Embryo implantation during the short luteal phase of the corn mouse, *Calomys musculinus*, and the apparent lack of a lactational diapause in South American murid rodents

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As the corn mouse, *Calomys musculinus*, has a short luteal phase (2–3 days) that is not prolonged after copulation, it was hypothesized that (i) implantation would occur at the end of this phase, that is, earlier than it occurs in most murid species that have been studied, and (ii) a lactational embryonic diapause would not occur during the luteal phase. These hypotheses were tested in females that had copulated during postpartum oestrus and were either lactating or not lactating. Data were recorded from day 3 to day 5 of pregnancy (day 1 = day after coitus), at both 03:00–05:00 h and 17:00–19:00 h. Evidence of implantation in both non-lactating and lactating animals was apparent at 03:00–05:00 h on day 4 (endometrial 'blue reaction' in all cases and failure to recover free uterine embryos in some cases) and implantation swellings

appeared within 24 h in both groups. In another experiment, the increase in duration of interbirth intervals in continuously mated females and their correlation with the number of suckling young were compared among *C. musculinus*, *C. laucha*, *Akodon molinae* (South American murid species) and *Peromyscus maniculatus* (a North American murid in which a lactational embryonic diapause has been shown). The results were indicative of a lactational embryonic diapause in the North American species, but not in the South American species. It was concluded that in *C. musculinus* (i) implantation occurs at the end of the spontaneous luteal phase, and (ii) that a lactational embryonic diapause does not occur: the absence of a lactational embryonic diapause may be common to other South American murid rodents.

Introduction

Calomys musculinus is a South American vesperal–nocturnal mouse, which is the main reservoir of Argentine haemorrhagic fever (Weissenbacher and Damonte, 1983). It is the most opportunistic of the common rodents of the Argentine pampa, as indicated by several reproductive parameters: frequency of postpartum oestrus (72%), duration of the oestrous cycle and gestation (5–6 days and 21 days, respectively), mean embryo count in the field (7.8), size of litter in the laboratory (5.4–7.6), a long breeding season (September–June, austral spring to winter) and a high percentage of adult pregnancies during the breeding season (65%) (de Villafañe, 1981; Hodara *et al.*, 1984; Cutrera *et al.*, 1988, 1992; Mills *et al.*, 1992).

South American murid rodents (that is, the neotropical division of the subfamily Sigmodontinae; Musser and Carleton, 1993) are a diverse monophyletic group that probably entered South America in late Oligocene to early Miocene times and has evolved in isolation ever since (Reig, 1984). Several unique reproductive features distinguish this

group from its North American counterpart (Hooper and Musser, 1964; Castro-Vazquez *et al.*, 1987; Spotorno *et al.*, 1990; Yunes and Castro-Vazquez, 1990; Pearson, 1992; Cutrera *et al.*, 1998). These differences have raised the question of the phylogenetic meaning of the 'North–South dichotomy' within the subfamily Sigmodontinae (Smith and Patton, 1993), which is the second most diverse of the 17 murid subfamilies (Musser and Carleton, 1993).

In many species of murid rodent, gestation may overlap with the lactation of the previous litter. The partitioning of resources and possibly the conflicting hormonal functions of these two physiological events is resolved through a mechanism known as embryonic diapause (or delayed implantation: Renfree and Calaby, 1981; Renfree and Shaw, 2000). A lactational embryonic diapause has been reported in seven species of three North American genera of sigmodontine rodents (Layne, 1968; Gilbert, 1984). However, it has been reported for only one South American species (*Calomys laucha*: Hodara *et al.*, 1989) as indicated by an increase in duration of the interbirth interval in continuously mated female mice.

In the laboratory rat, in which a lactational embryonic diapause is well known (Lataste, 1891), the maintenance of plateau concentrations of progesterone between day 4 and day 11 after copulation (Pepe and Rothchild, 1974) allows

the embryos still to encounter a functional corpus luteum to sustain their growth after some days of diapause. However, *C. musculus* has a short (2–3 days) luteal phase that is not prolonged either by copulatory or lactational stimuli (Cutrera *et al.*, 1998).

