- 37 adult nulliparous domesticated female pouched mice (between 102 and 459 days of age)
  - 12 adult females conceived, born and reared under long day conditions (14L : 10D)
  - 11 adult females conceived, born and reared under short day conditions (10L : 14D)
  - 14 adult females of unknown parity, birth photoperiod or age

*Experimental design:*

This study formed part of Section 5.2.1.1 in which all the litter sizes throughout the four year experimental period were recorded. These data were collected from the results of 25 successful matings of domesticated female pouched mice under long daylength, and included 12 nulliparous females of known age born and reared in long daylength plus 13 females of unknown age, birth photoperiod or parity. Once the resultant litters were weaned the 25 female and male pairs were moved to short daylength, acclimatised for four weeks and allowed to rear a second litter. A further group of 12 domesticated females (11 nulliparous females of known age born and reared under short daylength and one female of unknown parity, birth photoperiod or age) were successfully mated under short daylength and allowed to rear their litters under the same photoperiod. Once the young were weaned the same female and male pairs were moved to long daylength, acclimatised for four weeks and allowed to rear a second litter. The total number of litters was 74 (37 in long daylength and 37 in short daylength).

The sex of the young was assessed at two days of age by visual inspection of the ano-genital distance (in males the ano-genital distance is greater than in females) and, in the case of the males, the presence of a penal protrusion.

Sex ratio = number of male pups / the total number of pups

### *5.4.1.2 The effect of photoperiod on the sex ratio of wild-caught and F1-generation female pouched mice*

*Animals:*

- 4 wild-caught females of unknown parity or age
- 3 adult nulliparous F1-generation female pouched mice born and bred under short day conditions (10L : 14D) (between 191 and 437 days of age)

*Experimental design:*

This section of the study formed part of Section 5.2.1.2 in which wild-caught pouched mice were mated in order to provide enough F1-generation mice for the experiments establishing the effect of domestication on reproduction. A group of 4 wild-caught female pouched mice were mated, as shown in Table 5.1, and allowed to raise the resultant litters in the same photoperiods. Once a litter was weaned the females were either mated again in the same photoperiod or moved to the alternate photoperiod, acclimatised for four weeks and successfully mated with the same male as previously done. A further group of 3 F1-generation adult nulliparous female pouched mice were successfully mated under short day conditions and allowed to raise the resultant litter until weaning. The female and male pairs were then moved to long day conditions, allowed a four week acclimation period and successfully mated again.

The sex of the young was assessed as described previously.

### *5.4.1.3 Data Analyses*

A one-way ANCOVA was carried out to determine the effect of photoperiod on the sex ratio of domesticated and wild-caught pouched mice. As the size of the litters could affect the sex ratio litter size was the co-variate. The sex ratio data were arcsine transformed as the data, for both the domesticated and wild-caught females, were not normally distributed (Underwood, 1997).

In order to discover whether the sex ratio deviated from a 1:1 ratio Chi-squared tests were carried out on the treatments data separately for both domesticated and F1-generation pouched mice.

Finally to determine whether domestication affected sex ratio, a two-way ANOVA was carried out comparing domesticated and F1-generation sex ratios in both long and short daylength. The sex ratio data were arcsine transformed as the data, for both the domesticated and wild-caught females, were not normally distributed (Underwood, 1997).

### 5.4.2 Results

#### *5.4.2.1* ***The effect of photoperiod on the sex ratio of domesticated female pouched mice***

**Table 5.9 and Figure 5.7**

Litter size did not have a significant effect on sex ratio of domesticated female pouched mice ($p > 0.05$; $F = 0.29$) (Figure 5.7). Sex ratio of domesticated females in long daylength was not significantly different from those of females in short daylength ($p > 0.05$; $F = 0.47$) (Table 5.9).

The Chi-squared tests indicated that litters from domesticated females had sex ratios which did not deviate from a 1:1 ratio in either photoperiod (LD: $p > 0.05$, $\chi^2 = 2.0$, DF = 36; SD: $p > 0.05$, $\chi^2 = 3.4$, DF = 36).

**Table 5.9** The mean ± standard deviation of the number of males, females and sex ratios from all the data of domesticated female pouched mice in both long and short daylength (n = 37).

| | LD | SD | Significance |
|---|---|---|---|
| Average litter size | 7.1 ± 2.3 | 6.0 ± 2.5 | NS |
| Total number of males | 3.5 ± 1.4 | 3.0 ± 1.7 | NS |
| Total number of females | 3.3 ± 1.6 | 2.8 ± 1.3 | NS |
| Overall sex ratio | 0.5 ± 0.2 | 0.5 ± 0.2 | NS |

LD = long daylength (14L : 10D); SD = short daylength (10L : 14D)
NS = non significant

