



Tasmanian bettong *Bettongia gaimardi*

REPRODUCTIVE BIOLOGY OF THE TASMANIAN BETTONG

(*BETTONGIA GAIMARDI*)

by

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DECLARATION

I declare that except as stated herein this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that, to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material published or written previously by another person, except where due reference is made in the text of the thesis.

R. Roy

22/10/1984

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ABSTRACT

The Reproductive Biology of the Tasmanian Bettong (*Bettongia gaimardi*)

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The Tasmanian bettong is a rat-kangaroo found only in Tasmania. Information on this macropodid is extremely scant and the thesis presents a detailed study of the species' reproductive biology.

Techniques for accurately estimating the age of the pouch young are reported, together with their application to the analysis of breeding season and growth rates. The bettong is able to breed throughout the year. The growth rate of pouch young is faster than that of other kangaroo species and this mirrors the findings that prenatal growth is also so rapid that the bettong has the shortest gestation of any macropodid.

The reproductive biology of the bettong conforms to the pattern found in most kangaroos. Gestation and the oestrous cycle are of similar duration (21-22 days), and a post-partum oestrus may result in the formation of a blastocyst that remains quiescent (embryonic diapause) throughout most of the pouch life. Oestrous cycles including a gestation are shorter (by 1.5 days) than those cycles without a pregnancy.

The presence of a fetus also has morphological effects upon the uterus. The uterine glands in the pregnant uterus remain enlarged for a longer period than do those of the non-pregnant uterus.

Pouch life is always terminated when a new young is born. In the absence of birth, final pouch emergence is associated with oestrus. Pouch vacation is apparently a consequence of a dramatic tightening of the pouch musculature. Data are presented to support the conclusion

that, contrary to expectations based on previous work, it is the physiology of the mother that is most important in the determination of the duration of pouch life. It appears that the duration of pouch life, in effect, may be a compromise between the wellbeing of the existing pouch young and the mother's capacity to maximise reproductive output during her life span.

The bettong displays a circadian rhythm in body temperature. The daily fluctuations in body temperature of females vary according to the phases of the reproductive cycle. This is a consequence of a relatively stable daily maximum temperature but a more labile basal body temperature (BBT). Computer analysis of the variations in the BBT demonstrate a cyclical variation whose period corresponded closely with the duration of the oestrous cycle. A profile of the fluctuations in the sex hormone progesterone appears to mirror the changes in basal body temperature.

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CHAPTER 1

INTRODUCTION

CHAPTER 1 INTRODUCTION

Although mammals evolved approximately 200 million years ago during the Triassic era, the two major groups (the Eutheria and Metatheria) most probably arose some time during the late Cretaceous (Lilliegraven 1979). The common ancestor to these two therian groups of mammals probably lived on the continent of America and the present-day marsupials and eutherians appear to have evolved at about the same time. Nothing is known of the common ancestor, though speculation as to its mode of reproduction tends towards the view that it was marsupial-like (Parker 1977; Lilliegraven 1979) although Sharman (1970) believes that both groups arose from an egg-laying ancestor. From a basic American stock, the didelphids, arose all other marsupial groups including those now present in Australia (Keast 1968). Fossils of a type, intermediate between Australian and American forms, are unknown although Archer (1976) puts forward the view that the Tasmanian tiger (*Thylacinus cyanocephalus*) shows marked similarities with an extinct group of South American marsupials, the Borhyaenoidea.

It is unclear how marsupials reached Australia. For many years a 'northern entry' hypothesis was accepted by most biogeographers (e.g. Darlington 1968; Keast 1968). This hypothesis involved the assumptions that marsupials arrived in Asia via the Bering Straits and subsequently travelled to Australia by island hopping and temporary land bridges. Acceptance of this view required that the existence of Wallace's line be virtually ignored.

During the past 15 years the so-called 'southern entry' hypothesis has gradually replaced the earlier 'northern entry' hypothesis in

popularity. It is now generally accepted by most biologists that marsupials moved on to the giant 'super' continent known as Gondwanaland and then migrated from South America via Antarctica to the area that eventually became Australasia. The recent discovery of a marsupial fossil from the Antarctic (Woodburn and Zinsmeister 1982) gives greater credence to this hypothesis.

1.2 REPRODUCTION: THE MARSUPIAL/EUTHERIAN DICHOTOMY

In view of the long separation (since the late Cretaceous) of the marsupials and eutherians it is not surprising that important differences have appeared in the reproduction of these two groups of mammals.

One fundamental difference between eutherian and metatherian mammals lies in the possession of a pouch by the majority of marsupials. The birth of marsupial young in an altricial state, albeit with some precocial development to facilitate the journey to the pouch and attachment to a teat, is another difference that has no counterpart in eutherian reproduction.

These differences and others associated with the structure of the reproductive tract led Sharman (1970, 1976) to suggest that both eutherians and marsupials evolved from a common ancestor (perhaps below the reptile/mammal boundary) and that viviparity evolved independently in each group. Acceptance of this view requires that one also accepts that considerable convergent evolution of physiological functions and reproductive patterns has occurred and as a result Lilliegraven (1969, 1975, 1979) argues that the many similarities between eutherian and marsupial reproduction suggest a more recent common ancestor (perhaps 100 million years ago). He proposes (Lilliegraven 1969, 1975) that this common ancestor was more likely to have had a marsupial mode of

reproduction (i.e. altricial young reared in a pouch). However, Lilliegraven (1979) remains somewhat cautious in his interpretation of the reproductive characteristics of Mesozoic mammals, "many pitfalls await the worker who is willing to over-extend the certainty of these interpretations".

It seems improbable that one could obtain corroborating palaeontological evidence for the presence of a pouch, though it is conceivable that a fossil might be found with evidence of the presence of a pouch young. On the basis of present knowledge it seems prudent to suspend judgement on the reproductive characteristics of the common ancestor of marsupial and eutherian mammals.

1.3 MACROPODID REPRODUCTION

Macropodid reproduction is characterised by ovarian cycles of medium length; in all but one case (*Wallabia bicolor*); these are of longer duration than that of the gestation period. Parturition is closely followed by movement of the neonate towards the pouch and its 'attachment' to one of a variable number of teats. A species dependent period of time is spent wholly within the pouch until the young emerges. The young is then able to move back and forth from the pouch until 'events' occur that see the young permanently vacate the pouch. These 'events' are a particular concern of part of this thesis (Chapter 7).

While the young is in the pouch it makes an ideal subject for studies on growth and the development of techniques for the estimation of age.

1.4 GROWTH AND AGE ESTIMATIONS

The ability to accurately determine the age of animals is an

invaluable tool for field workers involved in ecological research as well as laboratory-based studies on animal physiology including reproduction. For example, knowledge of age is important in determinations of when animals mature, how long they live, or how many young can be produced in a life-time. Age determinations of marsupial pouch young by extrapolation allows the definition of the extent of the breeding season and, as a consequence, enables one to conduct experiments investigating the reproduction of seasonal breeding species at the appropriate time of year. For example, the finding (see Chapter 4) that the Tasmanian bettong can breed throughout the year allowed experiments to continue without regard to the seasons.

The early attempts at age estimations in marsupials by Lyne and Verhagen (1957), Guiler (1960) and Shield and Woolley (1961) made use of simple extrapolations from the size of known-aged animals. The advent of easy access bench top computers saw the consolidation of the use of mathematical models to describe the growth of marsupials and this permitted age estimation by the substitution of various parameters into mathematical formulae. However, sophisticated models are only useful if they provide more information or a better estimate of age than simpler methods. In Chapter 3 of this work a comparison is made of several methods of age estimation; some of these are simple (i.e. they involve obtaining an estimate of age directly from a curve) whilst others are based on mathematical models.

After pouch life, growth slows and individual variation (including sexual dimorphism) increases and it becomes necessary to use other methods to 'age' individuals. The most commonly applied techniques involve the use of the sequence of molar eruption and dental wear. An elaboration of this, 'molar progression' (Kirkpatrick 1965) has been successfully used with the Macropodinae but does not apply to other

marsupials. Although the technique of age estimation based on the stage of molar eruption has been applied to several members of the genus *Bettongia* (Tyndale-Biscoe 1968, Christensen 1980), these studies used few animals and were not based on individuals of known age. This thesis presents data for the first three years of the life of Tasmanian bettongs using animals whose dates of birth were known.

The study of growth rates in marsupials has been a neglected area. Lyne and Verhagen (1957) made an attempt to compare the growth rates of the possum *Trichosurus vulpecula* with a number of eutherian species (including man) and more recently Maynes (1972, 1976) studied the growth rates of the parma wallaby *Macropus parma*. This thesis will present data on growth rates in the Tasmanian bettong and compares them with the data from the studies of Maynes and other published information.

Recent studies of growth rates are generally linked to an understanding of energetics and milk production and assimilation (Green, Newgrain and Merchant 1980; Fleming, Harder and Wukie 1981). However, this line of investigation was largely beyond the scope of this thesis, though some attempt has been made to relate growth rates with the stage of lactation. In particular the effect on the growth rate of experimental alteration of the stage of lactation and/or the sucking pattern is presented in Chapter 7.

Low (1978) has correctly pointed out that it is the marsupial strategy of extended lactation (within and outside the pouch) that differs most from eutherian parental strategies. She suggests that lactation is less energy efficient than trans-placental transfer of nutrients so that on this criterion eutherian reproduction is more efficient. However, Fleming *et al.* (1981) have shown that in the opossum (*Didelphis virginiana*) the metabolic costs of rearing marsupial

young are not substantially different from those of rearing similar sized eutherians. This type of comparative investigation should be extended to other species of marsupial as opossum young are reared at a small size relative to their mother (5-10% of maternal weight) compared to the 20% found in many macropodids (Chapter 3).

An important consequence of the ability to accurately estimate the age of pouch young is the opportunity to calculate the day of birth and hence obtain reliable information about the breeding season.

Many marsupials living in temperate regions have regular breeding seasons (Tyndale-Biscoe 1973) that are separated by periods of seasonal anoestrus. This often results in a greater proportion of young being released from the pouch in the spring/early summer when food abundance is greatest. The breeding seasons of five species of macropodid found in Tasmania are presented in Chapter 4.

1.5 OESTROUS CYCLES AND GESTATION

No study of the reproduction of a mammal would be complete without details of the oestrous cycle and some of its component parts: the follicular and luteal phases. Macropodid oestrous cycles vary in length from 21 to 42 days (Tyndale-Biscoe *et al.* 1974). In general, the luteal phase (as defined in Chapters 4 and 5) comprises the greater proportion of the oestrous cycle. The gestation length in most of those non-macropodids for which information is available is approximately equivalent to the length of the luteal phase. This implies that gestation in those species is shorter than the length of one oestrous cycle and also is primarily controlled by secretions from the corpus luteum. After parturition subsequent oestrous cycles in most marsupials are inhibited due to the sucking of the pouch young (lactational anoestrus).

If the neonate is prevented from reaching the pouch and sucking, the subsequent period of oestrus will occur on time as if the animal had not been pregnant (Sharman 1970). In other words, the duration of the oestrous cycle in a marsupial is independent of whether or not the animal is pregnant.

This fact has long been cited as evidence that there is no additional hormonal secretion (other than that occurring during a normal oestrous cycle) to allow pregnancy to be extended much beyond the luteal phase (Sharman 1970, 1976).

For example, Harder and Fleming (1981) have measured hormonal levels during these periods in the opossum and failed to find significant differences between pregnant and non-pregnant animals.

The reproduction of most macropodid marsupials differs from that found in other marsupials in that gestation occupies the greater part of the oestrous cycle i.e. it is extended beyond the luteal phase into the post-luteal/ follicular phase. This usually results in birth being closely followed by a post-partum oestrus. How gestation might be extended beyond the luteal phase in macropodids including the Tasmanian bettong is discussed in Chapters 4 and 5. The mating at post-partum oestrus can result in a fertilised egg that develops to the blastocyst stage and then follows one of two pathways. If the pouch young born near post-partum oestrus is lost or removed experimentally (removal of pouch young = RPY) the blastocyst will develop normally. However, if the pouch young successfully reaches the pouch and continues to suck, the blastocyst will enter 'embryonic diapause' and usually remains in this condition until sucking of its predecessor becomes intermittent near the end of pouch life.

Sharman and Calaby (1964) found that in the red kangaroo fertilisation, pregnancy, birth and attachment of the young to the teat did not

affect the timing of the onset of the next oestrus. However, Merchant (1975, 1979), Merchant and Calaby (1981) and Hinds and Tyndale-Biscoe (1982) have shown small but statistically significant differences in the lengths of the oestrous cycle of pregnant and non-pregnant kangaroos. Differences of this nature have not been described previously for any member of the Potoroinae. The search for their existence forms part of the studies reported upon in Chapter 4.

