The role of photoperiod on the initiation of the breeding season of the brushtail possum (*Trichosurus vulpecula*)

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Summary. The role of photoperiod on the initiation of the breeding season of brushtail possums was investigated in possums housed in three light regimens: a short-day, a natural and a long-day photoperiod. Seven possums were housed in a natural photoperiod. Four possums were transferred to a short-day photoperiod (10 h light, 14 h dark) and eight possums to a long-day photoperiod (14 h light, 10 h dark) on 22 November, when the daylength was 13.34 h. The first rises in plasma progesterone concentrations were observed on 9 January $\pm$ 9 days ($n = 4$), 11 March $\pm$ 6 days ($n = 7$) and 6 May $\pm$ 6 days ($n = 8$), for possums held in short-day, natural or long-day photoperiods respectively. Similarly, births were observed on 12 January and 14 February in the short-day group, from 3 March to 8 May for the natural photoperiod group, and from 5 May to 8 August for the long-day group. These results suggest that photoperiod is important in the timing of the breeding season. However, annual breeding will commence in a nonstimulatory long-day photoperiod. Thus a long-day photoperiod does not prevent breeding activity.

Keywords: seasonal breeding; daylength; marsupial; brushtail possum; captivity

Introduction

Marsupials can be separated into two groups on the basis of the presence or absence of embryonic diapause. Embryonic diapause occurs in most members of the family Macropodidae and in the honey possum, *Tarsipes rostratus* (Renfree, 1981; Russell, 1982). In these marsupials, ovulation and oestrus occur soon after parturition, yet the suckling by the newborn young prevents the full development of the corpus luteum and arrests the growing embryo at the unilaminar blastocyst stage. This mechanism, a lactational inhibition of reproduction, is known as lactational quiescence. Seasonal quiescence is not merely a prolongation of lactational quiescence but a response to environmental factors such as photoperiod (Tyndale-Biscoe & Renfree, 1987). Most studies of seasonal breeding in marsupials have been carried out with two macropodid species: the tammar wallaby (*Macropus eugenii*) and Bennett's wallaby (*Macropus rufogriseus*). In these marsupials photoperiod is the major factor in the reactivation of the quiescent corpus luteum and the dormant blastocyst and in terminating seasonal quiescence (Loudon & Curlewis, 1987; Tyndale-Biscoe & Renfree, 1987).

The brushtail possum (*Trichosurus vulpecula*) is a member of the family Phalangeridae and, along with families Didelphidae, Peramelidae and Dasyuridae, does not display embryonic diapause. In South Australia and in the Northern Territory births of *T. vulpecula* have been recorded throughout the year (Pilton & Sharman, 1962; Kerle, 1984; Sanderson & O'Driscoll, 1986). In other regions of Australasia, births are more seasonal (Kerle, 1984). In Queensland, the Australian Capital Territory and New South Wales, a peak of births in March and April has been reported, followed by a second peak during September and October (Lyne & Verhagen, 1957; Dunnet, 1964; Winter,

1976; Gemmell *et al.*, 1987). This second peak was not observed in New Zealand and Tasmania (Tyndale-Biscoe, 1955; Lyne & Verhagen, 1957). Thus, the breeding season of *T. vulpecula* is variable within Australasia and the environmental cues that regulate the onset and duration of the breeding season are poorly understood.

The brushtail possum is a short-day breeder i.e. begins breeding as the days shorten. In Queensland, births in the breeding colony are first seen during March and continue throughout the year until November, no births being observed in December, January and February (Gemmell *et al.*, 1987; Gemmell, 1987, 1990). Melatonin implants or housing in a short-day photoperiod (10 h light:14 h dark) have been shown to hasten the start of the breeding season (Gemmell, 1987, 1990) and those observations support the view that photoperiod influences the breeding activity of *T. vulpecula*. In this study, the influence of photoperiod on the initiation of the breeding season of *T. vulpecula* was investigated by comparing the breeding activity of female possums in a natural photoperiod with those kept in a long-day or short-day photoperiod.

Materials and Methods

Animals and treatments

Nineteen adult female possums that were known to have given birth previously were used in this study. Seven possums were housed in a natural photoperiod. Three or four female possums, with one adult male, were housed in each of two fully enclosed holding yards measuring $9 \times 3 \times 2 \text{ m}^3$, and were supplied with running water, fruit and vegetables. The major portion of these two yards was open and the possums were thus kept in a natural photoperiod. The remaining 12 adult female possums were transferred from similar outside holding yards to light-controlled rooms on 22 November, 1989 when the daylength was 13.34 h. The light-controlled room for the eight female possums held in a long-day photoperiod was divided into two sections by a chain wire partition. In each section, $3 \times 3 \times 4 \text{ m}^3$, four female possums and one male were housed and supplied with water, fruit and vegetables. Light was supplied by one 40 W fluorescent tube. The level of illuminance measured from light reflected from the walls was $140 \pm 15 \text{ lx}$ ($n = 6$). Light levels were determined with a National Digital Lux tester (Matsushita Electric Industrial Co. Ltd, Japan). Lights were turned on at 05:00 h and off at 19:00 h (14 h light, 10 h dark). The room had separate air intake and exhaust ducts which were baffled to prevent light leaks. Temperature was not controlled. The four possums held in a short-day photoperiod (10 h light, 14 h dark) were housed in another room under similar conditions (Gemmell, 1990). Lights were switched on at 07:00 h and off at 17:00 h.