The present study was designed to test the hypothesis that a lactational embryonic diapause does not occur in *C. Musculus* or in any species that shares this unusual luteal control mechanism. The study also included the South American sigmodontines *Calomys laucha* and *Akodon molinae*, and *Peromyscus maniculatus* (a North American sigmodontine). Published data on *Clethrionomys glareolus*, *Rattus norvegicus*, *Mus musculus* and *Microtus ochrogaster* were also analysed for comparison.

Materials and Methods

Animals, housing and feeding

The current study was carried out on adult (at least 2 months of age) laboratory bred individuals of three South American sigmodontines (*Calomys musculus*, *Calomys laucha* and *Akodon molinae*) and one North American sigmodontine (*Peromyscus maniculatus bairdii*). The approximate body weights of these South American species were 15–20 g, 12–16 g and 60–80 g, respectively. The original stocks of *C. musculus* and *A. molinae* were from wild mice captured in the Ñacuñán Biosphere Reserve (Mendoza, Argentina), whereas the original stock of *C. laucha* was captured in the area of Manfredi (Córdoba, Argentina). The *Peromyscus* colony originated from animals of the *Peromyscus* Genetic Stock Centre (University of South Carolina).

Male–female pairs of both *Calomys* species and of *P. maniculatus* were housed in wire-topped plastic cages (12 cm × 20 cm × 30 cm) and *A. molinae* pairs were housed in wire-topped metal cages (20 cm × 20 cm × 30 cm). In all cases, wood shavings were provided as bedding and an aluminium tin as shelter. All animals had free access to a commercial pelleted rodent diet (Cargill) (supplemented with sunflower and wheat seeds) and water. Animal housing conditions included a controlled photoperiod (14 h light:10 h dark) and temperature ($24 \pm 2^\circ\text{C}$). The middle of the dark period was considered as ‘midnight, colony time’, and all times of the day were reported in relation to this reference point. In addition, as most females (72%) become receptive immediately after 19:00 h (Cutrera *et al.*, 1988), the number of hours after mating was calculated from this time. It should be noted that ovulation also occurs at approximately 19:00 h in this species (Cutrera *et al.*, 1992) and, therefore, the number of hours after mating and the number of hours after ovulation are approximately equivalent.

Experiment 1: timing of embryo implantation in *C. musculus*

Two groups of pregnant females were studied: (i) seventy-one female mice were allowed to copulate at

postpartum oestrus (during the first hours of the dark photoperiod after parturition: Cutrera *et al.*, 1988; Laconi and Castro-Vazquez, 1998), that is, females in this group were simultaneously pregnant and lactating; and (ii) sixty-one female mice were also allowed to copulate at postpartum oestrus, subsequent to the removal of their young between 17:00 and 19:00 h on the day of parturition, that is, females in this group were pregnant, but not simultaneously lactating. In all cases, males were removed from the cage at 23:00 h (when spermatozoa were observed in vaginal flushing) and the subsequent day was considered as day 1 of gestation.

Animals were killed by quick decapitation with a small guillotine at 04:00 h (± 60 min, at the end of the dark photoperiod) and at 18:00 h (± 60 min, at the end of the light photoperiod) on day 3, day 4 and day 5 of gestation. After the animals had been killed, the genital tract was excised by ventral laparotomy to determine the passage of developing embryos. Fallopian tubes were cut into serial sections (5 μm), fixed using Bouin’s fixative, embedded in paraffin wax and stained with haematoxylin–eosin to determine the presence of oviductal ova. The presence of free embryos in the uterine cavity was determined by flushing the uterine horns with saline solution and collecting the effluent on slides for examination under a microscope. Implantation swellings were observed directly under the stereoscopic microscope ($\times 10$). Two additional groups each of eight females (lactating and non-lactating) were injected i.v. with a macromolecular dye (1% (w/v) Evans blue, 150 μl per female) on day 4 at 04:00 h and were killed 20 min later, when the uterine horns were examined for the presence of endometrial blue spots (Psychoyos, 1960; Gosden *et al.*, 1981).