Although Flynn (1930) was unable to breed either the potoroo (*Potorous tridactylus*) or the related Tasmanian bettong in captivity, he suggested that both species had similar gestation lengths (6 weeks). Tyndale-Biscoe (1968, 1973) points out that length of gestation may vary considerably between related species, and makes the comparison between the potoroo and the boodie (*Bettongia lesueur*). The adults of both species are similar in size and give birth to young that weigh approximately 300 mg but the gestation period of the boodie is 21 days and that of the potoroo 38 days (close to Flynn's estimation). As a consequence of these observations, Tyndale-Biscoe (1968) observed that there is a need for the re-examination of the duration of the gestation length of the Tasmanian bettong.

The first bettong obtained in the present study was a female with a small pouch young (later estimated as aged 6 weeks). The pouch young was removed (RPY) and the female isolated from males. Sixteen days later a new young was present in the pouch, and a vaginal smear taken from the mother indicated oestrus. This single set of observations provided the initial impetus for the whole study on the reproduction of the Tasmanian bettong.

1.6 UTERINE CYCLE AND INTRA-UTERINE DEVELOPMENT

Tyndale-Biscoe (1973) queries why the potoroo required 17 days more than *B. lesueur* to produce the same sized offspring? He also pointed out that at that time 'embryonic development of neither species is known'. Shaw and Rose (1979) have since described the intra-uterine development of the potoroo and this thesis presents details of the embryonic growth of the Tasmanian bettong (Chapter 5).

Following ovulation in marsupials, a uterine 'secretory' phase is initiated as a result of the presence initially of an active corpus luteum. This phase is spontaneous and hence develops whether or not the animal mates (and becomes pregnant). Since pregnancy in most species of marsupials has little if any effect upon the length of the oestrous cycle and the development of the luteal phase, Hill and Donoghue (1913) used the term pseudo-pregnancy to distinguish the non-pregnant cycle from the pregnant cycle. Flynn (1930) studied the uteri of the Tasmanian bettong during various phases (of unstated and presumably unknown stages) of pregnancy. He found that there were few histological differences between the pregnant and contralateral non-pregnant uteri during early pregnancy, but that from mid-pregnancy, morphological and histological changes became evident. Some of these changes were merely a result of uterine distension by the expanding embryo but others, e.g. the rehabilitation of the uterine epithelium in the non-pregnant uterus and its maintenance in a 'well-stimulated condition', were found by Flynn to be 'peculiar and significant'.

The bettong is monovular but has paired uteri and Flynn used the terms 'pregnant' and 'pseudo-pregnant' to distinguish between the two uteri of the one individual during pregnancy. He did not study pseudo-pregnant animals in the sense used by Hill and Donoghue (1913). The

present study repeated some of Flynn's work but with known-age stages of gestation and concurrently also observed ovarian and uterine changes during pseudo-pregnancy (*sensu* Hill and Donoghue). The similarities and differences between the two conditions are detailed in Chapter 5.

Tyndale-Biscoe (1973) proposed that there are three phases of intra-uterine development in marsupials: (1) an autonomous phase in which the embryo exists mainly on its limited endogenous reserves, this preliminary phase may be extended during embryonic diapause; (2) an absorptive phase during which the embryo grows as a result of nutrient transfer from the secretory uterus via the bilaminar yolk sac; and (3) a respiratory phase during which the vascularisation of the yolk sac allows the rapidly growing embryo to cope with its increased respiratory demands; this final phase usually coincides with the dissolution of the shell membrane. Subsequently Tyndale-Biscoe^{et al} (1974) observed that much of the variation in gestation lengths between marsupials is due to differences in the first two stages which he amalgamated into the 'free vesicle or progestational' phase. Organogenesis occurs mainly after the shell membrane has ruptured (Hughes 1974) and although implantation involving disruption of the maternal uterine epithelium may not occur, close apposition of maternal and embryonic membranes is restricted to the rapid final phase of intra-uterine development.

The early phase of development proceeds slowly (Hughes 1974; Lyne and Hollis 1977; Selwood 1981) and Tyndale-Biscoe (1973) has suggested that this phase occurs in order to promote synchrony of embryonic and uterine development as there is a variable period after ovulation until a secretory uterus develops.

Embryonic growth is initiated in the potoroo within 2-3 days after the removal of the pouch young (Shaw and Rose 1979). This is associated with an early luteal phase. Chapter 5 reports an early luteal phase

(after RPY) in the Tasmanian bettong also and considers how this might be related to the animal's relatively short gestation period.

1.7 TEMPERATURE CHANGES DURING THE REPRODUCTIVE CYCLE

The well known and simple use of body temperature to monitor the human menstrual cycle and to pin-point ovulation has been applied to few animals apart from domestic species. Peters and Rose (1979) were the first to use bio-telemetric techniques to correlate changes in body temperature with the oestrous cycle in a marsupial. Their 'pilot study' on the common wombat (*Vombatus ursinus*) has been expanded considerably in this thesis with the bettong and in addition the periodic regression of both the diurnal rhythm and the variation associated with the reproductive cycle has been analysed by computer.

Although vaginal smears and/or the actual observation of mating will probably remain the preferred method for obtaining data on the length of the oestrous cycle and the timing of oestrus, little is known of the effect of daily capture and scraping of the vaginal wall. A simple method that avoids animal handling may have some part to play in reproductive studies on 'nervous' animals (such as Tasmanian bettongs) as with animals in the field. The possibility that bettongs might demonstrate temperature changes that could be correlated with different phases of the reproductive cycle (in particular, oestrus) was investigated and the results obtained are presented in Chapter 6.

1.8 POUCH LIFE AND VACATION

Sharman (1965) commented that Flynn's (1930) estimate of 6 weeks for the duration of pouch life in the bettong was most unlikely. This study has examined in detail the duration of pouch life and has

investigated some of the events that immediately precede pouch vacation.

Several species of kangaroos, e.g. *M. rufus* (Sharman and Calaby 1964) and *Thylogale billardierii* (Rose and McCartney 1982a), exhibit a precise sequence of events on the night the young finally leaves the pouch. There is a contraction of the pouch associated with the occurrence of birth and a post-partum oestrus. Sharman and Calaby (1964) implied that circulating levels of the sex steroid, oestrogen (associated with the approach of oestrus), played a role in the final vacation of the pouch. The present study has followed up this possibility and has examined the hypothesis that final pouch vacation in the bettong is a result of changes in maternal physiology.

1.9 CONSERVATION

For many years, including the early part of this study, the eastern bettong (=Tasmanian bettong) was on an international list of endangered species (Fisher 1969). This alone was sufficient reason for a detailed study of the reproduction and growth of captive members of the species. During the course of the study, the conservation status of the species eased to 'vulnerable' (Johnson and Rose 1983; Rose 1983). After the development of a small breeding colony more animals were available for experimental purposes and study, and this allowed the more detailed studies in Chapters 5, 6 and 7 to be initiated.

1.10 SUMMARY OF AIMS

1. A detailed study of reproduction of the bettong, in particular an examination of the gestation length in order to check the estimate of 6 weeks suggested by Flynn (1930);

2. the provision of data to allow the reliable estimation of ages of both pouch young and adults in order to assist field studies of this vulnerable species;
3. a comparison of growth rates of a member of the Potoroinae with other Macropodinae;
4. a histological comparison of the uteri from pregnant and non-pregnant animals to determine differences brought about by the presence of a fetus;
5. a study of the embryonic growth (in conjunction with 4. above) to determine whether growth provides a key to the understanding of the disparities between gestation lengths in the sub-family Potoroinae;
6. the development of a telemetry system that would allow a study of the temperature changes during the reproductive cycle, and the application of this system to the determination of the timing of oestrus in animals in captivity, and perhaps in the wild also;
7. an investigation of the cause of final pouch vacation in the Tasmanian bettong.

CHAPTER 2

GENERAL BIOLOGY AND MAINTENANCE

OF THE BETTONG IN CAPTIVITY

CHAPTER 2 GENERAL BIOLOGY AND MAINTENANCE OF THE BETTONG IN CAPTIVITY

2.1 INTRODUCTION: TAXONOMY

There are three major groupings of Australian marsupials: the herbivorous diprotodontids which include the Macropodids (kangaroos); the carnivorous dasyurids, and the intermediate peramelids.

Although the kangaroos comprise a diverse group of some 50 species, most authors have included them in the one family, Macropodidae, within which there are two major sub-families, the Potoroinae and the Macropodinae. The potoroines are considered to be the more primitive of the two groups.

Pearson (1946) elevated the sub-family Potoroinae to that of a family (Potoroidae) in which he included the musky rat kangaroo (*Hypsiprymnodon moschatus*). Few authors accepted this elevation until the recent papers by Archer (1981) and Johnson and Strahan (1981). There are obvious differences in the dentition between the two groups, the Potoroidae having bunodont and the Macropodidae lophodont dentition (Archer 1981). However there are intermediate fossil types (Archer 1981) so that the actual relationship between the groups is somewhat blurred. In this thesis the term Potoroinae will be retained as it has been in common usage for some time.

2.2 THE GENUS *BETTONGIA*

This genus was established by Gray in 1837. Prior to 1967 the genus comprised four species: *Bettongia lesueur*, *B. penicillata*,

B. gaimardi and *B. cuniculus*.

In 1967, Wakefield, on the basis of skull morphology synonymised the Tasmanian species *B. cuniculus* with the Australian mainland form, *B. gaimardi*. The mainland form is believed to be extinct (Poole 1979). In his review Wakefield (1967) also erected a new species from Queensland based solely on museum collections of skulls. This species, which he called *B. tropica*, was until recently thought to be extinct. However, Sharman *et al.* (1980) after rediscovery of a Queensland specimen attributable to *B. tropica*, have studied its chromosomes but found no reason to distinguish it from the west Australian form of *B. penicillata*. The specimen obtained by Johnson and Sharman may well have been *B. penicillata* although this does not exclude the possibility that *B. tropica* once existed.

2.2.1 Nomenclature and Taxonomy

Bettongia gaimardi was originally described by Desmarest (1822) as *Kangurus gaimardi*, so named as it had been first found by Gaimard near Port Jackson. The same specimen was also named *Kangurus lepturus* (Gaimard 1824) and *Hypsiprymnus whitei* (Quoy and Gaimard 1824).

2.2.2 The Tasmanian Bettong: *Bettongia gaimardi cuniculus*

The taxonomic history of this sub-species has been summarised by Wakefield (1967). Ogilby (1838) described a specimen, which he cited as from "Hunter's River, New South Wales" as *Hypsiprymnus cuniculus*. Waterhouse (1846) suggested that an error of locality had been made and that the specimen was in fact from Tasmania. He based his suggestion on the morphological measurements of the type skull. Although the basal length of the skull of the Tasmanian species is 10% longer than

in the mainland sub-species, only one of many skull proportions (measured by Wakefield 1967) is statistically different between the two forms.

Because of the larger body size, the proportionally less developed bullae and the less developed tail crest of Tasmanian specimens, Wakefield considered that the Tasmanian population should be given sub-specific status: *B. gaimardi cuniculus*. The extinct mainland form was assigned sub-specific status as *B. g. gaimardi*. *Bettongia gaimardi* is distributed over much of the eastern half of Tasmania. Its currently known distribution is illustrated in Figure 2.1. The mainland species of the genus *Bettongia* have suffered a dramatic decline in range and numbers since the advent of European man and his introduced eutherian mammals; fire and fox in particular have been considered as having had a major role in the decreased distribution of the genus (Christensen 1980). Only in Tasmania are bettongs relatively abundant.

The disappearance of bettongs from the mainland was coincident with the dramatic population expansion of rabbits and foxes, and because foxes have not become established in Tasmania it is tempting to identify them as a principal cause of the decline. However, rabbits apparently never reached the abundance in Tasmania that they did on the mainland and consequently may not have been so destructive of the grassy habitat required by the bettong for cover. This in turn may have allowed the bettong to maintain levels of population in Tasmania not found on the mainland (Johnson and Rose 1983).