Blood samples

Blood samples (2 ml) were obtained once a week from the seven control possums, until a young was first observed in the pouch. Blood samples were obtained once a week from the four possums held in a short-day photoperiod, until a young was first observed in the pouch or until 1 May. Samples were obtained from the eight possums housed in a long-day photoperiod every two weeks from November to 1 May, and every week from 1 May until a young was first observed in the pouch or until 17 July. The size and morphology of the teats of the females were noted as these structures enlarged close to parturition. The date of birth of pouch young was determined either by observing the day of birth or estimating the age of pouch young from the length of the head and the total body weight according to the method described by Lyne & Verhagen (1957).

Progesterone assay

Progesterone concentrations in 50–100 μl plasma were determined by radioimmunoassay using the method described previously (Gemmell *et al.*, 1987) using sheep anti-progesterone-11-hemisuccinate-bovine serum albumin (Antiserum No. 334) kindly donated by R. I. Cox, CSIRO, Prospect, New South Wales, Australia. The anti-serum was highly specific for progesterone, the only significant crossreaction being with 11β -hydroxyprogesterone (11.9%). The limit of sensitivity of the assay was 25 pg per tube and at a progesterone concentration of 10 ng ml^{-1} the intra- and interassay coefficients of variation were 10.5% ($n = 5$) and 13.0% ($n = 10$), respectively. The assay buffer blank was less than 0.1 ng ml^{-1} and the efficiency of extraction was 85.0% ($n = 10$).

Statistical analysis

One-way analysis of variance was used to determine whether the mean day of first rise in progesterone concentration differed significantly across the three groups, that is short-day, natural and long-day photoperiods.

Results

The first increase in plasma progesterone concentration greater than 5 ng ml⁻¹ occurred on the mean date of 9 January for the four possums held in a short-day photoperiod. One of the possums gave birth on 12 January and one on 4 February (Table 1). Possums 3 and 4 were displaying

Table 1. Reproductive events in the three groups of possums held in differing light regimens

Photoperiod	Mean day of first rise in progesterone concentration (over 5 ng ml ⁻¹)	Mean day of first birth
Short day (10 h light:14 h dark) <i>n</i> = 4	9 Jan ± 9 days ^a range 26 Dec–6 Feb	12 Jan, 4 Feb (two possums did not give birth)
Natural photoperiod <i>n</i> = 7	11 Mar ± 6 days ^a range 26 Feb–10 Apr	1 Apr ± 10 days range 3 Mar–8 May
Long day (14 h light:10 h dark) <i>n</i> = 8	6 May ± 6 days ^a range 3 Apr–22 May	5 May, 1 and 22 Jun, 8 Aug (4 possums did not give birth)

^aOne-way analysis of variance shows that groups were significantly different from each other, *P* < 0.001.

nonpregnant or oestrous cycles (Figs 1 and 4). Possum 2 had one nonpregnant cycle before giving birth on 4 February (Figs 1 and 4).

The mean date of the first rise in plasma progesterone concentration for the seven possums held in a natural photoperiod was 11 March and the mean date of the first births of the breeding season was 1 April (Table 1). All seven possums gave birth, possums 5, 6, 7 and 8 on the first ovulation of the breeding season and the other three possums displayed cyclic activity before becoming pregnant (Figs 2 and 4).

Only four of the eight possums held in the long-day photoperiod gave birth. Possums 12, 13, 14 and 17 gave birth on 5 May, 1 and 22 June and 8 August, respectively. The mean date of the first rise in progesterone concentration was 6 May (Table 1), although possums 16 and 19 only showed one slight increase in plasma progesterone concentration, 3.6 and 5.1 mg ml⁻¹, respectively. The plasma progesterone profiles of possums 15, 17 and 18 indicated that several oestrous cycles had occurred during the period of observation (Fig. 3). Possums 18 and 19 died on 6 and 12 June, respectively. No obvious reasons for the deaths were observed.

One-way analysis of variance demonstrated that the mean day of first rise in progesterone concentration for each group was significantly different from every other group (*P* < 0.001) (Table 1).