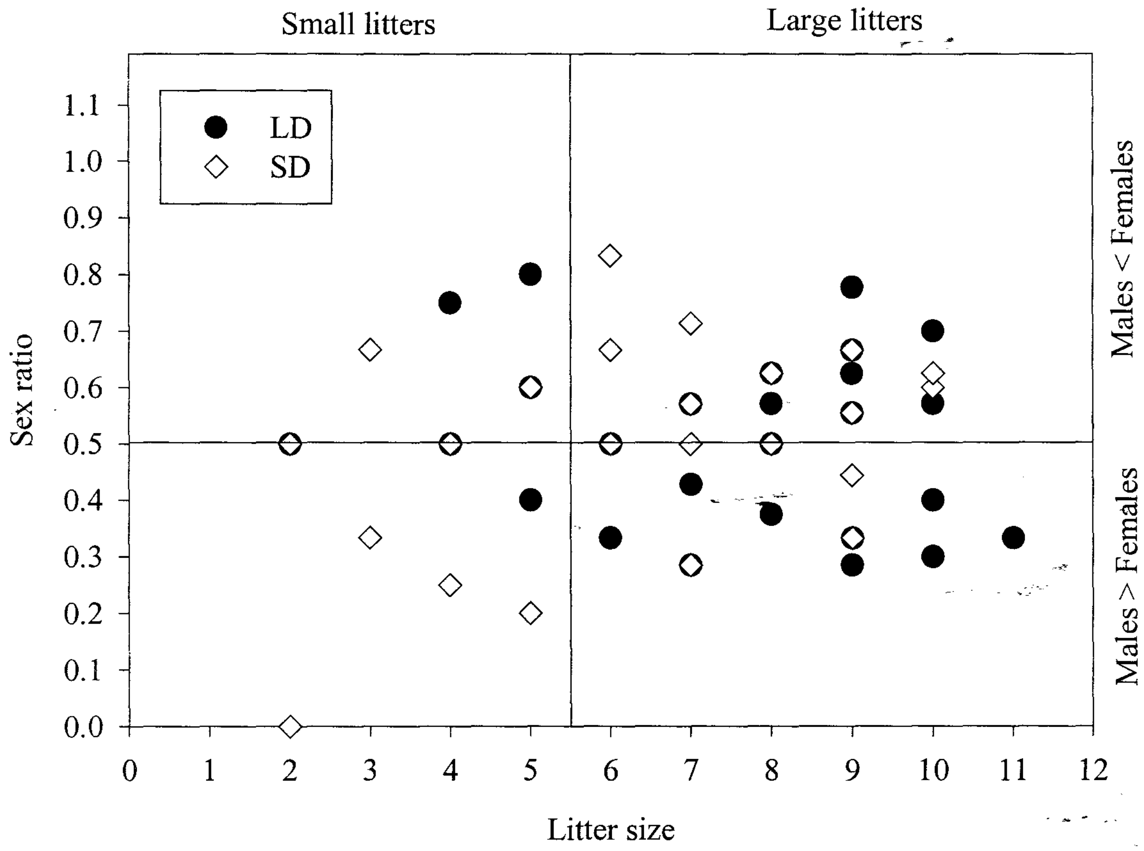

**Figure 5.7** Sex ratios from the data of all domesticated female pouched mice in both long and short daylength (n = 37).

### *5.4.2.2 The effect of photoperiod on the sex ratio of litters from wild-caught and F1-generation female pouched mice*

**Table 5.10 and Figure 5.8**

Photoperiod did not significantly affect the sex ratio of the litters of wild-caught and F1-generation female pouched mice ($p > 0.05$, $F = 0.61$) (Table 5.10) and these sex ratios were not affected by litter size ($p > 0.05$; $F = 0.085$) (Figure 5.8).

The sex ratios of litters did not deviate from a 1:1 ratio in either long daylength ($p > 0.05$, $\chi^2 = 0.8$, DF = 10) or short daylength ($p > 0.05$, $\chi^2 = 1.1$, DF = 12).

**Table 5.10** The mean ± standard deviation of the number of males, females and sex ratios of litters from all the data of wild-caught and F1-generation female pouched mice in both long and short daylength.

| | LD (n = 11) | SD (n = 13) | Significance |
|---|---|---|---|
| Average litter size | 5.73 ± 1.8 | 4.46 ± 1.8 | NS |
| Total number of males | 2.91 ± 1.4 | 1.92 ± 1.2 | NS |
| Total number of females | 2.73 ± 1.7 | 2.46 ± 1.1 | NS |
| Overall sex ratio | 0.51 ± 0.2 | 0.41 ± 0.2 | NS |

LD = long daylength (14L : 10D); SD = short daylength (10L : 14D)
NS = non significant