2.3 DISTRIBUTION

In Tasmania *B. g. cuniculus* is found over a large area of eastern Tasmania extending from the north-east to the southern regions (Figure 2.1). The species extends to parts of the central highlands at altitudes

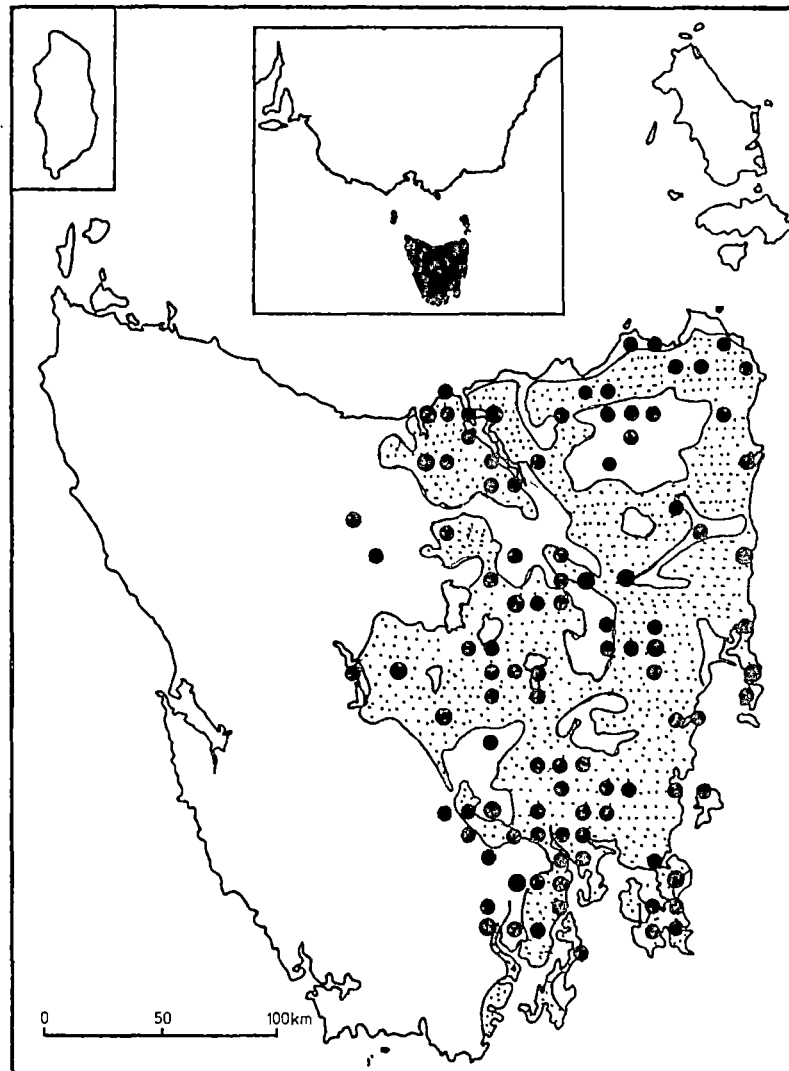


Figure 2.1. The present distribution of the Tasmanian Bettong.
The stippled area represents the distribution of open forest in this state.

greater than 1000 m. Island populations are found on Bruny and Maria Islands. There are no bettongs on the large Bass Strait islands King and Flinders, nor is there any evidence of bettongs having been on these two islands in the recent past as Hope (1973) was unable to find sub-fossils of the species. However, Le Soeuf (1924) states that "in addition, another species of Rat-Kangaroo, probably *Bettongia cuniculus* (= *gaimardi*) is reported as still being occasionally seen on Flinders and Barren Islands". *Aepyprymnus rufescens* has been found as a sub-fossil on Flinders Island and it is possible that Le Soeuf misidentified *Aepyprymnus* as *Bettongia*.

2.4 HABITAT (Figure 2.2)

The distribution of the Tasmanian bettong is very similar to the distribution of open forest in Tasmania as defined by Specht (1970); such forest was previously referred to as dry sclerophyll (Jackson 1965). In Figure 2.1 the distribution of the bettong is superimposed upon the distribution of open forest. Those points in Figure 2.1 that appear outside the general region of open forest are in fact in smaller pockets of this type of forest (Jackson pers. comm.). The understorey is usually low (Figure 2.2b) and is comprised of either grass species, e.g. *Poa rodwayi* (Tussock grass), *Danthonia* sp. (Wallaby grass) or *Agrostis* sp. (Browntop). The most prominent understorey species is the common sag (*Lomandra longifolia*) which could be designated as an indicator species for the bettong. In recently burnt areas the bracken fern (*Pteridium* sp.) may be common. Other ground storey species may include *Lomatia tinctoria* (guitar plant) and in wetter areas, *Gahnia* sp., *Restio* (Cord rush) and *Empodisma minor* (Sedge). Heath species such as *Epacris* sp. and *Leucopogon collinus* are present in sandy areas. Medium-

a) Koonya, south-east Tasmania



b) Kempton, eastern Tasmania



Figure 2.2 Typical bettong habitat. Medium height shrubs are absent and the ground cover can be sparse.

sized shrubs consist mainly of tea-tree (*Leptospermum lanigerum*). *Eucalyptus viminalis* and *E. obliqua* are the dominant tree species (Figure 2.2a), but in higher and more extreme habitats this role is taken by *E. pauciflora* (weeping or snow gum). *Acacia dealbata* (silver wattle) is also a common tree species.

Most bettong habitats are situated on dolerite soils, although they are occasionally found on granite in some areas in the north-east and east coast and in regions of sandstone in the north-east. The soils are not fertile and are often leached (podzolic). With the exception of the central highlands, much of which can be covered with snow for extended periods, the bettong is found only in the warmer parts of Tasmania with relatively low rainfall (mean 50 to 75 mm annum⁻¹).

2.5 NESTS

The Tasmanian bettong has a slightly prehensile tail with which it is able to carry nest material for some distance. One bettong was shot in the wild with a tail full of suitable nest material: *Selaginella* (pteridophyte); grass (*Tetrahena* sp., Hairy rice grass) and cord rush; the whole package weighed about 100 g. One nest at Kempton contained three species of grass (*Danthonia* sp., *Poa rodwayi* and *Agrostis* sp.) and was 'wrapped' in a basket of leaves and bark of *Eucalyptus obliqua*. The nest contained seeds from a number of commercial crops including millet, sorghum, oats, maize, wheat and sunflower. As the nest was some distance from a farm, the bettong presumably carried the seeds to the nest. These seeds may have been stored as animals in captivity sometimes stored food.

The Tasmanian bettong builds densely-woven nests of dry grass and bark which they conceal with remarkable skill. Nests are commonly sited

under fallen limbs, along clumps of short bushes or tussocks, or under fallen branches from which the leaves have not dropped. The nest is an oval shaped hollow structure about 300 mm long by 200 mm wide. The walls are 20-30 mm thick and a small opening is made at one end. The nest is constructed in a shallow depression about 150 mm in diameter and 50 mm deep which the animal digs using its strongly clawed forelimbs.

The long and prehensile tail is used to carry nest material. This is collected while the animal sits on its hindlegs with the tail forward under the body. Nest material is gathered with the front paws and placed across the tail. When enough is collected the tail is wrapped around and the bettong hops forward over the load and proceeds to the nest site holding the tail well clear of the ground.

Bettongs are nocturnal and usually rest in their nests for the entire daylight period. They generally leave the nest just on dark and return before first light. Departure from the nests is usually rapid and the bettong travels some distance before pausing. The animals normally remain hidden until one approaches to within a few metres whereupon they erupt suddenly from the nest, often uttering a sharp hiss, and hop rapidly from sight. Nests may be used regularly for periods of at least 30 days, and some may be re-occupied after being vacant for a similar period. In captivity, bettongs made numerous nests out of whatever material was available. In the summer, on hot days, they often dismantled their nests.

Home ranges of between 65 ha and 135 ha may be used and bettongs sometimes travel as much as 1.5 km between a nest site and a feeding area (Johnson and Rose 1983). In contrast with the larger macropods of Tasmania they are rarely seen on pasture land.

2.6 DIET

Qualitative analysis of the faeces from seven wild caught animals indicated a diet that consisted predominantly of hypogean fungi. Other material found included fibrous rootlike material and seeds (including those of *Lomandra longifolia*). Insects were found in a number of stomach contents obtained from animals killed in the wild, but as they were whole it appears that they might have been ingested by accident. A gummy substance was often present in the stomach contents (3 animals) and the faeces; this was probably an *Acacia* exudate as bettongs were seen to eat the exudate from such trees.

2.7 CAPTURE AND MAINTENANCE

Cook (1973) has described briefly the maintenance of *B. penicillata*, and Tyndale-Biscoe (1968) that of *B. lesueur*. Although *B. gaimardi* is far more common than the other species, little work has been reported since the early paper by Flynn (1930). The following information is summarised from Rose (1982).

2.7.1 Capture

Medium-sized treadle traps (46 cm x 30 cm x 30 cm) baited with bread and apple smeared with peanut butter were placed along the 'runs' of *B. gaimardi* and checked the following day. In the wild *B. gaimardi* are so nervous that traps containing them were approached with caution (lest the animals damage themselves), and the animals were quickly transferred to thick hessian bags before being transported to the holding areas. The animals were usually left in the opened bag until they found their own way out after nightfall. Pouch young were removed

routinely after it was noted that newly caught females usually lost their young within a week of capture. This phenomenon was also noted by Flynn (1930).

2.7.2 Enclosures

The animals were kept in three cages in an enclosed area of bush near the Zoology Department of the University of Tasmania. The cages (4 m x 4 m x 3 m) were made from wire mesh covered with an outer layer of aviary wire to keep out birds. The framework of the cages was embedded in concrete but the floors of the cages were bare earth, since *B. gaimardi* showed no signs of digging. Roofs were necessary to prevent the animals from climbing out; these were made of corrugated fibreglass. Water taps were installed in each cage. Nest boxes were provided in the form of teachests or fruit boxes although the animals preferred to construct their own nests out of bales of hay or straw. Nests of straw appeared to be sufficient to conserve the animals' body temperature in winter and to protect them from the heat of the day during summer. The animals constructed many nests and sometimes used more than one during one night.

Several animals were placed in the enclosed area of bush.

2.7.3 Feeding and Diet in Captivity

In captivity, the animals were given a standard diet of apples, bread, rolled oats and solid dog pellets. Lucerne, grass and kangaroo pellets were originally provided but only rarely were they eaten and never in preference to any of the standard diet. The animals rarely ate green vegetables although they did eat carrots, cooked potatoes and peanuts. They gnawed cooked meat and chicken from bones and also ate mealworms. Although water was always available, the animals drank rarely.

Only small water containers were provided after one juvenile drowned in a bucket of water. A salt-lick was provided and vitamin drops were occasionally added to the bread, as suggested by Tyndale-Biscoe (1968), but these supplements appear unnecessary as the animals grew and bred without them.

2.7.4 Handling

Recently caught *B. gaimardi* were nervous, aggressive and hence difficult to handle. They attempted to bite and sometimes kicked strongly with their long, powerful hind legs. Animals reared in captivity were much tamer and easier to handle. *B. gaimardi* in cages were either caught by hand from the nest, if tame, or with a small hand-net. They were held by the tail with their hind legs pointing away from the handler and then placed into a sack within which they could be more easily manipulated. Even within the sacks, the animals had to be held firmly as they struggled, often violently.

A female's pouch was checked by placing her in the bag between the handler's knees, grasping her tail and hind legs and turning her onto her back. Stretching the hind limbs facilitated examination of the pouch and the taking of vaginal smears.

Halothane (Astra Chemicals, N. Ryde, N.S.W.) was used as an anaesthetic for most surgical procedures following an intra-muscular injection of Ketalar (Ketamine hydrochloride) at a dose of 10 mg kg^{-1} . Blood samples were most commonly obtained by heart puncture as suitable superficial veins were difficult to find.

2.8 BEHAVIOUR

Little is known about the behaviour of *B. gaimardi* but it appears similar to that which Stodart (1966) described for *B. lesueur*.

B. gaimardi were aggressive, and adult males had to be separated.

Occasionally there was intersexual aggression but usually only between newly caught animals. Young males could be housed in the same cages as their fathers, but only until they approached maturity.

Aggression between females was infrequent and took the form of kicking with hind limbs while lying on one side; the opponent's pouch could be damaged slightly. *B. gaimardi* are nocturnal and were rarely seen out of their nests during the day. At night even nervous animals were approached more easily than during daylight. Young animals remained close to their mothers in the one nest until they approached maturity.

2.9 DISEASES AND PARASITES

In captivity *B. gaimardi* appeared remarkably free from disease and ecto-parasites. No adult died from disease during 3 years, although some small pouch young did die. Tyndale-Biscoe (1968) also noted that pouch young died in captivity.

Some animals damaged their long nails when fighting or climbing the wire mesh walls, but these wounds cleared up after the application of disinfectant and the topical antibiotic powder, tetracyclin. One animal that had been maintained with other species of marsupial in a zoo, was brought in suffering from a skin complaint that had caused it to lose large areas of fur. Examination showed this to be associated with a small mite. Ticks were occasionally found on *B. gaimardi* and some wild populations had both fleas and lice.

CHAPTER 3

GROWTH AND AGE ESTIMATION

CHAPTER 3 GROWTH AND AGE ESTIMATION

3.1 INTRODUCTION

The short gestation period of marsupials determines that most growth occurs outside the uterus. This is initially accomplished within the stable environment of the pouch where the young are readily accessible and make an excellent subject for the study of growth.

Studies of growth are usually undertaken to enable an understanding of the energetics of a particular species to be obtained or for the purposes of estimating the age of individuals. The accurate ageing of animals is necessary in order to obtain information on breeding seasons and population dynamics.

