Breeding of *Philander frenata* (Didelphimorphia, Didelphidae) in captivity

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Summary

Data on the reproductive biology and laboratory management of *Philander frenata* are presented here. Sensory contact before pairing increased breeding success and reduced aggression between pairs. Gestation period was 13–14 days, with a post-lactation oestrous. Births occurred only between August and February. Mean litter size at birth and at weaning was 5.5 (SD = 2.4) and 4.0 (SD = 1.9), respectively. Sex ratio at birth was statistically biased toward males. The timing of weaning was established at between 70 and 80 days old, with the young weighing from 24 g to 49 g.

Marsupials have been a subject of interest to biomedical scientists since their discovery in South America in the 17th century, because of their unique developmental process (Barnes 1968). During the last 20 years, marsupials have become a subject of genetic studies (VandeBerg *et al.* 1987) and reproductive biology (Kirsch 1977). South American marsupials are small and some reproduce in captivity, *Monodelphis domestica* being the most commonly used as a laboratory animal (VandeBerg 1990). The importance of studying their reproduction in captivity has increased since the discovery of snake venom inhibition by the sera of *Didelphis albiventris* and *Philander frenata*, and their importance in the epidemiological cycles (Julien-Laferrière *et al.* 1989, Deane & Jansen 1990).

The animal

Philander frenata is a nocturnal, semi arboreal member of the Didelphidae family, inhabiting

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South American tropical rainforests (Patton *et al.* 1996). Its food habits consist of invertebrates (mainly insects), small vertebrates, fruits, and occasionally carrion (Santori *et al.* 1995). The adult male head and body length observed, in captivity, was 262.6 to 327.4 mm ($x = 300.4$ mm, SD = 24.2), and the prehensile tail length was 236.7 to 326.8 mm ($x = 293.0$ mm, SD = 28.9). Adult males usually weigh from 340.8 to 910.3 g ($x = 588.4$ g, SD = 209.1) and females from 341.0 to 466.5 g ($x = 395.9$, SD = 41.6). Female head and body length recorded was 268.4 to 284.4 mm ($x = 274.4$ mm, SD = 5.0), and tail length was 253.3 to 299.5 mm ($x = 274.2$ mm, SD = 16.4).

The face has a black mask around the eyes and a large white spot above each eye that accounts for its common name, four-eyed opossum. Females have a complete abdominal pouch, small and white in nuliparous ones and stained deep orange if they have young in their pouches. The number of teats is said to range from seven to nine (Walker 1975). However, our studies show that the modal number is seven, larger numbers

being seemingly rare. Adult males always have scrotal testes, and two inguinal orange spots. Dental formula is I5/4, C1/1, M3/3 and P4/4. This animal is polyoestrous and can produce from one to three litters in each breeding season (Fleming 1973).

Colony management

Housing

Animals utilized in this study were collected at Restinga de Barra de Maricá, Rio de Janeiro State, Brazil, and new individuals were regularly introduced in the colony, to maintain heterosis. The colony of *P. frenata* at the Laboratório de Vertebrados, Departamento de Ecologia, Universidade Federal do Rio de Janeiro, was established using 15 males and 20 females captured in the field and 11 males and 7 females born in the laboratory. Twenty-two litters were obtained between October 1988 and January 1991. Four females were captured pregnant.

The animals were kept individually in polypropylene cages of $45 \times 30 \times 20$ cm. A 1 cm layer of expanded vermiculite and shredded paper were used for cage bedding. Mothers and young were kept in the same cage until weaning.

Temperature and humidity were monitored. Temperature varied from $22°$ to $26°C$ and relative humidity from 55 to 75%. The light/dark cycle was the natural cycle of Rio de Janeiro. Water was provided *ad libitum*.

Cages were washed twice a week and disinfected with a solution of potassium permanganate with insecticide. Drinking bottles and food containers were autoclaved. Once a day sodium hypochloride was sprayed in the air of the animal house. Newly arrived animals were pulverized with insecticide talc (1 Naphthyl-N-Methylcarbamete) to control ectoparasites, and remained isolated from the colony for two weeks.

The food of the colony consisted of bananas, oranges, quails' eggs, chicken or beef meat, supplemented twice a week with commercial dog meal, codfish oil, Tenebrionidae larvae and live mice (Périssé *et al.*

1989). To improve the colony management we developed a dried pellet meal following the nutrient proportion of the diet balanced by Périssé *et al.* (1989). We tested this ration for 22 days using seven individuals, weighed each second day. The increase in weight during the period tested was significant (Wilcoxon's test, $P = 0.02$; Zar 1984). For seven months our colony was fed using these dried pellets supplemented once a week with codfish oil. We detected neither large weight variation nor any increase in mortality while using this meal.

The colony was kept in the same room as other marsupial species and wild rodent species.