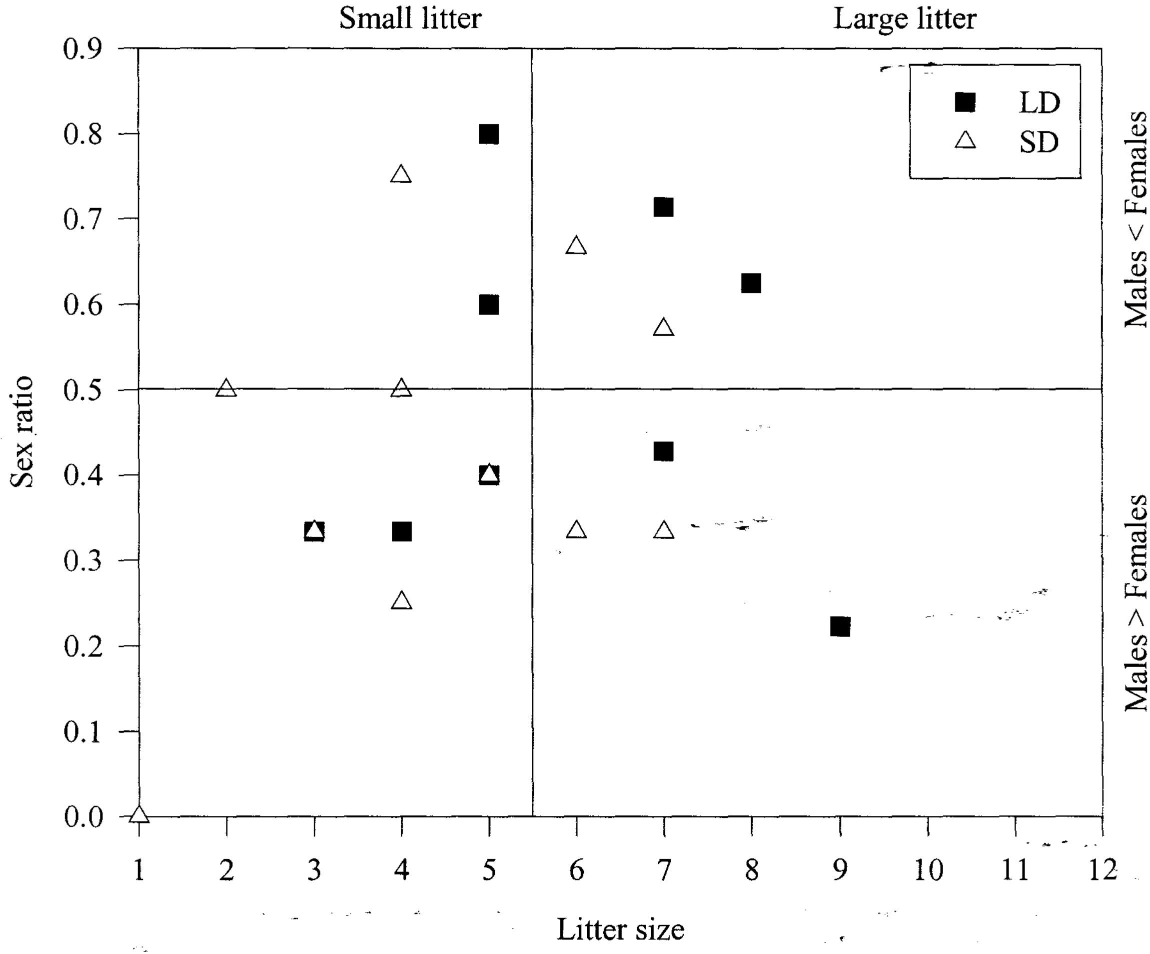

**Figure 5.8** Sex ratios of wild-caught and F1-generation female pouched mice in both long and short daylength.

#### *5.4.2.3 The effect of domestication and photoperiod on the sex ratio of female pouched mice.*

As reported in Section 5.2, both domestication and photoperiod had a significant effect on the average litter size (domestication: $p < 0.01$, $F = 7.4$; photoperiod: $p < 0.05$, $F = 5.1$), however, the interaction of domestication and photoperiod was not significant ($p > 0.05$, $F = 0.02$). The only difference was that domesticated females in long daylength were significantly lighter than those in short daylength.

On comparing the sex ratio of litters born to domesticated and F1-generation females, it was found that neither domestication or photoperiod significantly affected the sex ratio of the litters (domestication: $p > 0.05$, $F = 1.0$; photoperiod: $p > 0.05$, $F = 2.4$). The interaction of photoperiod and domestication was also found to be non-significant ($p > 0.05$, $F = 0.6$). Therefore, the number of males and females born to domesticated and F1-generation females' litters conformed to the 1:1 ratio.

### 5.5 Summary:

1. Photoperiod did not affect litter size of domesticated or wild-caught pouched mice, although larger litters occurred in long daylength than in short daylength.
2. Litter size was highly variable in domesticated and wild-caught pouched mice, with ranges of 1 to 11 in domesticated pouched mice, and 1 to 9 in wild-caught pouched mice.
3. Ovulation rate was not significantly affected by photoperiod in neither domesticated or F1-generation female pouched mice.

4. Photoperiod did not affect sex ratio in domesticated and wild-caught pouched mice, thus pouched mice did not preferentially select one sex above the other in short daylength.
5. Sex ratio was not affected by litter size of domesticated and wild-caught pouched mice.

## 5.6 Discussion

Small mammals are generally short-lived surviving only a year or two so they have to maximize their reproductive efforts (Eisenberg, 1981; Bronson, 1989; Read and Harvey, 1989; Promislow and Harvey, 1990). Litter size is therefore important as is the number and quality of litter produced (Mountford, 1968; Perrin, 1986; Innes and Millar, 1987; Sikes, 1995). Most small mammals display a natural variability in litter size, which arises from differences in litter size between individuals of a population and between litters of the same individual (Mountford, 1968). They tend to have litter sizes smaller than the optimum litter size, *i.e.* the litter size that contributes the most surviving individuals to the population (Mountford, 1968; Sikes, 1995). The most frequent litter size is not always the most productive but represents the one with minimal energetic cost and the maximum size and number of young that would survive that reproductive event (Millar, 1977; Sikes, 1995).

The results of the present study indicate that photoperiod did not significantly affect the litter size of the domesticated female pouched mice or wild-caught and F1-generation female pouched mice. Litter sizes of both domesticated and wild-caught pouched mice were highly variable in long daylength and this variability was only slightly reduced in short daylength. Even though the trend was for slightly reduced litters in short daylength, reproduction continued in the presence of *ad libitum* food. The variability in litter size meant that although the average litter size in short daylength was 5.97 ± 2.5 pups, the largest litter was 10 pups so therefore individual female pouched mice showed different degrees of sensitivity to the decrease in daylength.

Pouched mice in the wild show highly variable litter sizes of between one and 13 young per litter (Smithers, 1971; Smithers and Wilson, 1979; Earl, 1980; De Graaff, 1981; Westlin-van Aarde, 1988; Skinner and Smithers, 1990). In the present study both the domesticated and wild-caught pouched mice displayed a highly variable litter size, although the maximum size of wild-caught female's litters was 9 pups while the domesticated females had a maximum of 11 pups. The domesticated females were significantly larger than the wild-caught and F1-generation females and this can affect the maximum number of young the female can have (Millar, 1977; Eisenberg, 1981; Myers *et al.*, 1985; Dobson and Myers, 1989; Read and Harvey, 1989; Promislow and Harvey, 1990). Neonatal body mass of pouched mice at birth is similar in both the domesticated (2.83 ± 0.08 g) and wild-caught pouched mice (2.78 ± 0.12g) (Pers. obs.). There is an upper limit to the number of neonates a female of a certain body size can carry and still be able to forage for food or escape predators (Eisenberg, 1981; Bronson, 1989). The larger body size of the domesticated mice may thus explain their larger litters.