Experiment 2: variation in the duration of gestation in lactating females caged continuously with a male

The duration of the interbirth intervals associated with concurrent lactation was estimated from birth records of our colonies of *C. musculus*, *C. laucha*, *A. molinae* and *P. maniculatus*, in which delivery of offspring was observed each day at the end of the light photoperiod (17:00–19:00 h) and was recorded as having occurred on that day. The numbers of offspring observed at birth and at weaning were recorded, and only those intervals during which no loss of young was observed were used for analysis. Intervals that exceeded the species ‘typical’ duration of gestation by > 10 days were not included in the analysis as they were unlikely to be due to a lactational embryonic diapause. Computations were made on a total of 150 interbirth intervals corresponding to 48 couples of *C. musculus*, 246 intervals corresponding to 55 couples of *C. laucha*, 177 intervals corresponding to 55 couples of *A. molinae* and 99 intervals corresponding to 35 couples of *P. maniculatus*.

Similar published data from murid rodents that have a lactational embryonic diapause (*Mus musculus* and

Rattus norvegicus: Mantalenakis and Ketchel, 1966; *Clethrionomys glareolus*: Clarke and Hellwing, 1983) and those that do not have a lactational embryonic diapause (*Microtus ochrogaster*: Richmond and Conaway, 1969) were also analysed for comparison.

For computations, the species typical duration of gestation in non-lactating females was defined as the modal duration in such conditions (or the mean duration when the mode was not available). These values were estimated from our records and from those of Mantalenakis and Ketchel (1966), Richmond and Conaway (1969) and Clarke and Hellwing (1983): 19 days for *C. glareolus*; 20 days for *M. musculus*; 21 days for *C. musculus*, *C. laucha* and *M. ochrogaster*; 22 days for *R. norvegicus*; and 25 days for *A. molinae* and *P. maniculatus*.

The variation from the typical duration of gestation that was observed in females with concurrent lactation was calculated by deducing the species typical duration from each interbirth interval. The variation from the species typical duration of gestation was correlated with the number of lactating young in mice of our colonies (*C. musculus*, *C. laucha*, *A. molinae* and *P. maniculatus*). Since the distributions were not Gaussian, Spearman's correlation test was used.

Results

Experiment 1: timing of embryo implantation in *C. musculus*

In all non-lactating females, oviductal embryos (from four-cell embryos to morulae) were observed on the morning of day 3 (57 h after mating). In one case, a single morula was also observed in the uterine flushing. In the evening of day 3 (71 h after mating), blastocysts surrounded by a zona pellucida were recovered in uterine flushings of all females. The subsequent morning (day 4; 81 h after mating) blastocysts were recovered by uterine flushing in approximately half of the cases (6 of 11 females). The blastocysts were usually zona-free; however, in one female, four zona-free blastocysts were recovered together with three blastocysts that were still surrounded by a zona pellucida. In addition, oocytes surrounded by a zona

pellucida were recovered by uterine flushing in four females (in two cases, the oocytes were found together with blastocysts). From the evening of day 4 (95 h after mating), distinct implantation swellings were observed in all non-lactating animals. These results are summarized (Table 1).

A similar pattern emerged from the study of lactating females. However, in addition, embryos were recovered in a few females on the evening of day 4 (95 h after mating) and on the morning of day 5. The differences in the timing of implantation (in lactating and non-lactating animals) were not significant (Fisher's exact probability test, $\alpha = 0.05$). A summary of the timing of parturition, copulation, embryo migration and implantation in *C. musculus* is presented (Fig. 1).

Two additional groups each of eight females (non-lactating and lactating, not shown; Table 1) were injected with Evans blue on the morning of day 4 (about 81 h after mating). Blue uterine spots (indicating decidual reaction) were observed in both uterine horns of all non-lactating and lactating animals and free embryos were recovered in uterine flushings in four of eight cases in both non-lactating and lactating groups. There was no significant difference in the number of lactating young in the lactating females (Table 1; Kruskal–Wallis test).