Growth of pouch young is usually monitored quantitatively by measuring the increase in body weight and the growth of various major body parts such as the head, foot and tail. The correlation between age and growth at this stage is high because of the rapid rate of development but qualitative observations on structures such as eyes and ears are generally used to supplement quantitative data. Numerous studies of the growth of macropodid pouch young are available, e.g. *Potorous tridactylus* (Guiler 1960); *Setonix brachyurus* (Shield and Woolley 1961); *Macropus rufus* (Sharman, Frith and Calaby 1964); *M. giganteus*, *M. robustus*, *M. rufogriseus* (Kirkpatrick 1965; Ealey 1967; Poole (unpublished, cited by Poole and Pilton 1964); *M. agilis* (Maynes 1972); *M. eugenii* (Murphy and Smith 1970); *M. fuliginosus* (Poole 1976); *Aepyprymnus rufescens* (Johnson 1978); *Petrogale penicillata* (Johnson 1979) and *Thylogale billardierii* (Rose and

McCartney (1982b).

The rate of growth usually declines after the young leave the pouch (Maynes 1972) and so other methods must be employed to provide a reliable estimate of age. These commonly involve analysis of the eruption and progression of molar teeth (Kirkpatrick 1965).

In the genus *Bettongia*, Tyndale-Biscoe (1968) studied the growth of six pouch young of *B. lesueur* and he has provided details of qualitative changes and tooth eruption. The brush-tailed bettong *B. penicillata* has been studied by Sampson (1971) and Christensen (1980) but in both of these studies there was a paucity of data on known-aged specimens. Qualitative and behavioural aspects of pouch life in a small colony of *B. penicillata* in the Peabody Museum at Yale University were examined by Cook (1973, 1975). There has been only one previous study on the growth of the Tasmanian bettong (*B. gaimardi*). That study was carried out by Lukshanderl and Lukshanderl (1969) and information is limited to the development of a single pouch young (150 g) of unknown age that had been rejected by its mother.

This study presents for the first time details of the growth and development of *B. gaimardi* of known age from birth to sexual maturity. Investigations involved both quantitative and qualitative parameters and allowed the estimation of the age from birth to approximately 3 years.

3.2 METHODS AND MATERIALS

3.2.1 Measurements

Data were obtained at regular intervals from 23 pouch young born in captivity whose dates of birth were known to within 24 hours. Weekly measurements and observations were made on seventeen of these

pouch young and later used for computational purposes. Observations on young once they had left the pouch were less regular but generally continued until maturity. The measurements obtained were those of the length of the head, right foot and tail; body weight was measured simultaneously. Measurements were obtained following the procedure described by Sharman, Frith and Calaby (1964). A number of young (5) were measured and weighed shortly after birth but thereafter young were not usually removed from the teat until week 5 except for experimental reasons. Prior to week 5 young could be removed from the teat and returned, although the return often took some time to accomplish.

Measurements of length were made to the nearest mm with vernier calipers and weight could be obtained to within 1 g of body weight by use of a field pan balance. Small pouch young could be weighed with greater precision in the laboratory.

Maturity in males was defined as occurring when abundant sperm were present in the urine and/or the animal sired a young; females were considered mature when a mating resulted in the birth of a young. Once maturity had been attained most animals were no longer measured.

3.2.2 Tooth Eruption

Data on the eruption of molar teeth and the replacement of premolars were obtained regularly from 10 animals of known birth date. Additional data were obtained from the cleaned skulls of 20 bettongs. In live animals the number and degree of eruption of molar teeth were observed by use of an otoscope while the jaws were held apart with loops of nylon cord. The criteria used to denote the stage of molar eruption were based on Sharman, Frith and Calaby (1964). The molar stages of .0 and .1 could not be distinguished in live specimens.

TABLE 3.1 Notation used to designate degree of molar eruption

Notation	Position of Anterior Loph	Position of Posterior Loph
.0	below jaw	below jaw
.1	through jaw below gum	through jaw below gum
.2	through gum	below gum
.3	partly erupted	just through gum
.4	fully erupted	completing eruption

Roman numerals preceded by M were used to indicate fully erupted teeth; thus MIII denotes that the third molar was fully erupted and MIII.2 denotes that the third molar was fully erupted and the anterior loph of the fourth molar had broken through the gum.

3.2.3 Age of Appearance of Certain Body Characteristics

The information summarised in Figure 3.4 was obtained from observations on the live pouch young as well as 36 preserved young obtained and stored in formalin during the study. Most observations were by the unaided eye, though a 10X lens was used to determine the sex of very small preserved young.

3.2.4 Suckling

After vacating the pouch, young continue to suck milk from the enlarged teat for some time. Although actual sucking by young out of the pouch was seen infrequently (as it occurs either within the nest or at night), this activity was inferred by the presence of a protruding teat (through the pouch entrance) and/or an enlarged teat from which

fluid could be manually expressed with ease.

3.2.5 Growth Rates

The mean instantaneous relative growth rate was obtained for the linear portions of the semi-logarithmic graph (log.weight against age) using the following formula:

$$K = \frac{\text{natural log.W2} - \text{natural log.W1}}{\text{age 2 (days)} - \text{age 1 (days)}} \log_e \text{ g.day}^{-1}$$

K is the instantaneous relative growth rate

W = weight in grams

(Huxley 1932; Brody 1945)

The linear equivalent (= cube root of weight) has been calculated to allow comparison of the growth of the head, tail and foot against a generalised body growth factor. The term 'linear equivalence' was introduced by Lyne and Verhagen (1957) to permit the comparison of the growth of various body parameters of *Trichosurus vulpecula* to be made against some standard. Although in theory the linear equivalence is defined as 'the length of the side of a cube whose volume is equal to that of the animal', in practise it is adequate to work with the cube root of the body weight (Lyne and Verhagen 1957).

3.2.6 Field Data

Morphological measurements of adult bettongs were obtained from various localities in eastern and south-eastern Tasmania. A sample of 14 animals from a population of bettongs at Kempton was trapped monthly over a six month period. Multiple capture of four females with pouch young in this population allowed some estimate of growth in the field; however, in most cases young manipulated for the purpose of

measurement were found to be missing when the mother was subsequently recaptured. (These data are included in Appendix A.)

3.2.7 Calculations and Statistical Treatment

(a) Students t-test were applied to the measurements of head length and weight of male and female pouch young at ages 4, 8, 12, and 16 weeks as well as to the measurements from adult bettongs in order to determine if sexual dimorphism occurs in the development of these parameters. Similar computations were applied to test for morphological differences between wild and captive bettongs.

Multiple regressions for the parameters measured during pouch life were obtained and the equations of best fit are presented. The accuracy of these regression equations has been tested by their use in the estimation of the ages of new individuals that were not involved in the construction of the equations. In this way a graph could be obtained of estimated age against actual age.

A chi-square test was used to test whether or not the sex ratio differed significantly from unity.

(b) Five stages of molar eruption could be recognised, as for the red kangaroo (Sharman *et al.* 1964). However, for the statistical analysis the stages of eruption were given decimal notations accumulating in fifths as described by Newsome *et al.* (1977) for the agile wallaby (*M. agilis*). This method has the advantage over the step/jump scale of the older notation of providing an almost continuous scale (up to 5) for statistical analysis. Equations are presented for the regression of the natural log of age against molar eruption following the procedure of Dudzinski *et al.* (1977).

3.3 RESULTS

3.3.1 Pouch Young

Neonates

Bettong young shortly after birth appear similar to other kangaroo neonates (Plate 3.1). The head is prominent with an enlarged olfactory region and a gaping mouth. The ears are fused with the skin of the head and pigmented eyeballs are visible beneath the undifferentiated skin. The forelimbs are relatively well developed in comparison to the paddlelike hindlimbs which are small and apparently useless as locomotory organs. The forelimbs possess deciduous claws that are shed 2-3 weeks later and replaced by permanent ones. A small tail, the free end of which extends on to the ventral surface of the body between the hindlimbs, obscures the pouch anlage or scrotal region ^(visible by low power microscopy) and makes the determination of sex difficult in live neonates.

Although all but one of the neonates (more than 50 births in captivity) had attached themselves to a teat by the time of observations at mid-morning, on those occasions when removal was attempted the young could be returned with little difficulty. In replacing young manually it was necessary to restrict the sinuous side to side (teat-searching?) motion of the head. Young removed on the day of birth were able to reattach without assistance.

The weights of five neonates were 0.2977, 0.2982, 0.3067, 0.3010 and 0.3325 g (\bar{X} = 0.3072 g). The lightest of the five was unattached in the pouch and it is likely that it had not as yet had a drink.

The mean mother to young weight ratio was 5542 to 1 (N = 5).



Adult bettong 1.8Kg.



New born bettong 0.3g. Crown-rump length 1.4mm.

3.3.2 Teat Selection

No systematic data were collected on the selection by neonates of one of the four teats. However, it was obvious that neonates avoided teats still in use by young 'at foot'. It was also clear that recently used teats that were enlarged were also not utilised.

3.3.3 Growth

The increases in mean weight and the mean lengths of the head, foot and tail over weekly intervals are presented in Table 3.2 together with the sample number (N), standard deviation and range. Figures 3.1, 3.2 and 3.3 illustrate these mean values together with their 95% confidence limits. The regression equations of best fit are presented in Table 3.3 for the fifteen week pouch life. The confidence limits increase towards maturity (Figure 3.3) since the sample size has decreased and individual variation increases as animals approach their adult size.

It is apparent that each of the measured parameters increases with increasing age and that the growth curves flatten out as maturity approaches. Most animals reach their adult size by 240 days.

There is a slightly different curve for each of the measured parameters. Head length increases in a linear fashion until the end of pouch life after which there is a gradual slowing and progressive overlapping of the confidence limits. There is a sigmoidal increase in foot and tail length with increasing age and the steepest part of the curves occurs near the end of pouch life. Confidence limits for the estimates of foot and tail lengths start to overlap after the termination of pouch life. This indicates that, although measurements of these parameters are likely to be useful for the estimation of age

TABLE 3.2 Weekly measurements of head, foot, tail and body weight of *B. gaimardi*
Values are mean \pm standard deviation and range

Age (days)	N	Head (mm)	Foot (mm)	Tail (mm)	Weight (g)
0	3	7.3 \pm 0.5 (7.0–8.0)	–	5.0	0.3
7	6	10.0 \pm 0.9 (8.5–11.0)		5.7 \pm 1.2 (5–7)	1.1
14	8	12.6 \pm 0.9 (12.0–14.0)	6.2 \pm 0.8 (5.0–7.5)	10.6 \pm 1.5 (9–12)	2.2 \pm 0.8* (1.66–2.75)
21	9	15.8 \pm 1.5 (13.0–17.0)	9.2 \pm 0.7 (8–10)	14.8 \pm 1.9 (11–17)	4.1 \pm 0.4* (3.8–4.4)
28	9	17.9 \pm 1.2 (16.5–20)	13.2 \pm 1.2 (12–15)	21.8 \pm 2.0 (19–25)	5.7 \pm 1.8† (3.0–6.9)
35	7	21.1 \pm 0.9 (20.0–22.0)	16.6 \pm 1.7 (14–19)	30.1 \pm 2.7 (26–34)	11.9 \pm 2.4† (9.6–14.9)
42	11	24.9 \pm 2.7 (20.0–30.0)	22.4 \pm 3.7 (18–30)	37.1 \pm 3.5 (33–44)	17.6 \pm 2.6 (12–21)
49	12	28.1 \pm 1.7 (25–30)	27.5 \pm 2.6 (22–32)	43.5 \pm 3.2 (38–47)	26.1 \pm 4.3 (17–31)
56	10	30.8 \pm 1.8 (27–33)	32.3 \pm 2.9 (26–33)	53.4 \pm 4.0 (46–58)	35.4 \pm 5.0 (25–39)
63	12	34.9 \pm 2.1 (31–38)	40.8 \pm 3.4 (33–47)	62.8 \pm 5.8 (53–73)	50.0 \pm 6.7 (33–60)
70	12	38.6 \pm 2.2 (34–41)	50.5 \pm 4. (41–61)	75.6 \pm 6.6 (62–83)	68.1 \pm 8.7 (47–82)
77	12	43.0 \pm 2.3 (38–46)	60.3 \pm 4.0 (51–64)	93.3 \pm 10.4 (75–108)	93.9 \pm 11.3 (66–111)
84	15	46.5 \pm 2.3 (42–50)	71.9 \pm 5.3 (61–79)	116.1 \pm 8.5 (95–125)	128 \pm 13 (92–144)
91	12	50.6 \pm 2.1 (46–53)	81.2 \pm 5.1 (72–88)	139.7 \pm 10.1 (121–155)	173 \pm 20 (128–205)
98	14	55.9 \pm 2.8 (50–60)	90.9 \pm 4.5 (83–95)	169.5 \pm 9.8 (145–180)	247 \pm 23 (191–289)
105	15	60.9 \pm 2.4 (57–63)	96.4 \pm 4.2 (87–102)	190.5 \pm 12.1 (170–205)	333 \pm 33 (279–370)
112	12	66.3 \pm 1.7 (63–69)	99.8 \pm 4.5 (92–106)	210.5 \pm 15.5 (190–245)	433 \pm 43 (370–488)