During periods of limited food supply small mammals can utilise one of a number of strategies to ensure that reproduction continues. Firstly, by reducing litter size they may be able to maintain growth rate and the age to weaning of the young. Or, by reducing the growth rate of the young they may be able to maintain litter size and the age of weaning of the young and finally, by increasing the age of weaning they may be able to maintain litter size and growth rate of the young (Millar, 1977). In the wild, pouched mice have a distinctly seasonal breeding pattern only producing litters in the wet, warm summer months (De Graaff, 1981; Skinner and Smithers, 1990). Under laboratory conditions pouched mice will breed all year round in both long and short daylength (Westlin-van Aarde, 1988; Bernard and Hall, 1995). The pouched mouse has therefore adopted a more opportunistic strategy by retaining the ability to remain reproductively active during the non-breeding part of the year.

Litter size in several species of voles (*Microtus pennsylvanicus*, *M. agrestis*, *Clethrionomys glareolus*) (Beer *et al.*, 1957; Kott and Robinson, 1963; Clarke, 1985; Lee *et al.*, 1987; Gorman *et al.*, 1994), prairie deer mice (*Peromyscus ochrogaster*) (Beer *et al.*, 1957; Myers *et al.*, 1985), house mice (*Mus musculus*) (Pelikán, 1981), and woodland mice (*Apodemus sylvaticus*) (Smyth, 1966; Clarke, 1985) decreases through the breeding season with the largest litters found in summer after which mean litter size decreases and remains smaller during winter if they breed throughout the year. Climate and food tend to be more unpredictable in tropical and subtropical regions (Perrin, 1986; Bronson, 1989; Bronson and Heideman, 1994) and therefore if females reproduce during periods of low rainfall and food availability this will probably reduce the size of their litters. It is beneficial for the female to raise a smaller litter during winter, or period of climatic unpredictability, in order to give herself and the neonates a greater chance of surviving. Many studies of small mammals have investigated the energetic costs of different litter sizes. The results have differed considerably due to the allometric relationship between energetic cost and the optimal litter size. These differences range from little or no difference in energetic cost between large and small litters to significant differences so that as litter size increases the energetic costs increase (Smith and McManus, 1975; Millar, 1977; Sikes, 1995). It has been reported that a single pouched mouse utilized its own fat reserves to meet their energetic costs of pregnancy, instead of increasing intake of food during pregnancy and lactation (Perrin and Clarke, 1987; Speakman *et al.*, 1992). However, in the present study lactating females increased their food consumption (Pers. obs.). It was not, however, noted in the present study whether an increase in litter size caused an increase in food consumption and further experimental work would be required to verify this.

Manipulation of litter size can start at ovulation and photoperiod did not affect the number of ova released by the domesticated or wild-caught female pouched mice. Domesticated females did ovulate significantly more ova than the wild-caught females but this could again be attributed to differences in body size. In order to maximise the reproductive fitness of the female mammal it would be beneficial for her to ovulate and implant a larger number of ova than the average litter size of that species. If conditions were favorable and food was plentiful the female would be able to sustain and rear a larger than average litter and still survive. In the present study it was found that although pre-implantation loss was not affected by photoperiod it did account for a large proportion of prenatal loss. Resorption accounts for the rest of the prenatal loss (Westlin *et al.*, 1995). Westlin *et al.* (1995) found that most prenatal loss was due to resorption of fetuses, which in pouched mice can occur until two days prior to parturition. The fetuses of pouched mice grow and develop normally until 18 days of age when growth is retarded between 18 and 19 days of age. By 17 days of age, although pouched mice fetuses had reached Carnegie Stage 23 (Butler and Juurlink, 1987) with the morphological features typical of this stage, no ossification of the bones had occurred. Ossification continued after 17 days of age thus giving the pregnant female a last chance to adjust litter size if conditions suddenly became unfavourable (Westlin *et al.*, 1995).

Manipulations of sex ratio can be caused by different types of stress (food deprivation, social factors, and environmental factors) (Trivers, and Willard, 1973; Rivers and Crawford, 1974; Myers, 1978; McClure, 1981; Clutton-Brock *et al.*, 1985; Myers *et al.*, 1985; Meikle and Drickamer, 1986; Bazhan *et al.*, 1996) plus timing of insemination in the oestrous cycle (Huck *et al.*, 1990; Hornig and McClintock, 1994; James, 1996, Havelka and Millar, 1997), hormonal influences (Hahn and Hays, 1963; Pratt and Lisk, 1991; Gorman *et al.*, 1994; James, 1996), age and parity of mothers (Huck *et al.*, 1988; Krackow and Hoeck, 1989; Krackow, 1992). Litter size in some small mammals has also been found to affect the sex ratio with the reduction in litter size affecting the sex ratio by biasing litters towards one or the other sex (Rivers and Crawford, 1974; McClure, 1981; Huck *et al.*, 1988, 1990; Krackow and Hoeck, 1989; Pratt and Lisk, 1989; 1991; Peaker and Taylor, 1996). The seasonal change in litter size in guinea pigs (Peaker and Taylor, 1996) and prairie deer mice (Myers *et al.*, 1985) is reflected in sex ratio as litters are biased towards males in spring and, as litter size decreases in autumn, litters become biased towards females. Sex ratios of domesticated and wild-caught female pouched mice were not affected by photoperiod nor did they deviate from a 1:1 ratio. Litter size of both the domesticated and wild-caught female pouched mice did not significantly affect sex ratios nor was there a seasonal change as litter sizes reduced in short daylength. A trend was not found between sex ratio and litter size in the wild house mouse (Krackow, 1997) or the golden hamster (Pratt and Lisk, 1991). Manipulation of sex ratio may not always be advantageous. The selection of gametes and fetuses of specific sex may be hidden (Reiss, 1987; Hendriksen *et al.*, 1996; Zwingman *et al.*, 1993) so by the time the sex of the fetus is identified considerable investment has already been made in the young (Maynard Smith, 1980) or the mechanism to select the sex to abort may be highly prone to error (Reeve, 1987). Alternatively, *Saccostomus campestris* may not have evolved the ability to manipulate sex ratio.