Experiment 2: variation in the duration of gestation in lactating females caged continuously with a male

The species typical duration of gestation and the variation from the species typical duration were calculated. The distribution of values for the variation from the species typical duration of gestation in *M. ochrogaster*, *C. musculus*, *C. laucha* and *A. molinae* are presented (Fig. 2). Corresponding values for *C. glareolus*, *M. musculus*, *R. norvegicus* and *P. maniculatus* are also presented (Fig. 3). Modal values for the variation from the species duration of gestation were zero in *M. ochrogaster*, *C. musculus*, *C. laucha* and *A. molinae* (Fig. 2) (that is, the modal interbirth interval was equal to the corresponding species typical duration of gestation), whereas the modal variation value was 2–5 days greater than the corresponding typical value in *C. glareolus*, *M. musculus*, *R. norvegicus* and *P. maniculatus* (Fig. 3).

Fig. 1. Timing of parturition (P), copulation (C), passage of embryos into the uterus (E), and implantation, from endometrial attachment of embryos to the development of a distinct decidual nodule (I), in lactating and non-lactating female *Calomys musculus*. Black vertical lines indicate midnight; grey bars extend throughout the period of each process. The light grey bar indicates the extended duration of implantation that was observed in some lactating females.

Table 1. Uterine migration and endometrial attachment of embryos in female corn mice, *Calomys musculinus*, that had copulated during postpartum oestrus and were either lactating or not lactating

Simultaneous lactation	Time of day (h)	n	Presence of oviductal embryos	Number of mice with embryos in uterine flushing	Number of mice with uterine swellings	Number of uterine embryos and uterine swellings (mean ± SEM)
No	Day 3					
	03:00–05:00	10	10	1	0	1 ^a
	17:00–19:00	10	–	10	0	4.0 ± 0.5
	Day 4					
	03:00–05:00	11	–	6	0 ^b	4.3 ± 1.3
	17:00–19:00	10	–	–	10	8.3 ± 0.3
Yes	Day 5					
	03:00–05:00	10	–	–	10	7.4 ± 0.6
	17:00–19:00	10	–	–	10	9.7 ± 0.7
	Day 3					
	17:00–19:00	10	–	10	0	4.6 ± 0.5
	Day 4					
	03:00–05:00	11	–	6	0 ^b	1.6 ± 0.9
	17:00–19:00	10	–	3	7	8.3 ± 1.2
	Day 5					
	03:00–05:00	10	–	1	9	8.4 ± 0.7
	17:00–19:00	10	–	–	10	8.8 ± 0.6

^aOne embryo was recovered by uterine flushing in one animal, which also had several embryos in the oviduct.
^bAlthough no distinct nodules were observed in these animals, both transparent and opaque zones alternated along the uterine horns in most animals. The number of these zones was not considered in the calculation of the mean number of embryos and swellings.

Fig. 2. Variation from the species typical duration of gestation in female *Calomys musculinus* (■), *C. laucha* (□), *Akodon molinae* (*) and *Microtus ochrogaster* (●) that were caged continuously with males and had lactating young (data for *M. ochrogaster* are from Richmond and Conaway, 1969). The species typical duration of gestation (that is, the duration in non-lactating females) was estimated at 21 days for *C. musculinus*, *C. laucha* and *M. ochrogaster* and at 25 days for *A. molinae*.

Fig. 3. Variation from the species typical duration of gestation in female *Peromyscus maniculatus* (◆), *Clethrionomys glareolus* (○), *Mus musculus* (▲) and *Rattus norvegicus* (▽) that were caged continuously with males and had lactating young (data for *C. glareolus*, *M. musculus* and *R. norvegicus* are from Clarke and Hellwing, 1983; and Mantalenakis and Ketchel, 1966). The species typical duration of gestation (that is, the duration in non-lactating females) was estimated at 19 days for *C. glareolus*, 20 days for *Mus musculus*, 22 days for *Rattus norvegicus* and 25 days for *P. maniculatus*.