TABLE 3.2 (continued)

119	13	69.2±3.1 (65-73)	103.0±4.8 (91-109)	221.5±16.0 (22-250)	530 ± 68 (399-638)
126	12	72.6±3.5 (67-79)	105.1±4.5 (95-110)	236.3±17.9 (205-265)	647 ± 82 (500-775)
133	14	75.0±2.7 (71-78)	106.7±4.4 (97-112)	249.6±19.4 (225-285)	780 ± 88 (631-910)
140	13	77.2±2.6 (73-81)	107.2±4.1 (98-113)	259.8±15.2 (240-290)	878 ± 101 (707-1005)
147	13	78.8±2.7 (75-82)	110±4.2 (105-119)	269±14.1 (250-290)	1009 ± 104 (816-1160)
154	13	80.4±3.0 (76-85)	10.3±4.5 (105-119)	272.2±15.1 (255-295)	1107 ± 107 (912-1270)
161	9	81.3±3.0 (77-85)	110.7±3.8 (105-117)	283.1±12.8 (260-295)	1174 ± 109 (1046-1350)
168	12	82.4±2.2 (78-86)	111.3±5.2 (101-120)	291.7±19.0 (265-310)	1279 ± 106 (1135-1400)
175	11	83.6±1.9 (81-85)	113.2±5.3 (100-120)	301.3±13.7 (280-320)	1325 ± 103 (1175-1530)
182	9	86.4±2.4 (83-90)	113.2±4.8 (105-120)	307.5±14.4 (290-325)	140 ± 117 (1190-1580)
189	11	85.8±1.9 (83-90)	112.4±5.0 (108-117)	307.2±16.4 (290-325)	146 ± 122 (1308-1650)
196	10	85.6±1.4 (83-87)	114.4±5.5 (111-121)	306.1±12.4 (285-320)	147 ± 123 (1270-1675)
203	8	86.5±3.1 (82-93)	115.8±3.0 (111-120)	311.9±13.6 (290-330)	1530 ± 143 (1330-1750)
210	7	87.0±4.5 (82-93)	115.3±3.3 (111-119)	305.7±8.4 (290-310)	1577 ± 129 (1350-1775)
217	4	89.8±2.9 (86-92)	114.5±2.7 (111-117)	301.7±7.6 (295-310)	161 ± 139 (1400-1775)
224	5	90.2±2.7 (87-93)	115.6±4.3 (112-122)	319.0±24.9 (285-350)	1670 ± 157 (1475-1850)
231	4	89.3±1.5 (88-91)	116.3±4.3 (111-121)	330.0±19.6 (310-350)	1645 ± 148 (1450-1850)
238	2	88.0 (87-89)	113	302.5±3.5 (300-305)	1670 ± 110 (1590-1850)
245	3	89.0±3.4 (85-91)	116.5±2.1 (115-118)	317.5±24.7 (300-335)	

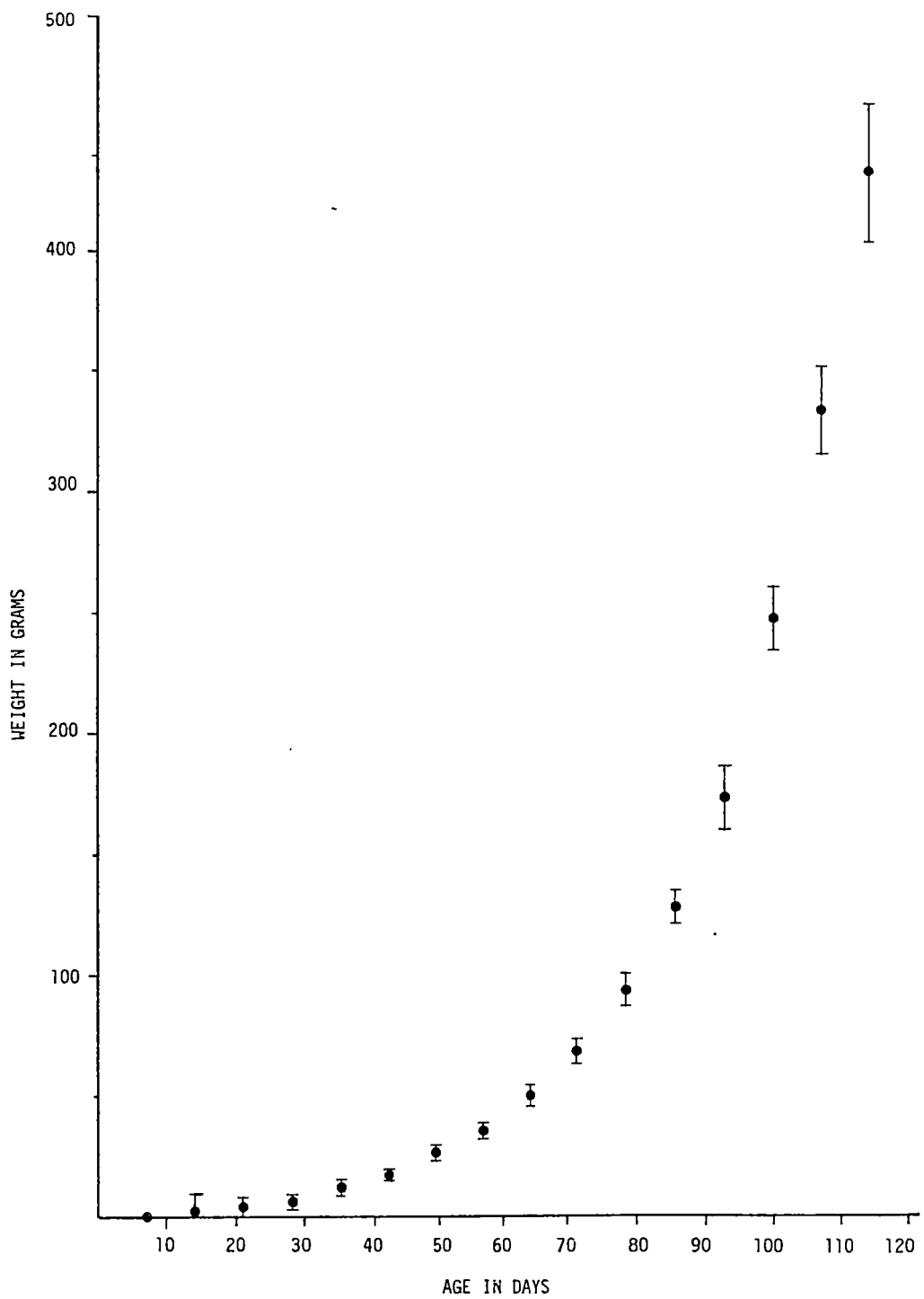


Figure 3.1 Mean weight ($\pm 95\%$ confidence limits) against age during pouch life.

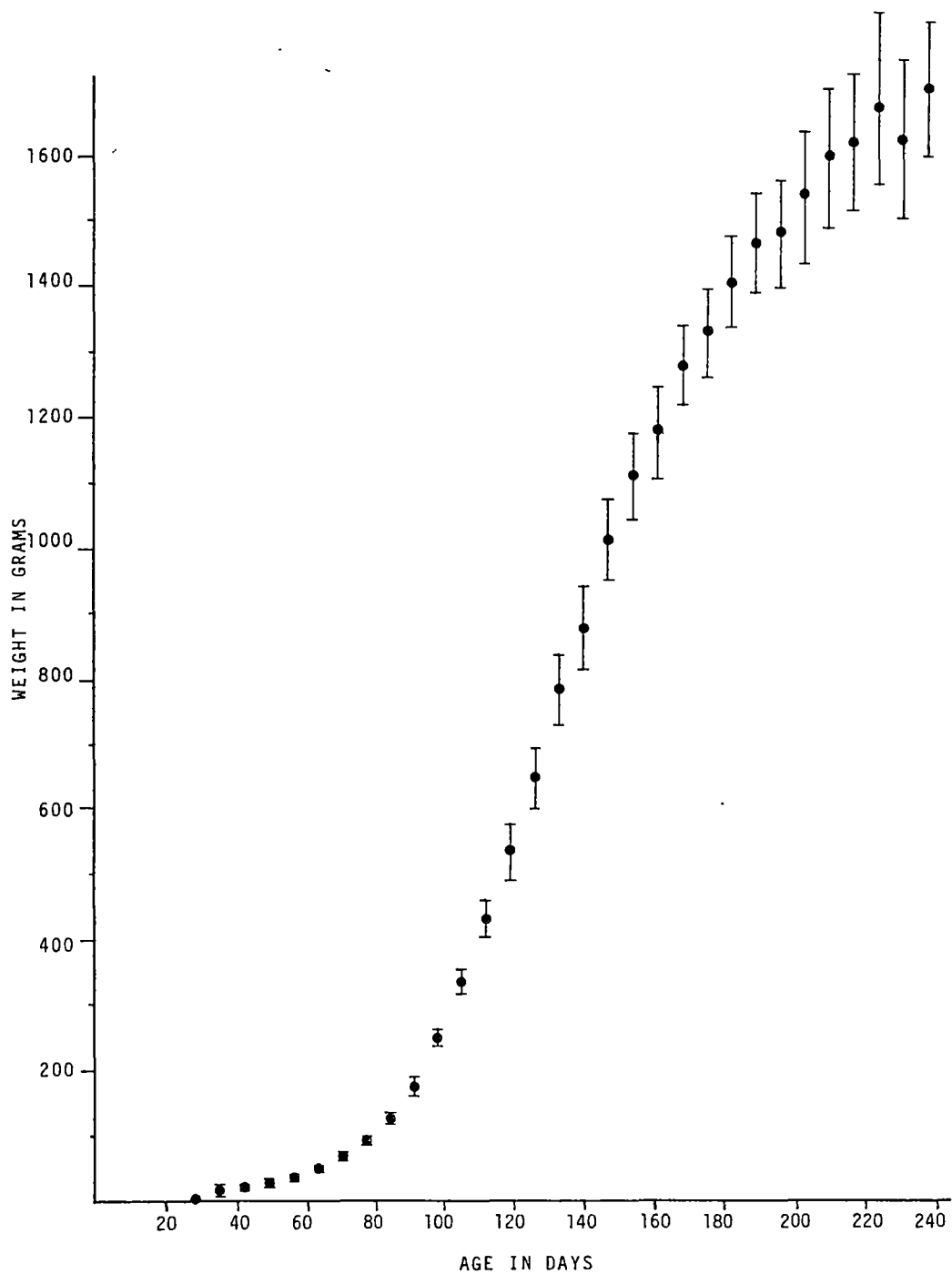


Figure 3.2 Mean weight ($\pm 95\%$ confidence limits) against age from birth to maturity.