## 5.7 Summary

1. Pouched mice had a highly variable litter size, as do many short-lived small mammals.
2. Litter size was not significantly affected by photoperiod, however, the litter size in long daylengths tended to be larger than in short daylength.
3. Although litter size tended to be smaller in short daylength, some pouched mice had the ability to have larger than average litters in short daylength.
4. Ovulation rate did not account for smaller litters in short daylength or the variation in litter size.
5. Sex ratio was not manipulated by litter size or by daylength.
6. Domestication was associated with a significant increase in litter size and ovulation rate, however, this did not affect the sex ratio in the litters.

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# Chapter 6

# General Discussion

## 6.1 Discussion

Reproduction is the ultimate objective of all organisms, ensuring the continuance of the organism's genetic material and the continuation of the species (Sadlier, 1969; Eisenberg, 1981). The different aspects of reproduction respond to selective pressures which together ensure that mating, gestation, parturition, lactation and weaning occur at the optimum time of year to ensure reproductive success (Millar, 1977; Glazier, 1985; Perrin and Swanepoel, 1987; Gittleman and Thompson, 1988). Many factors, both physical (body size, condition, longevity) and environmental (rainfall, ambient temperature, food quantity and quality, photoperiod), may influence the reproductive strategies used by a population to guarantee the successful production and survival of young. Most habitats in which mammals are found, from the polar regions to the tropics, have seasonal changes in some component of the climate, and this seasonality dictates the reproductive strategy (Bronson, 1985; 1988; 1989; Bronson and Heideman, 1994).

There are three main small mammal reproductive strategies with some variation within each strategy;

1. Seasonal reproduction with both sexes limiting their reproductive effort to a part of the year.

2. Seasonal reproduction with only one of the sexes reproducing seasonally while the other sex remains reproductively active throughout the year.
3. Aseasonal reproduction with both sexes remaining reproductively active throughout the year (Bronson, 1989).

These patterns of reproduction should not be seen as species specific, and the use of a reproductive strategy will vary between species, between populations of the same species and even between individuals within the same population at the same locality (Bronson, 1989; Bronson and Heideman, 1994). The reproductive strategy adopted by species may vary latitudinally (Figure 6.1) (Bronson, 1985; 1989; Bronson and Perrigo, 1987; Bronson and Heideman, 1992; 1994; Heideman and Bronson, 1993). In temperate latitudes, factors such as ambient temperature and food availability are predictably unfavourable during winter with temperatures dropping very low and food becoming scarce. In this region most small mammals breed seasonally and reproductive activity of both sexes is inhibited in winter. The most beneficial cue to use to regulate this seasonality is photoperiod as it is highly predictable and the changes are easily detected thus enabling an animal to physically prepare itself for the oncoming season (Reiter, 1980; Bronson, 1985; 1989; Bronson and Heideman, 1994). In temperate latitudes, (45° - 60° latitude) most small mammals will breed seasonally, e.g. *Peromyscus* spp breeds for only 2 to 3 months of a year, and as latitude decreases to 45° latitude the breeding season lengthens (5 to 7 months). Between 45° latitude and 30° latitude both seasonal and aseasonal strategies are used while below 30° latitude *Peromyscus* spp tend to breed all year (Bronson and Pryor, 1983; Bronson, 1985; 1989; Bronson and Heideman, 1994). The male Syrian hamster (*Mesocricetus auratus*) is reproductively photoresponsive above 13° latitude as reproduction is inhibited in short daylengths. Below 13° latitude they tend to be nonphotoresponsive (Heideman and Bronson, 1993; Heideman *et al.*, 1996).

The value of photoperiod, as a cue, decreases with decreasing latitude since differences in daylength between seasons, decreases with decreasing latitude. In the lower latitudes, especially the tropical latitudes, there is little variation in daylength as well as in ambient temperature. However, there are some small mammals in the subtropics and tropics that breed seasonally and in most of these cases the seasonality is due to other environmental factors, such as food availability and rainfall (Heideman *et al.*, 1992; O'Brien *et al.*, 1993; Heideman *et al.*, 1996; Demas and Nelson, 1998). Rainfall is seasonal in the tropics and therefore dictates the availability of food. A few seasonally breeding small mammals in the tropics (musk shrew, *Suncus murinus*) do use photoperiod as a cue (Rissman *et al.*, 1987).