Table 2. Relationship between the variation from the species typical duration of gestation and the number of lactating young in *Calomys musculinus*, *Calomys laucha*, *Akodon molinae* and *Peromyscus maniculatus*

Species	<i>n</i>	<i>P</i> value	Rank	Number of lactating young (mean $\pm$ SEM)
<i>C. musculinus</i>	150	NS	-0.0845	7.33 $\pm$ 2.72
<i>C. laucha</i>	246	NS	-0.0418	4.97 $\pm$ 1.69
<i>A. molinae</i>	177	NS	0.0582	4.72 $\pm$ 1.94
<i>P. maniculatus</i>	99	< 0.0001	0.4002	4.13 $\pm$ 1.52

Spearman's rank test; significant at $P < 0.05$.

NS: not significant.

Table 3. Difference in the variation from the species typical duration of gestation in female mice with one to four versus five or more lactating young in *Calomys musculinus*, *Calomys laucha*, *Akodon molinae* and *Peromyscus maniculatus*

Species	Number of lactating young (1–4)		Number of lactating young (≥ 5)		<i>P</i> value
	<i>n</i>	Variation (mean $\pm$ SEM)	<i>n</i>	Variation (mean $\pm$ SEM)	
<i>C. musculinus</i>	26	0.4 $\pm$ 0.4	124	0.2 $\pm$ 0.2	NS
<i>C. laucha</i>	106	0.5 $\pm$ 0.1	140	0.5 $\pm$ 0.1	NS
<i>A. molinae</i>	81	0.9 $\pm$ 0.3	96	1.1 $\pm$ 0.3	NS
<i>P. maniculatus</i>	61	1.6 $\pm$ 0.3	38	4.3 $\pm$ 0.5	< 0.0001

Mann–Whitney U test; significant at $P < 0.05$.

NS: not significant.

Maximum numbers of lactating young were: 14 for *C. musculinus*, 9 for *C. laucha* and *A. molinae*, and 8 for *P. maniculatus*.

A significant relationship (Table 2; Spearman r test; $P < 0.05$) between the variation of the species typical duration of gestation and the number of lactating young was observed only in *P. maniculatus* (no such data were available for the three Palearctic species and *M. ochrogaster* studies by Mantalenakis and Ketchel, 1966; Richmond and Conaway, 1969; and Clarke and Hellwing, 1983). The increase in the duration of the interbirth interval was also significant in *P. maniculatus* when females with one to four lactating young were compared with those with five or more lactating young (Table 3; Mann–Whitney U test; $P < 0.05$), whereas no difference was observed in either *C. musculinus*, *C. laucha*, or *A. molinae* as expected. In *P. maniculatus*, the duration of gestation increased together with the number of lactating young, indicative of a lactational embryonic diapause, but this increase was not seen in the three South American species.

Discussion

In postpartum *C. musculinus* females implantation occurs on day 4 of gestation, that is, at a time similar to that in

nulliparous females (Montoro *et al.*, 1987). The prediction that a coitus-induced prolongation of luteal function is not needed for pregnancy to be established in this species (Cutrera *et al.*, 1998) is supported by the fact that implantation occurs at a time when the corpus luteum formed at ovulation has not yet regressed (Cutrera *et al.*, 1992).

The hypothesis tested in the present investigation was that a lactational embryonic diapause does not occur in *C. musculinus* because of the lack of coitus-induced sustained concentrations of progesterone in females with no implanted embryos (Cutrera *et al.*, 1998). Because of their phylogenetic affinities, it is possible that this hypothesis could be extended to other South American sigmodontine species, as supported by evidence in the present study.