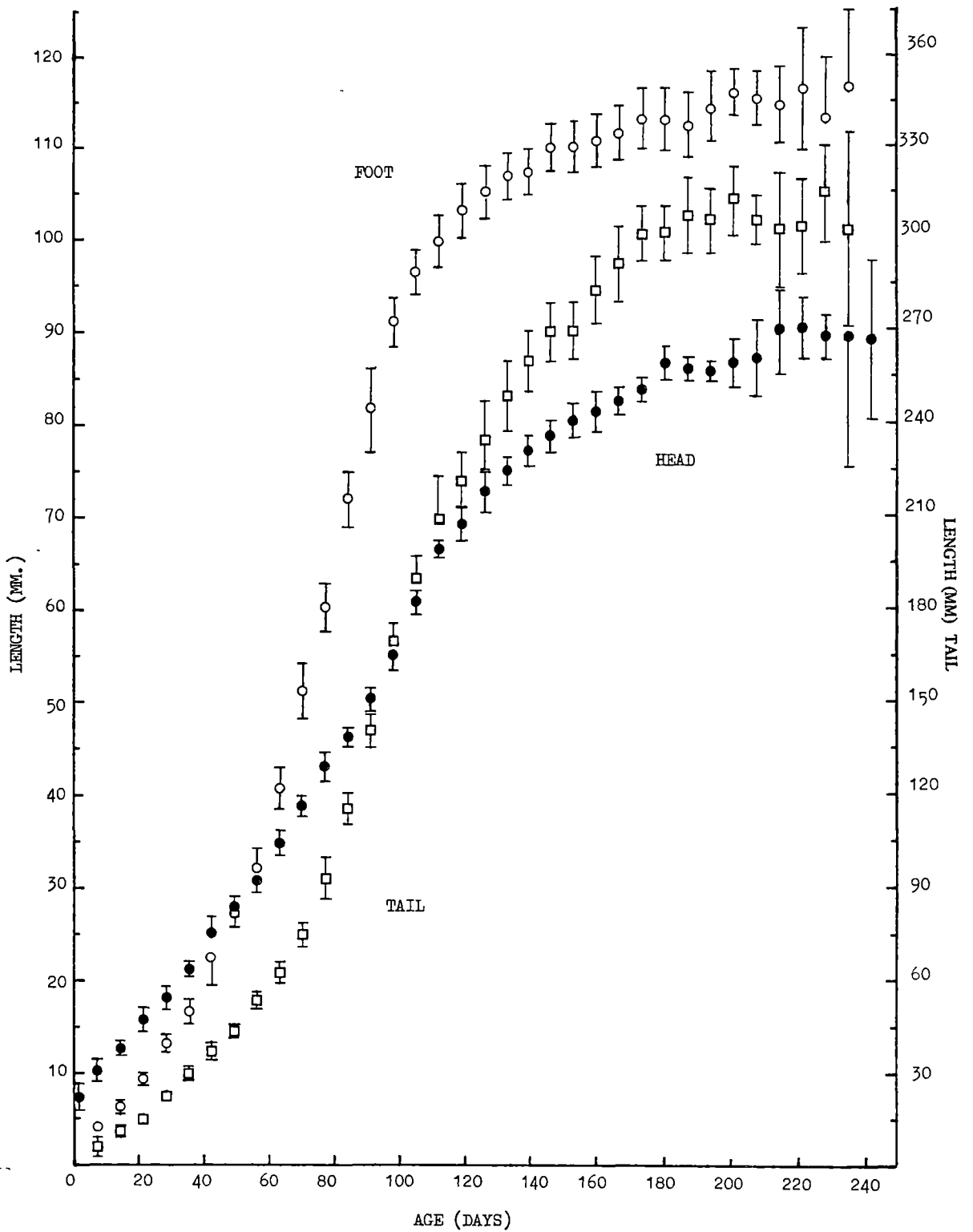


Figure 3.3 Head, foot and tail length against age. Mean values are given $\pm 95\%$ confidence limits.

during pouch life, accuracy of estimation will decline after this period.

3.3.4 Developmental Stages of Pouch Life

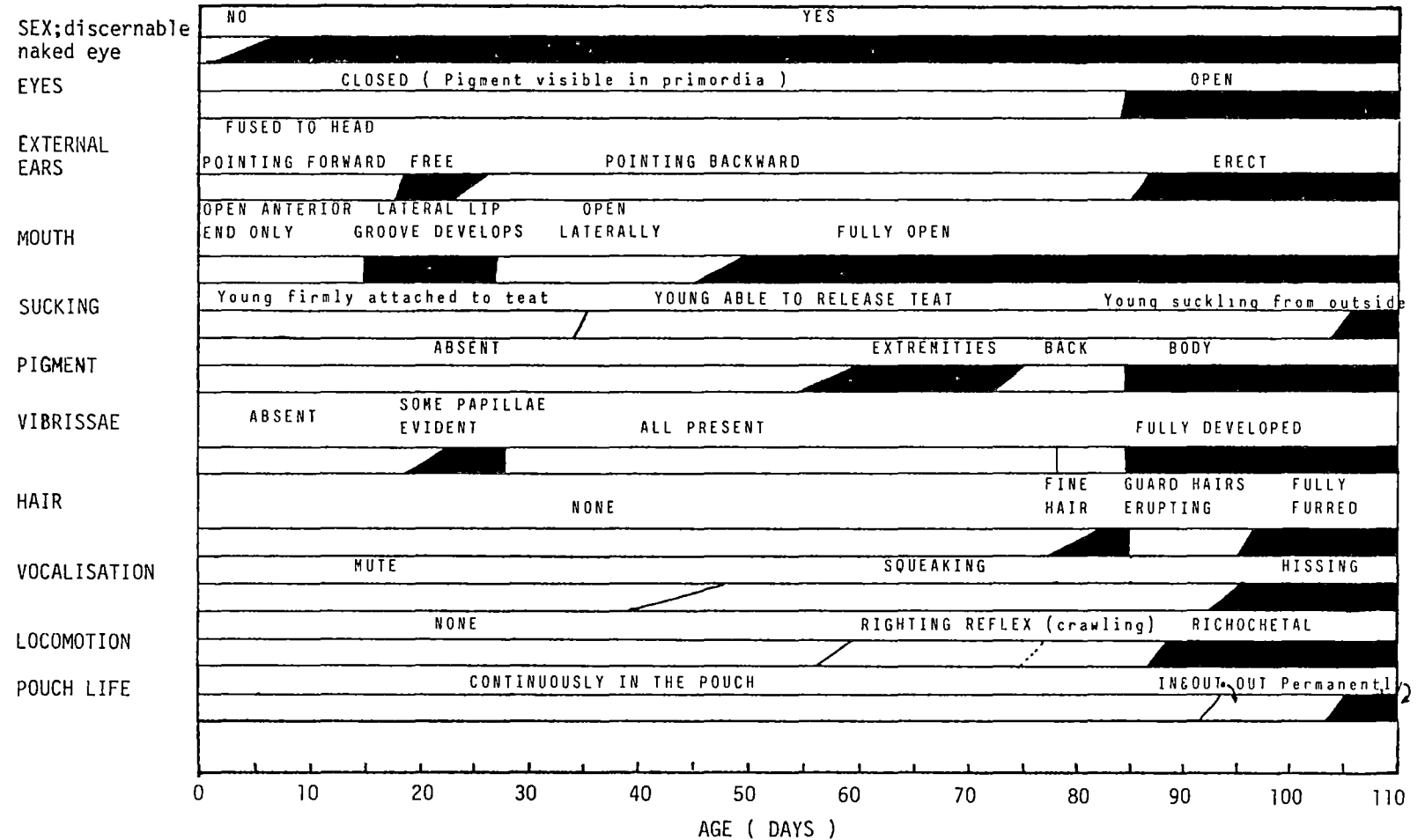
Figure 3.4 summarises the appearance of various characters. In preserved newborn young the pouch anlage or scrotum could be determined with a 10X lens. In live newborn young, this region is obscured by the tail and difficult to view in the pouch but by day 7 after birth the tail was sufficiently elevated for the sexually differentiated region to be observed using the unaided eye.

The first appearance and variety of facial vibrissae was determined from the 36 preserved specimens. The terminology of Lyne (1959) has been adhered to in describing this aspect of development. The first vibrissae to be observed are the mystacial and the supraorbital; these are apparent from about day 28 although their pre-emergent papillae could be distinguished at day 22. The genal vibrissae first appear at day 49, and submental, ulnar, carpal and interramal were first seen in a preserved specimen 63 days of age. Although actual numbers of vibrissae were not counted it is interesting to note that Lyne (1959) found that the Tasmanian bettong generally had more vibrissae than sub-species of the same animal from the Australian mainland.

3.3.5 Sexual Dimorphism

Table 3.3 summarises measurements obtained from male and female bettongs at various ages. Although the sample size is not large, males were generally larger than females. However, student t-tests showed that there were no significant differences between male and female at

FIGURE 3.4 AGE AT APPEARANCE OF BODY CHARACTERS.



any age, indicating that at least in these parameters there is no sexual dimorphism. In fact, apart from the obvious difference due to the presence of a scrotum or pouch, male and female bettongs are not easily distinguished.

TABLE 3.3 Head length and weight of males and females of various ages ($\bar{X} \pm \text{S.E.}(N)$ in each case)

Age	Parameter	Female $\bar{X} \pm \text{S.E.}(N)$	Male $\bar{X} \pm \text{S.E.}(N)$
4 weeks	Head length (cm)	$1.8 \pm 0.06(5)$	$1.8 \pm 0.06(3)$
8 weeks	Head length (cm)	$3.1 \pm 0.08(7)$	$3.2 \pm 0.05(4)$
Pouch	Weight (g)	$33.7 \pm 2.4(7)$	$37.0 \pm 1.8(4)$
12 weeks	Head (cm)	$4.6 \pm 0.08(11)$	$4.8 \pm 0.06(4)$
	Weight (g)	$123.1 \pm 6(8)$	$132.7 \pm 1.6(4)$
16 weeks	Head (cm)	$6.67 \pm 0.04(9)$	$6.67 \pm 0.15(3)$
Post-Pouch	Weight (g)	$416.6 \pm 18.6(7)$	$436.3 \pm 18.9(4)$
Adult	Head (cm)	$9.1 \pm 0.13(17)$	$9.4 \pm 0.12(17)$
	Weight (kg)	$1.7 \pm 0.04(17)$	$1.7 \pm 0.06(17)$

3.3.6 Tooth Eruption

The dental formula of *B. gaimardi* is similar to that of other potoroides:-

$$I \frac{1-3}{1}, C \frac{1}{0}, P \frac{2-3}{2-3}, M \frac{1-4(5)}{1-4(5)}$$

The actual number of teeth present varies with age. During pouch life the first teeth to be observed are the lower incisors. By the time young vacate the pouch, P2 is erupting with dP3 shortly following. No molar teeth have pierced the gum at this stage.

Molar teeth erupt in the manner usual for other macropodids: the anterior cusp erupts prior to the posterior cusp. The age of first appearance of the various stages of molar eruption is presented in Figure 3.5 and Table 3.4. These data are based on captive animals of both sexes of known day of birth. It can be seen that each stage of molar eruption lasts for a proportionally longer period than the previous one. Molar stage MIII is a relatively stable period that may last for three months before the anterior cusp of MIV appears through the gum (stage MIII.2). Prior to this occurrence, however, P2 and dP3 are replaced by a single large fluted tooth, P3. This occurs after 12 months. Maynes (1972) states that P3 absorbs the roots of P2 and dP3 and may in fact be in position (i.e. erupted) before the other teeth have been shed; this may account for its variable appearance. The animal which showed the earliest eruption of P3 (B801), though born in captivity, was released into the large enclosure (1 hectare) and fed mainly on natural food.

It is apparent from examination of cleaned skulls that P3 replaces the deciduous premolars after MIV has erupted through the jaw but before it has pierced the gum, i.e. at molar eruption stage MIII.1. The corresponding stage in *B. penicillata* is MIII.2 (Christensen 1980).

MIV is usually the last tooth to erupt through the gum and this occurs well into the second year of the life of the bettong. One skull had a molar stage of MIV.1. This animal had been tattooed as an adult four years previously and had been recaptured in the wild. It was probably therefore at least five years of age. The teeth were extremely worn. The presence of a fifth molar was not described for *B. lesueur* by Tyndale-Biscoe (1968) or for *B. penicillata* by Christensen (1979). However, Lord and Scott (1924) state that absolute suppression of the fifth molar has not been accomplished in the genus *Bettongia* and may

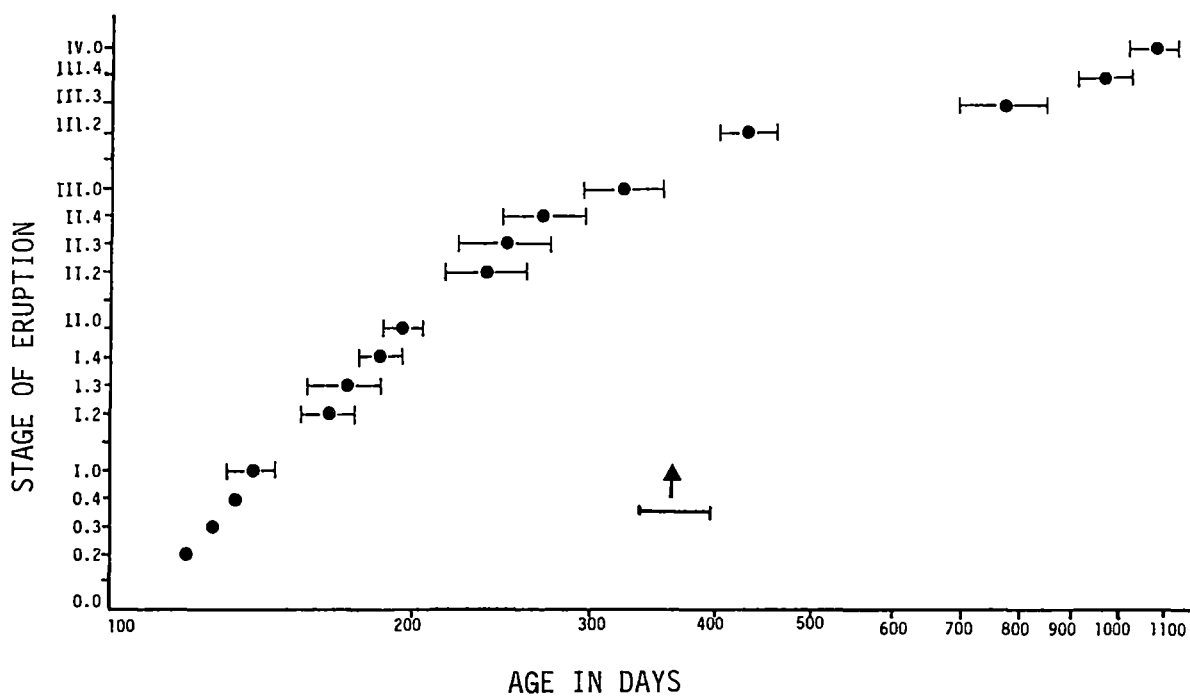


Figure 3.5 Stage of molar eruption against age ($\pm 95\%$ confidence limits). Arrow indicates eruption of P3.

reappear in individual specimens. Wood Jones (1924) describes two specimens of *B. penicillata* with a fifth molar.