The proximate factors affecting the reproductive strategy adopted are mainly environmental factors, such as rainfall, ambient temperature, food availability and food quality (Figure 6.1) (Perrin, 1986; Bronson, 1989; Bronson and Heideman, 1994). Food is the main limiting factor as all physiological processes are dependent on energy (Millar, 1977; Gittleman and Thompson, 1988). The energy obtained from the food has to maintain metabolic, thermoregulatory and locomotory demands before it can be either stored as fat or utilised for reproduction (Bronson and Perrigo, 1987; Bronson, 1989). Small mammals have a high surface area to volume ratio so they lose heat quickly and their thermoregulatory costs are high. To overcome the thermoregulatory costs of low ambient temperatures they need to increase food intake as most small mammals do not carry enough body fat to compensate for the energetic cost (Millar, 1977; Bronson and Marsteller, 1985; Glazier, 1985; Bronson and Perrigo, 1987; Gittleman and Thompson, 1988; Bronson, 1989). Rainfall and temperature influence the availability of food, although temperature primarily affects the mammal's thermoregulatory costs and thus the energy available for reproduction (Millar, 1977; Gittleman and Thompson, 1988; Bronson, 1989; Bronson and Heideman, 1994). There is a complex interaction between ambient temperature and food availability as many small mammals live in thermally insulated burrows ensuring that they are only subjected to the ambient temperatures when they leave their burrows to look for food or water. Food availability will determine the length of time spent foraging (Bronson, 1985; 1988; Bronson and Perrigo, 1987; Bronson and Heideman, 1994). If food becomes scarce and ambient temperatures are low the energy expended while foraging may not be balanced by the energy gained from the food (Millar, 1977; Bronson and Perrigo, 1987; Bronson and Heideman, 1994). Animals can overcome this to a certain extent by being generalist feeders, eating what is available at the time, or hoarding food for times of low food availability (Pettifer and Nel, 1977; Ellison, 1993).

In southern Africa the photoperiod differences between season are sufficient to be used as a cue for reproduction, but the climate is highly unpredictable. In particular, rainfall is highly variable and this is reflected in food availability (Neal, 1986; Perrin, 1986; Perrin and Swanepoel, 1987; White and Bernard, 1996; Cumming and Bernard, 1998; Jackson and Bernard, 1999). The climate of southern Africa is seasonal, in the same way that the climate in temperate latitudes is seasonal. But while the seasons at temperate latitudes are predictable, those in southern Africa are not (Bowland, 1986; Neal, 1986; Perrin, 1986; Lovegrove and Raman, 1998). It would therefore be advantageous for small mammals in southern Africa not to rely solely on photoperiod to control reproduction but to use some other environmental factor or a combination of factors to regulate reproduction.

Body size is a further factor influencing reproduction in small mammals as most small mammals have a short life-expectancy surviving only a year or two (Figure 6.1). The short life-span imposes strong selective pressures for the early attainment of sexual maturity and continuous reproduction thereafter thus maximising their reproductive output (Eisenberg, 1981; Perrin, 1986; Read and Harvey, 1989; Promislow and Harvey, 1990). Under laboratory conditions, pouched mice have been found to live for over 3 years (Pitchford and Visser, 1970; Westlin-van Aarde, 1988) and over 4 years in this study (Pers. obs.). In the wild, though, males will survive for approximately 150 days and females 425 days (Earl, 1980).

A more opportunistic strategy to use in unpredictable environmental regions would be for one sex to reproduce seasonally while the other remains sexually active throughout the year. The two sexes of pouched mice, both as adults and juveniles, exhibited different amounts of photosensitivity in some but not all aspects of reproduction. Adult male pouched mice showed very limited reproductive photoresponsiveness as they remained reproductively active irrespective of photoperiod and even under gradual changes in photoperiod the testes remained large and produce spermatozoa. Males are able to mate successfully resulting in litters in short daylength (Bernard and Hall, 1995; and in present study).

Female pouched mice tend to be more sensitive to photoperiod. Adult females from the domesticated stock are not affected by abrupt changes in photoperiod (present study). They retained a regular four-day oestrous cycle and successfully reared young in short daylength (Bernard and Hall, 1995; and in the present study). The females in the domesticated stock, however, became photosensitive when subjected to the gradual changes in photoperiod such as those they would experience in the wild. A larger proportion of the domesticated females became acyclic as daylengths decreased after the austral summer solstice with only 17% of females exhibiting a regular four-day oestrous cycle, however, by austral winter 1997 all the females had a regular oestrous cycle.

Female pouched mice from the wild stock were not affected by short-term changes in photoperiod. In the wild, pouched mice breed seasonally during austral spring and summer (wet, warm season) (Hanney, 1965; Smithers, 1971; Skinner and Smithers, 1990). The wild-caught females and their F1/F2-generation progeny therefore became reproductively active seasonally in a way similar to those in the wild but this seasonality was not due to photoperiod. This condition persisted throughout their life-time in the laboratory (approximately 2.5 years). It is therefore possible that these pouched mice exhibit a circannual endogenous rhythm that may be entrained by photoperiod or some other environmental factor, climatic or dietary (Figure 6.1). Endogenous rhythms can override the environmental factor which entrains them (Masson-Pévet *et al.*, 1994; Turek and van Cauter, 1994) and then they will free-run at intervals of 8 to 11 months. In the wild, changes in photoperiod, or other environmental factors, would entrain the circannual endogenous rhythm to a 12 month cycle (Woodfill *et al.*, 1991; Masson-Pévet *et al.*, 1994; Concannon *et al.*, 1997).

Strategies used by juvenile mammals may differ from those used by the adults or even between juvenile male and females within the same population (Bronson, 1989). Reproductive development of juvenile males was not affected by photoperiod as they attained sexual maturity at the same age in both long and short daylength. The onset of sexual maturity of juvenile female pouched mice from both the domesticated and wild stocks were retarded under short daylengths. Unlike the juvenile males, in many small mammals the young females need to acquire a certain body size before becoming reproductively active (Drickamer, 1975; Falconer, 1984; Foster *et al.*, 1988). This factor was more important in F1/F2-generation wild-caught females as they became sexually mature at approximately the same body mass, while in the young domesticated females weight-dependence was no longer important as this genotype was lost over successive generations. The age of sexual maturity of female pouched mice tended to be a labile event while genetic mechanisms rigidly programme reproductive development in the male pouched mouse. Although the stocks should not be directly compared due to the possible latitudinal differences, years of domestication can unintentionally select those individuals that breed quicker under laboratory conditions, which corresponds with the individuals with a larger body size (Bronson, 1984; 1989; Bronson and Heideman, 1994).