Despite the suggestion of a short diapause (< 1 day) in a small proportion of lactating female *C. musculinus* (10–30%), there were no significant differences in the proportion of lactating and non-lactating females bearing implantations at each time tested or at the time when the blue spots were detected in the uterus. In addition, no indication of an increase in duration of gestation concurrent with lactation was observed when the duration of the

Table 4. Occurrence of coitus-induced prolongation of the luteal phase (pseudopregnancy: PSP) and of lactational embryonic diapause (LED) in murid rodents

Subfamily	Species	PSP	LED	Reference
Arvicolinae	<i>Clethrionomys glareolus</i>	–	Yes	Andersson and Gustafsson (1979)
	<i>Dicrostonyx groenlandicus</i>	–	Yes	Manning (1954)
	<i>Lagurus lagurus</i>	–	No?	Whitney and Burdick (1966)
	<i>Microtus agrestis</i>	Yes	Yes	Breed (1969); Milligan (1975)
	<i>Microtus montanus</i>	–	No	Pinter and Negus (1965)
	<i>Microtus ochrogaster</i>	–	No	Richmond and Conaway (1969)
	<i>Microtus oregoni</i>	–	No	Cowan and Arsenault (1954)
	<i>Microtus pennsylvanicus</i>	–	No	Lee and Horvath (1969)
	<i>Microtus pinetorum</i>	–	No	Kirkpatrick and Valentine (1970)
Cricetinae	<i>Phodopus campbelli</i>	Yes	Yes	Newkirk <i>et al.</i> (1997); Edwards and Wynne-Edwards (1994)
	<i>Phodopus sungorus</i>	Yes	Yes	Newkirk <i>et al.</i> (1997); K. Wynne-Edwards (personal communication)
Gerbillinae	<i>Gerbillus simoni</i>	–	Yes	Lataste (1888)
	<i>Meriones crassus</i>	–	Yes	Marafie <i>et al.</i> (1978)
	<i>Meriones shawi</i>	–	Yes	Lataste (1888)
	<i>Meriones tristrami</i>	Yes	–	Dewsbury <i>et al.</i> (1978)
	<i>Meriones unguiculatus</i>	Yes	Yes	Norris and Adams (1971); Wu (1974)
	<i>Tatera indica</i>	–	Yes	Bland (1969)
Murinae	<i>Grammomys surdaster</i>	–	No	Bland (1973)
	<i>Hydromys chrysogaster</i>	–	Yes	Olsen (1982)
	<i>Mus musculus</i>	Yes	Yes	Long and Mark (1911); Diamond (1970)
	<i>Notomys alexis</i>	–	Yes	Breed (1979, 1981)
	<i>Notomys cervinus</i>	–	Yes	Crichton (1974)
	<i>Notomys mitchelli</i>	–	Yes	cited in Breed (1989)
	<i>Pseudomys australis</i>	–	No	Breed (1989)
	<i>Pseudomys nanus</i>	–	No	Breed (1989)
	<i>Pseudomys hermannsburgensis</i>	–	Yes	Finlayson (1941)
	<i>Pseudomys novaehollandiae</i>	–	Yes	Kemper (1976)
	<i>Rattus norvegicus</i>	Yes	Yes	Long and Evans (1922)
	<i>Rattus rattus</i>	Yes	–	Kenney <i>et al.</i> (1977)
Sigmodontinae (North American)	<i>Baiomys taylori</i>	–	No	Hudson (1974)
	<i>Neotoma cinerea</i>	–	No	Egoscue (1962)
	<i>Onychomys leucogaster</i>	–	Yes	Egoscue (1960)
	<i>Onychomys torridus</i>	–	No	Taylor (1968)
	<i>Peromyscus californicus</i>	Yes	Yes	Kenney <i>et al.</i> (1977); Gubernick (1988)
	<i>Peromyscus eremicus</i>	Yes	–	Dewsbury and Estep (1974)
	<i>Peromyscus gossypinus</i>	Yes	Yes	Kenney <i>et al.</i> (1977); Pournelle (1952)
	<i>Peromyscus leucopus</i>	–	Yes	Svihla (1932); Layne (1968); Hill (1972)
	<i>Peromyscus maniculatus</i>	Yes	Yes	Svihla (1932); this paper; Kenney <i>et al.</i> (1977)
	<i>Peromyscus polionotus</i>	–	Yes	Laffodan (1957)
	<i>Peromyscus yucatanicus</i>	–	Yes?	Lackey (1976)
	<i>Scotinomys teguina</i>	–	No	Hooper and Carleton (1976)
Sigmodontinae (South American)	<i>Akodon molinae</i>	–	No	This paper
	<i>Calomys laucha</i>	–	No?	Hodara <i>et al.</i> (1989); this paper
	<i>Calomys musculinus</i>	No	No	Cutreria <i>et al.</i> (1998); this paper
	<i>Oryzomys laticeps</i>	–	No	Worth (1967)
	<i>Ototylomys phyllotis</i>	–	No	Helm (1975)
	<i>Sigmodon hispidus</i>	–	No	Meyer and Meyer (1944)
	<i>Tylomys nudicaudus</i>	–	No	Helm and Dalby (1975)
	<i>Tylomys panamensis</i>	–	No	Helm and Dalby (1975)