Figure 3.6 illustrates several stages of molar eruption whilst Table 3.4 compares the time of eruption of various molar stages in the three extant members of the genus. It can be seen that the times of eruption are quite similar between *B. gaimardi* and *B. penicillata* but occur somewhat earlier in *B. lesueur*.

Molar eruption stage MIII.2 lasts for a considerable period (approximately 12 months) before the posterior cusp of the fourth molar erupts. The posterior cusp is very small and difficult to detect in the caudal region of the jaw of living animals. The fourth molar does not erupt completely until the start of the fourth year (158 weeks).

TABLE 3.4 Molar eruption in the genus *Bettongia*
(The data are in the form of mean time of first appearance (days) of each molar.)

Stage	<i>B. gaimardi</i>	<i>B. penicillata</i> *	<i>B. lesueur</i> **
MI	137	140	125
MII	195	200	180
MIII	323	275	260
MIV	1071	503	430-730 (approx. range)

* From Christensen (1980)

** From Tyndale-Biscoe (1968)

3.3.7 Age Estimation

Approximate estimates of the age of bettongs may be obtained by comparing the measurements of head, foot and tail lengths as well as weight from an animal of unknown age with the mean values in

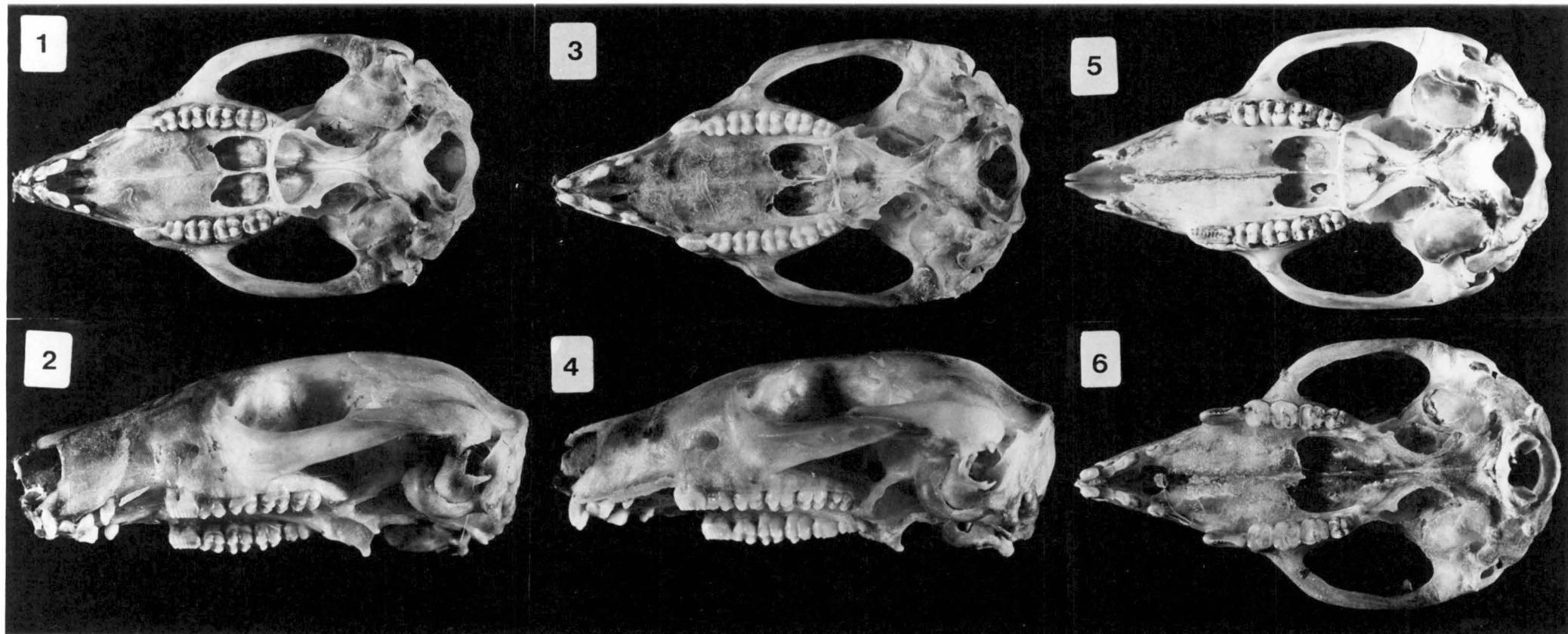


Figure 3.6 Photographs of cleaned skulls illustrating various stages of teeth eruption.
 1 and 2. p2 dp3 MIII.0; 3 and 4. p2 dp3 MIII.2; 5 MIII.2 p3 just erupted;
 6 MIV.0. Note worn molars.

Tables 3.2a and b. However, estimates of age may also be obtained by substituting values into the equations in Table 3.5. It can be seen from this Table that, based on the square of the correlation coefficient (R^2), the quadratic equation gives a better fit than the linear or exponential equations. In the case of weight estimations an exponential relationship gives a slightly better fit.

(a) Pouch Young

From the point of view of field workers, however, quadratic equations are cumbersome to solve and it may be that this inconvenience outweighs any inaccuracies introduced by the use of the simpler linear regression. In order to compare the usefulness and accuracy of these two equations the head length of an additional eight pouch young of known age was measured regularly. The actual recorded age of these young was compared with their estimated age using both the linear and quadratic equations and the results are plotted in Figure 3.7. The straight line indicates the situation when the estimated age equals the actual age. Two major points that come out of the comparison are (i) that during pouch life, estimates by either equation were generally accurate to within one week (usually closer) of the actual age, and also (ii) that little apparent increase in accuracy was obtained by use of the quadratic equation. Linear regressions of these data (Table 3.6) illustrate that linear regression of head length is in fact a slightly better estimator of age than the quadratic equation.

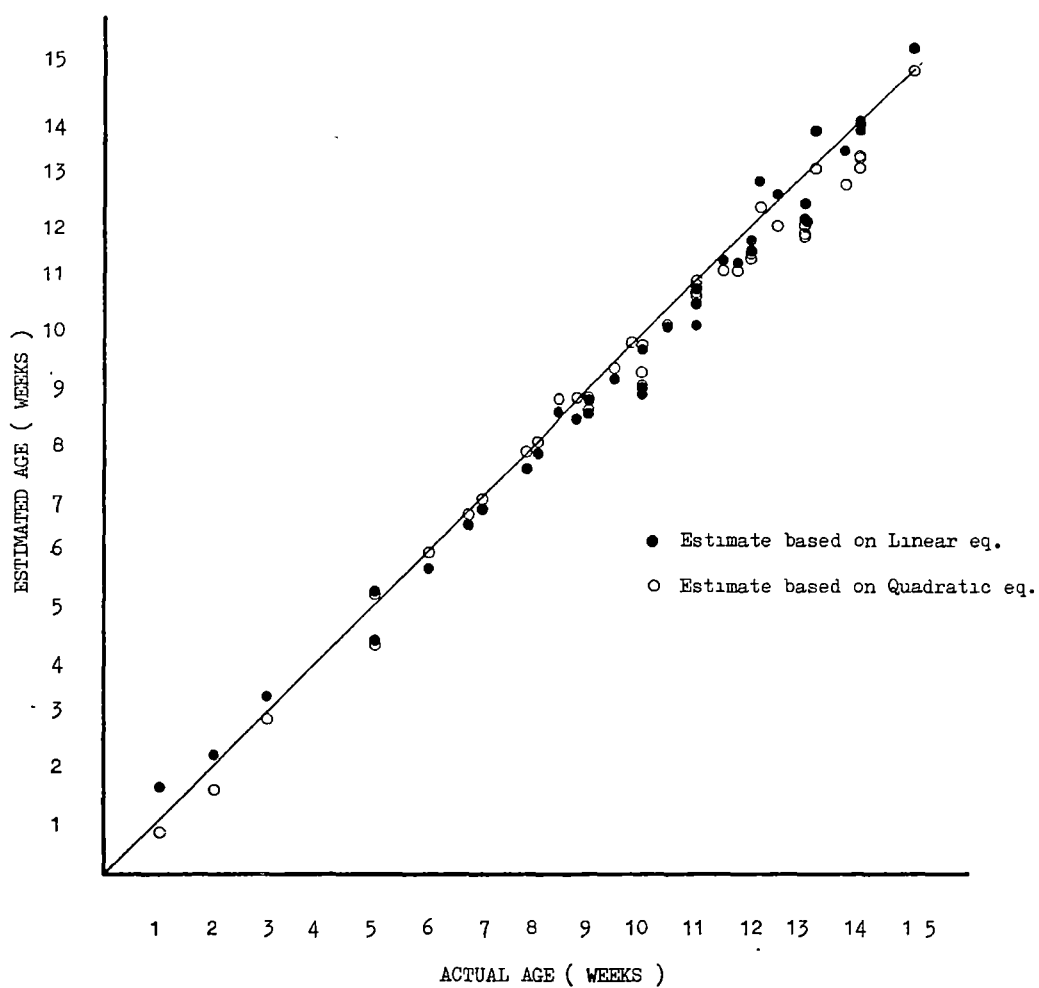


Figure 3.7 Actual age compared with estimated age.
Straight line indicates when actual age
and estimated ages coincide.

TABLE 3.5

Parameter	Equation	R ²	F
Head	Q Y = 0.7853+0.2265 X + 0.0081 X ²	0.9838	4939
	L Y = 0.3924+0.36X	0.7056	6564
	Ex. Y=1.0738 ^e (0.1221X)	0.9573	3673
Tail	Q Y=1.7809-0.3982X + 0.103 X ²	0.9800	3651
	L Y=-4.0372+1.3665X	0.9031	1397
	Ex. Y=0.8417 ^e (0.2172X)	0.9667	4352
Foot	Q Y=0.0992+0.1579 + 0.0338 X ²	0.9808	3872
	L Y=-1.8394+0.7378X	0.9504	2933
	Ex. Y=0.5667 ^e (0.2058X)	0.9568	3390
Weight	Q Y=48.9188-25.8393X + 2.8516 X ²	0.9457	1227
	L Y=-130.6931 + 24.5407	0.7475	420
	Ex. Y=1.3281 ^e (0.3821X)	0.9536	2921

Q - Quadratic equation	Y = A+BX+CX ²	R = correlation
L - Linear regression	Y = A+BX	coefficient
Ex.- Exponential regression	Y = A ^e (BX)	F = F-statistic
X - Age (weeks)		
Y - Parameter (cm/g)		

Regression equations of best fit for the parameters of head, foot and tail length (cm) and weight (g) during the 15 week pouch life. Analysis of data in Figures 3.1, 3.2 and 3.3 and Table 3.2.

TABLE 3.6 Accuracy of linear and quadratic equations in age estimation

Estimation based on:	Correlation coefficient	Slope	Standard error of slope
Linear equation	0.994	0.978	0.024
Quadratic equation	0.987	0.972	0.032

All three of the computations in Table 3.6 indicate that the line of age estimations based on the linear regression equation give the more accurate results, e.g. the closer the slope is to 1.0 the better the overall result.