The main parameter in the pouched mouse that was photosensitive was body weight during reproductive development of both male and females in both domesticated and wild juveniles. Juveniles in short daylength were consistently lower in body mass than those in long daylength. The pouched mice has been shown to reduce body mass in winter (Korn, 1989), however, this could be due to an increase in the number of juvenile mice recruited as only young pouched mice showed this reduction in body weight in the non-breeding season (dry season) (Ellison *et al.*, 1993a). The older animals retained a more or less constant body mass throughout the year (Ellison *et al.*, 1993a). Being hoarders, the older mice will have stored enough food to remove the need to reduce body mass (Pettifer and Nel, 1977; Ellison, 1993; Ellison *et al.*, 1993a). The younger animals on the other hand would have had insufficient time to collect a store of food and so the reduction in body mass in the harsher season is thought to be an adaptation to the scarcity of food by reducing the energy requirements (Ellison *et al.*, 1993a). Many small mammals reduce body weight in winter due to a lower intake of food as the animal metabolically prepares itself for the oncoming harsh conditions (Hoffman, 1973; Dark *et al.*, 1983; Perrin, 1986; Korn, 1989).

The pouched mouse is found in a number of biotic zones (Rautenbach, 1978) and has evolved an opportunistic strategy for the highly unpredictable environment in which it lives (Skinner and Smithers, 1990; Lovegrove and Raman, 1998; Tinney *et al.*, 2001). The use of photoperiod alone as a cue is inappropriate in southern Africa due to the unpredictability of rainfall, which in turn affects food availability and quality. The pouched mouse has evolved to use some other environmental cue or cues to trigger reproduction, possibly food quantity or quality, rainfall or ambient temperature, or a combination of factors to produce the seasonality in the wild. In these unpredictable climatic regions the onset of reproduction occurs when vegetative growth begins after high rainfall. Secondary plant compounds, such as 6 methoxybenzoxazolinone (6-MBOA) have been found to initiate reproduction in small mammals (Schadler *et al.*, 1988; Bronson, 1989; Nelson and Shiber, 1990; Linn, 1991; Bronson and Heideman, 1994). Other phenolic compounds, such as p coumaric acid (pCA) or ferulic acid, produced by senescing plants at the end of the wet season, have an inhibitory effect on the reproductive cycle (Batzli, 1983; Andersson and Jonasson, 1986). The age of sexual maturity of female pouched mice is not advanced by the secondary plant compound 6MBOA (White and Bernard, 1996) while the reproductive cycle of adult pouched mice is not inhibited by pCA (White and Bernard, 1999).

Results for *Rhabdomys pumilio* support the multiple cue idea with photoperiod playing a minor role (Jackson and Bernard, 1999). Multiple cues might cause inhibition in spermatogenesis in pouched mice (Tinney *et al.*, 2001). Interactions of environmental factors have been shown to inhibit reproduction in other small mammals or to facilitate the effects of short daylength (Nelson, *et al.,* 1992; Ruf *et al.*, 1993). Food deprivation, although causing a reduction in the mass of testes and accessory glands of male pouched mice, did not affect spermatogenesis (Tinney *et al.*,2001). Steroidogenesis is more sensitive to unfavourable environmental conditions, such as food deprivation and low temperatures, than spermatogenesis leading to a reduction in accessory gland mass initially (Pang *et al.*, 1979; Bronson, 1989; Carballada and Esponda, 1992; Bronson and Heideman, 1994). The removal of accessory glands, especially the seminal vesicles, did not, however, affect mating behaviour in male rats but did decrease fertility in mice (Pang *et al.*, 1979), rats (Carballada and Esponda, 1992) and hamsters (Chow *et al.*, 1986). The secretions of the accessory glands are important in maintaining the spermatozoa (Pang *et al.*, 1979). Normal fertilisation in the golden hamster can be achieved without accessory glands but development of the embryos was affected (Ying *et al.*, 1998). So the reduction in the mass of the accessory glands of the pouched mouse may not have a negative effect on reproduction.

The reproductive cycle in the female pouched mouse was a more labile event (Figure 6.1), which is affected by photoperiod to a certain extent. However, photoperiod probably only accentuates the affects of other environmental factors, such as temperature, rainfall and food availability. Once conditions become favourable an acyclic female rodent can be stimulated to cycle within 3 days if a fertile male is present (Dominic, 1969; Kenagy and Bartholomew, 1981; Bronson and MacMillan, 1983; Hamilton and Bronson, 1985; Bronson and Heideman, 1994). The spermatogenic cycle takes a number of weeks (Clermont, 1972), while only a few days are required for a female to achieve fertility (Hamilton and Bronson, 1985; Kenagy and Bartholomew, 1981; Bronson and Heideman,