Breeding

Pairs of animals were placed in wire mesh cages, $100 \times 40 \times 35$ cm, with wood nest boxes. Before pairing, sensory contact was allowed for some couples, with the animals separated by a wire grid. From 103 couples, 28 were allowed preliminary contact, and 75 were simply placed together. This contact between male and female increased breeding success and reduced aggression between pairs. Mating success was 28.6% with previous contact, and only 10.7% without that. We found no evidence of female induced oestrous by male presence in *P. frenata*, as described for another didelphoid marsupial, *M. domestica* (Fadem 1985). Thus, increased breeding success related to previous contact may be due to reduced antagonistic behaviour between pairs.

Pairs were kept together for seven days. A longer mating period did not increase the breeding success. Aggression among members of a pair was common, resulting in mutilation of the tail, but never in the death of an animal.

Mating was observed twice. The animals copulated several times in an interval of 15 min. After that, they returned to their aggressive behaviour towards each other.

After mating, females were inspected daily, showing a 13–14 day gestation period. We did not record post-partum oestrus or embryonic diapause, but the animals had a

post-lactation oestrous. Lactation inhibits the oestrous cycle in several marsupial species resulting in a post-lactation oestrous (Short 1972). Interruption of the lactation of *P. frenata* in the early stages resulted in a mating success rate of 50%.

Biological data

Reproduction

Seasonal reproduction is a general pattern for Neotropical marsupials (Fleming 1973, Streilein 1982, Cerqueira 1984, Cerqueira 1989, Cerqueira et al. 1993). This pattern seems to be triggered by the changes in photoperiod (Cerqueira & Bergallo 1993, Bergallo & Cerqueira 1994). In our field studies, at Restinga de Barra de Maricá, lactating females of *P. frenata* were found between July and March (Cerqueira et al. 1993). In laboratory conditions, we obtained pouch young between August and February. This suggests a seasonal pattern of reproduction for this species.

Litter size at birth ranged from 1 to 10 with a mean of 5.5 (SD = 2.4, $n = 26$). At weaning, mean litter size was 4.0 (SD = 1.9, $n = 14$). Russel (1982) pointed out that the maximum litter size in marsupials is limited by the number of teats, but the birth of excess young is common in many species. We observed three litters of *P. frenata* with more than seven young, seven being the number of teats observed in all the females. Due to their dependence on the teats, the extra neonates never survived their first day of life.

Sexing was first possible at an age of 11 to 20 days. At this age the sex ratio was significantly biased towards males: 59 males : 35 females ($\chi^2 = 6.12$, $P < 0.05$). At weaning, this trend was not observed, the proportion being 32 males : 24 females ($\chi^2 = 1.14$, $P = $ ns).

Fifty-five out of 101 animals born (54.5%) survived to weaning and beyond, in spite of being frequently handled for measuring and other procedures.

The timing of weaning was established at between 70 and 80 days of age, with the young weighing from 24 g to 49 g ($x = 35.7$ g,

SD = 4.8) and having a dental formula of $dP3M^1{}_2$, with the last molars not being functional. If the young were not individually separated from the mother at this age, cannibalism might occur. The same phenomenon was recorded for *M. domestica* (Fadem et al. 1982) and for *D. aurita* (Motta 1988).

Age at first reproduction, observed in a female born in captivity, was 352 days. For males, this age was 282 days. In the field, studies of lactating females whose age was estimated by the method of D'Andrea et al. (1994) indicated that the first reproduction most probably occurs after dental age $P3M^4{}_3$, which corresponds to a chronological age of 210 days.

Field animals whose age was estimated using the methods of D'Andrea et al. (1994) and kept in a laboratory lived for up to 3 years.

Morphological development

At birth the neonate mean weight was 0.1 g (SD = 0.04, $n = 7$), and crown/rump length was 9–11 mm. The ears were absent, and the eyes were little black spots covered by the skin. The young did not have eyelids and vibrissae. Internal organs were visible through the naked skin. Around day 55 they were completely furred, and their eyes opened at day 58. Table 1 shows the timing of the appearance of some morphological characteristics. Detachment from the nipples occurs at 60–68 days simultaneously with the onset of aggressive behaviour between siblings. Young removed from their mother's nipple at early lactation for weighing procedures had no problems in reattaching to the nipple.

Table 1 Timing of appearance (in days) of some developmental characteristics of *Philander frenata* pouch young, in captivity

Characteristics	Mean (±SD)	n
Sexual characteristics	15.8 ± 2.7	12
Vibrissae	30.3 ± 3.4	12
Ears open	52.5 ± 4.3	12
Mouth open	57.7 ± 3.0	13
Eyes open	62.5 ± 2.5	11

Handling procedures

Philander frenata are not tame animals. When disturbed, they open their mouths and hiss, and when threatened they can bite. So they must be handled by their tails, with frequent vigorous shaking movements while holding, to prevent them from climbing up their own tails to reach the handler's hand.

To catch and restrain this animal without anaesthesia, it must be grasped behind its neck, while exerting tension on the tail using a leather glove. Pouch young can be marked by toe clipping from the early days of their lives, without risk of rejection by their mothers, or infection. Adults can be marked by ear punching or by tail tattooing (Fernandez 1989) following light anaesthesia with intramuscular injection of about 0.8 ml of Ketalar™ (Parke Davis).

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