Data are mainly from Asdell (1964), Layne (1968), Breed (1979, 1981, 1989), Renfree and Calaby (1981), Gilbert (1984), Seabloom (1985) and Cutreria *et al.* (1998). Three murid species were excluded from this table: (i) *Mesocricetus auratus*, a species that undergoes pseudopregnancy after cervicovaginal stimulation (Diamond and Yanagimachi, 1968), but which does not show postpartum oestrus (Freeman and Goldman, 1999) and, therefore, simultaneous pregnancy and lactation cannot occur; and (ii) *Acomys cahirinus* and *Mesembriomys gouldii*, which have a long and functional luteal phase in the absence of coital stimulation (that is, a functional luteal phase occurs spontaneously; Crichton, 1969; Peitz, 1981).

interbirth intervals in females with five or more young was compared with that in females with smaller litters.

The values for the variation from the species typical duration of gestation in *C. musculinus* (Expt 2) did not show any indication of a lactational diapause. The same was true for *C. laucha* (despite the report of Hodara *et al.*, 1989), as well as for *A. molinae*, and for *M. ochrogaster* (as reported by Richmond and Conaway, 1969). However, a comparison of the curves of these four species revealed that both *Calomys* species show a steeper slope than those of *A. molinae* and *M. ochrogaster*. The shallower slope in *M. ochrogaster* may relate to variation in timing of mating (as shown for *M. agrestis* by Breed, 1969) as copulation may occur during lactation in *M. ochrogaster* (Richmond and Conaway, 1969) and this might also be the case for *A. molinae*. However, previous studies on oestrous behaviour in *A. molinae* have not been extended beyond the night after parturition (Yunes and Castro-Vazquez, 1990). The current study confirms the occurrence of a lactational embryonic diapause in *P. maniculatus* (Svihla, 1932), as occurs in other species of the genus (Layne, 1968; Gubernick, 1988).

The available evidence on the distribution of a coitus-induced prolongation of the luteal phase (pseudopregnancy) and of a lactational embryonic diapause in murid rodents is reviewed (Table 4). The evidence is fragmentary, in the sense that information regarding at least one of these mechanisms is available for only 50 species, and information regarding the occurrence of both mechanisms is available for only ten of these species. However, the information includes representatives of five subfamilies encompassing 92% of all extant species in the family Muridae. The data show some plasticity regarding the occurrence of a lactational diapause within some groups of closely related species. Furthermore, the information is in agreement with the hypothesis of the present study, in that a lactational embryonic diapause has been reported only in species in which coital stimulation prolongs the short luteal phase, as commonly found in murid rodents.

In conclusion, the apparent lack of pseudopregnancy and of a lactational embryonic diapause in South American sigmodontines may represent a major physiological difference from their North American counterparts. This observation supports the view of an early divergence of the South American and North American stocks of the Sigmodontinae (Reig, 1984; Gardenal *et al.*, 1990; Smith and Patton, 1993).

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