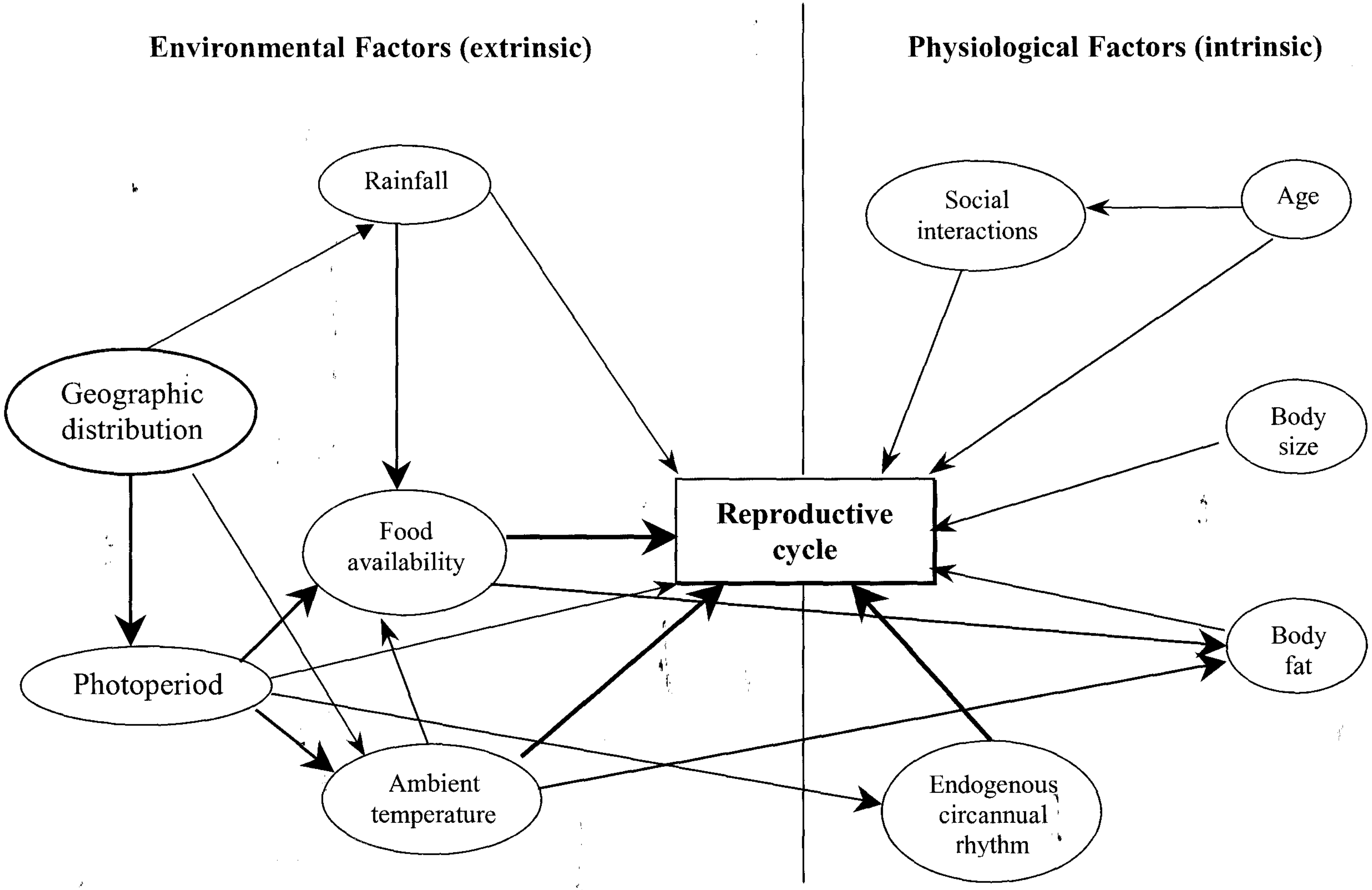

**Figure 6.1** A hypothetical model of extrinsic and intrinsic factors affecting reproduction in small mammals.

1994). Interactions of cues, such as low ambient temperatures, high humidity and poor quality diets, inhibit reproductive activity in adult female house mice without affecting adult males (Pryor and Bronson, 1981). House mice are seasonal in the wild but under laboratory conditions reproduction is not photoresponsive (Hamilton and Bronson, 1985). The pouched mouse also has the ability to resorb fetuses up to 2 days prior to parturition when stressed (Westlin *et al.*, 1995). Fetal resorption is the mechanism they use to vary litter size, as ovulation rates remain the same in both long and short daylength. If environmental conditions suddenly become unfavourable then all or some of the fetuses can be resorbed thus avoiding death and the loss of an energetically costly effort (Westlin *et al.*, 1995; and in present study).

The pouched mouse, *Saccostomus campestris*, from South Africa has the ability to breed throughout the year and the seasonality found in the wild could be mainly due to female pouched mouse. The reproductive seasonality of wild pouched mice reported by De Graaff (1981) and Skinner and Smithers (1990) was based on the collection of gravid females during January to April (Smithers, 1971) and October to Febuary in Kwa-Zulu-Natal, South Africa. However, little mention of the reproductive activity of male pouched mice was made, except that males in Malawi, became non-reproductive during the dry season (De Graaff, 1981). Photoperiod alone has only a small effect on the oestrous cycle of the female and other environmental factors, or an interaction of factors, are probably the main cue to seasonality in the wild. *Saccostomus mearnsi,* the second species of the pouched mouse, is found in central Kenya and displays a similar pattern of reproduction to *S. campestris* (Keesing, 1998). The reproductive cycle is highly seasonal occurring during the wet season, however, 10% of the population bred during the dry season. Most of the males (85%) remained sexually active throughout the year, while it was the female that dictated seasonality as only 10% of females were found in breeding condition in the dry season (Keesing, 1998).

The results of the present study suggest that the reproductive seasonality observed in wild populations of the pouched mouse, *Saccostomus campestris*, is driven principally by the photoresponsiveness of the female, whilst males of the species appear to be unaffected by changes in photoperiod. The oestrous cycle of the female has been found to be inhibited by decreasing photoperiods with the possibility of a circannual endogenous rhythm entrained by photoperiod or some other environmental factor. Spermatogenesis in the male, although nonphotoresponsive, may be more sensitive to interactions of photoperiod with additional environmental factors. The probable role of environmental factors in the seasonality of reproduction in *Saccostomus campestris* is undoubtedly of significant importance but further experimental work is required to fully understand the complex interaction between both exogenous and endogenous factors.

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