Discussion

The breeding colony from which the possums in this study were selected has been observed from 1983 to 1989 and found to begin producing young between 9 March and 30 April (Gemmell, 1990). In this study, the seven possums kept outdoors in natural photoperiod bred at the expected time, giving birth between 3 March and 8 May. When housed in a short-day environment breeding occurred ahead of the natural photoperiod group, with one possum giving birth on 12 January and one on 4 February and the remaining two showing oestrous cycles. A similar outcome was observed previously, with births on 11 and 30 January, 17 February and 21 March in short-day

Fig. 1. (a–d) Plasma progesterone profiles of four possums held in a photoperiod of 10 h light: 14 h dark (short days) on 22 November. (a) Date of birth was 12 January; (b) date of birth was 4 February; (c) and (d) were not pregnant.

conditions (Gemmell, 1990). Increasing the light period to 14 h delayed the onset of ovarian activity by nearly two months to $6 \text{ May} \pm 6 \text{ days}$ ($n = 8$). There are two possible explanations for this. The possums housed in a long-day photoperiod could have become refractory to the nonstimulatory photoperiod and ovulated. The second possibility is that the possums have a circannual rhythm and the nonstimulatory photoperiod cannot inhibit the breeding activity that occurs annually. Nevertheless, the data from this present study support the view that possums use decreasing photoperiod to initiate their breeding season.

In sheep, as in possums, breeding occurs naturally as daylength shortens (Legan & Karsch, 1980; Wayne *et al.*, 1988). When placed in a constant short-day photoperiod, sheep are stimulated to ovulate (Legan & Karsch, 1980; Wayne *et al.*, 1988) and breeding activity shows a circannual cycle when this photoperiod is maintained over several years (Robinson & Karsch, 1988; Karsch *et al.*, 1989). However, sheep differ from possums in that a constant long-day photoperiod does not delay ovarian activity beyond that seen in a natural photoperiod (Robinson *et al.*, 1985). Thus both sheep and possums have a circannual reproductive rhythm that is synchronized by photoperiod, except that the circannual rhythm in possums is clearly more labile to the influence of photoperiod.

Fig. 2. Plasma progesterone profiles of seven possums held in a natural photoperiod. The date of birth was (a) 3 March; (b) 5 March; (c) 11 March; (d) 29 March; (e) 8 April; (f) 30 April and (g) 7 May.

Long-day breeders such as voles, rats, hamsters and deermice also change their breeding season in response to manipulation of photoperiod and if placed under appropriate laboratory conditions will show continuous breeding (see reviews by Zucker *et al.*, 1980; Bronson, 1988). Sheep placed in a photoperiod of 12 h light:12 h dark have also been reported to breed continuously (Radford, 1961; Jackson *et al.*, 1990). Whether possums can also be induced to breed continuously by some suitable photoperiod regimen has not yet been investigated. An equatorial photoperiod needs to be tried since the experience with sheep indicates an almost total replacement of circannual breeding with continuous breeding (Jackson, 1990).

Whereas all possums in a natural photoperiod gave birth, those in either short or long day-lengths showed a decline in the birth rate, in spite of the changes in plasma progesterone concentrations which indicated a return to oestrus. The reasons for this lowered fertility are unclear. A pre-existing state of low fertility in the females is an unlikely explanation, since only possums of proven fertility were included in this study. The male possum shows a seasonal pattern of plasma testosterone concentration (Gemmell *et al.*, 1986) and the prostate enlarges sixfold during the breeding season, whereas the testes and epididymides remain unchanged (Gilmore, 1969). However, males housed in a nonstimulatory, that is, long-day photoperiod are fertile and were clearly so

Fig. 3. Plasma progesterone profiles of eight possums held in a photoperiod of 14 h dark:10 h light (long days) on 22 November. The date of birth was (a) 5 May; (b) 1 June; (c) 22 June and (f) 8 August; (g) and (h) possums died on 6 June and 12 June, respectively, and (d) and (e) did not give birth.

in this study because all groups, which consisted of three to four females housed with a single male possum, had females that gave birth. Nevertheless, housing males in photoperiod rooms may reduce their fertility and examination of the prostate and testes of these possums may assist in explaining this reduction in production of young. It is possible that oestrous behaviour was absent in the nonpregnant possums; perhaps these possums were displaying 'silent' oestrus, akin to those reported in sheep at the beginning of their breeding season (Walton *et al.*, 1977). Further studies are required to determine the cause of the decreased fertility in possums kept in artificial photoperiods.

Fig. 4. Summary of the reproductive responses of possums to (a) short-day photoperiod, (b) natural photoperiod and (c) long-day period. Arrows indicate the first rise in progesterone concentration. B: birth.

As stated previously, possums held in a long-day photoperiod ovulate and give birth. If this activation of the reproductive processes of the possum is due to refractoriness, then presumably the possum will continue to ovulate continuously. If the reactivation is due to a circannual rhythm then the possum should ovulate and give birth for up to 9 months and then enter a period of anoestrus. The possums in this study, housed in short-day and long-day photoperiods, will be monitored for several years to obtain this information.

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