Other workers (e.g. Maynes 1972) have concluded that greater accuracy can be obtained if the average of more than one morphological parameter is used to age marsupials. Shield and Woolley (1961) have stated that simple linear interpolation from growth curves give a better estimate of age than values obtained from substitution into formulae. Table 3.7 compares the actual age of nineteen different pouch young (chosen to provide a full range of ages) with four estimates. Two estimates are based on values obtained from the linear regression equations (Table 3.5) (columns 1 and 3). One of these is based simply on the head length measurement, the other is an average age estimation based on head, foot and tail length. The age estimation in columns 2 and 4 were obtained by simple linear interpolation from the graphs in Figure 3.3. The term 'Mean Error' at the base of each column has been calculated by subtracting the various estimates of age from the actual age. From this it can be seen that, in general, interpolation between the values in Figure 3.3 gives a better estimate of age than values substituted into the linear regression equations. On average the technique of simple interpolation allows an age estimation that is

TABLE 3.7 Age estimations based on linear equations, interpolation and averaging

Actual Age	Estimated Age (Days)			
	Based on Head Length		Based on Average of Head, Foot and Tail Length	
	using linear equation	using interpolation	using linear equation	using interpolation
21	24	21	26	21
28	29	32	30	29
35	35	37	34	35
42	39	41	38	41
47	45	48	43	49
56	53	56	50	57
63	59	61	55	61
63	60	64	56	62
70	64	74	61	69
72	71	73	68	73
77	74	79	74	79
77	76	79	73	78
84	82	85	80	83
84	82	85	86	87
91	85	87	85	85
98	99	98	103	98
101	99	98	106	100
105	103	100	106	100
107	109	107	110	1-9
Mean Error* \pm SD	2.2 \pm 1.9	1.8 \pm 1.65	4.58 \pm 2.1	1.3 \pm 1.77

All estimations used the linear regression equations from Table 3.5.

* The mean error is the average discrepancy in days (from the actual age) obtained using each of the four techniques

within one or two days of the actual age and there is only a marginal improvement in accuracy by averaging of age estimates from the three parameters. The simplest and most accurate method of age estimation in the field would involve similar interpolation based on the graph of mean head length against age.

(b) Age Estimations of Juveniles and Adults

The data in Table 3.4 and Figure 3.5 relate the stage of molar eruption to the age of bettongs from the end of pouch life (105 days) until the age of 3+ years. Simple interpolation or the use of the regression equation presented below can be used to estimate age. Once again the quadratic expression is the equation of best fit.

$$\text{Natural log age (days)} = 4.7119 \pm 0.1556 \text{ ME} + 0.0594 \text{ ME}^2$$

ME = molar eruption stage (scored in fifths) after Dudzinski *et al.* 1977

$$R^2 = 0.928$$

$$F\text{-statistic} = 296.5$$

3.3.8 Growth Rates

The weight increment ($\text{g}\cdot\text{week}^{-1}$) of growing animals increases until the nineteenth week after birth; weight increase peaks during this week (3-4 weeks after pouch vacation) with an average of $113 \text{ g}\cdot\text{week}^{-1}$ (Figure 3.8). Subsequently weekly increments slowly decrease as maturity approaches, although they remain at about $50 \text{ g}\cdot\text{week}^{-1}$ for some time.

If the weekly mean weight increment is expressed as a percentage of an animal's actual weight at the start of the week (Figure 3.9) it can be seen that after a dramatic weight increase in the first week of

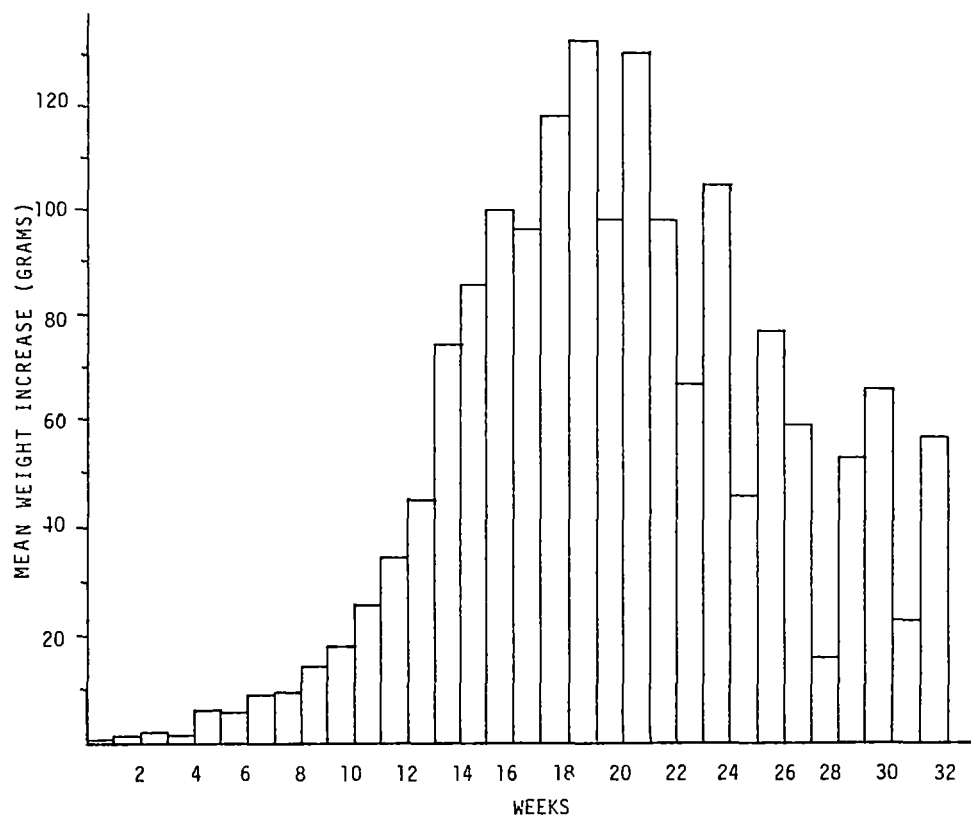


Figure 3.8 The increase in mean weight per week.

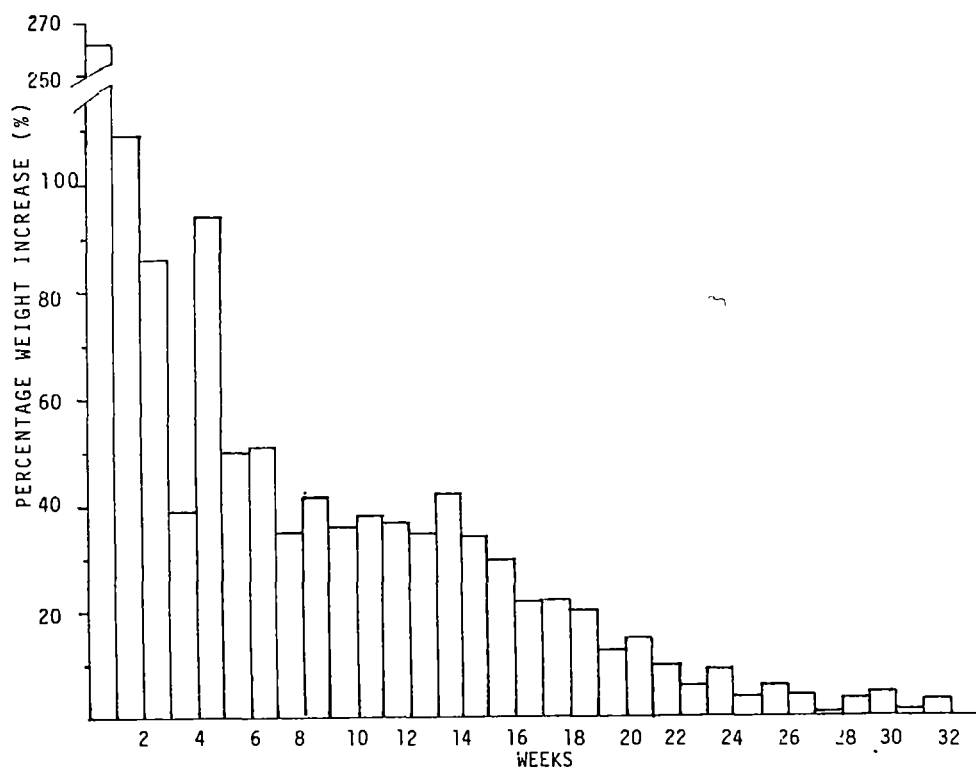


Figure 3.9 The percentage increase in mean weight per week.

life (266%) over the next three to four weeks percentage weight increase is approximately 100% per week. For most of the subsequent pouch life, weekly weight increase stabilises at about 35-40%. Following pouch vacation there is a gradual decrease in weekly weight increase to below 5% near maturity.

3.3.9 Linear Equivalence (see Figure 3.10)

Prior to the end of pouch life, the head length curve (Figure 3.10) is parallel to the linear equivalence curve but after pouch vacation the increase in head length as a percentage of adult size decreases in relation to the linear equivalence. During the first few weeks of life the foot is small and well below the linear equivalence. However, the growth of the foot is such that it is above the linear equivalence for about the second half of pouch life. Relative foot length shows its greatest divergence from the linear equivalence at the time of pouch emergence. Tail length remains below the linear equivalence for the majority of pouch life but approaches it just prior to pouch vacation. During the last weeks of pouch life the increases in tail length varies between 20 and 30 mm per week.

However, when the data are expressed using a logarithmic/linear scale (Figure 3.11) a different impression emerges: one of a decreasing rate of growth throughout life. Five linear segments can be discerned in Figure 3.11 (designated 1 to 5) and each of these represents periods during which growth occurs at a constant rate. These empirically determined segments correspond with identifiable phases of the life of the bettong (Table 3.8). The slope of these linear segments represents the mean 'instantaneous relative growth rate' (k).

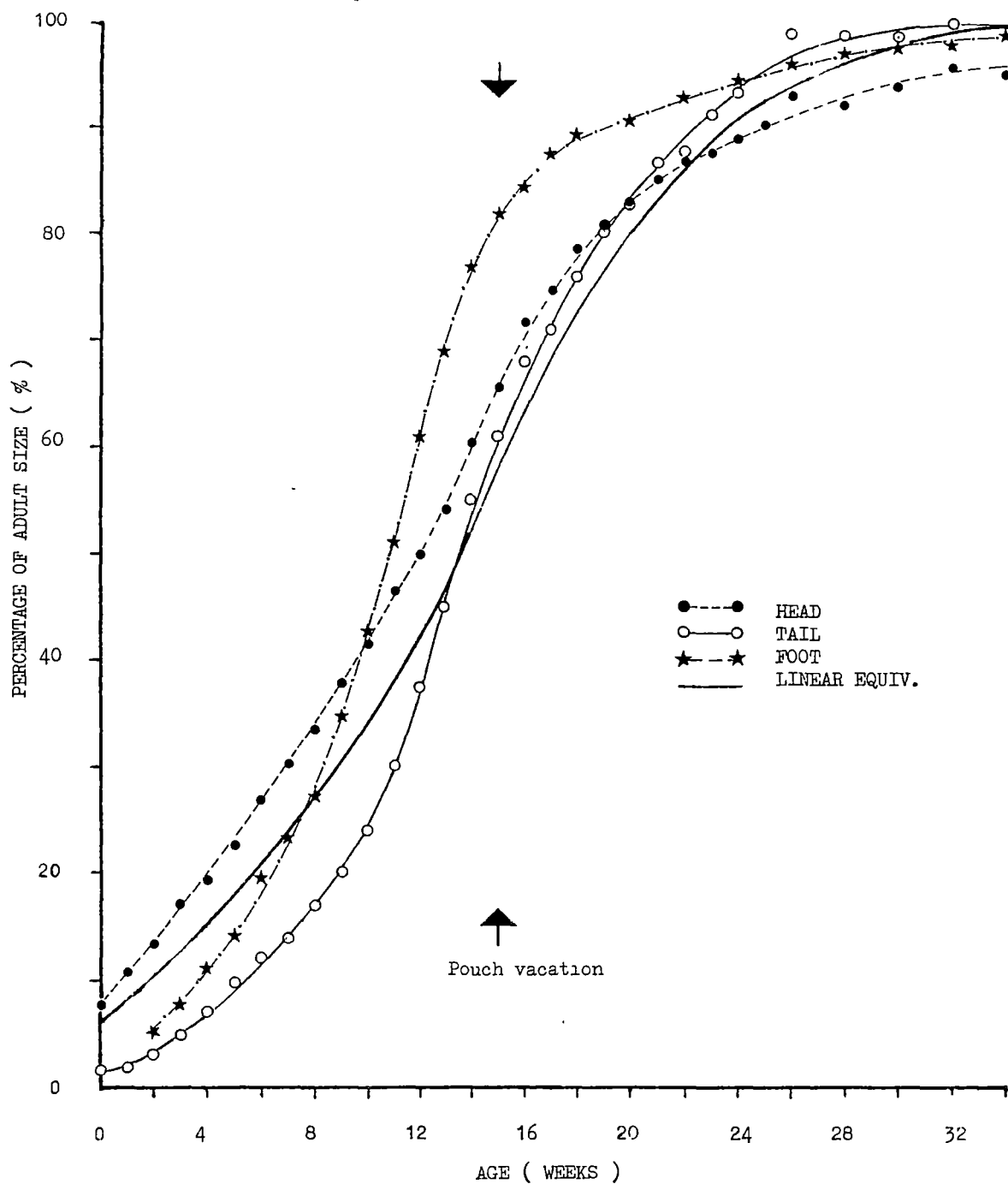


Figure 3.10 The weekly change in head, tail, foot and linear equivalence as a percentage of adult size (= 100%).

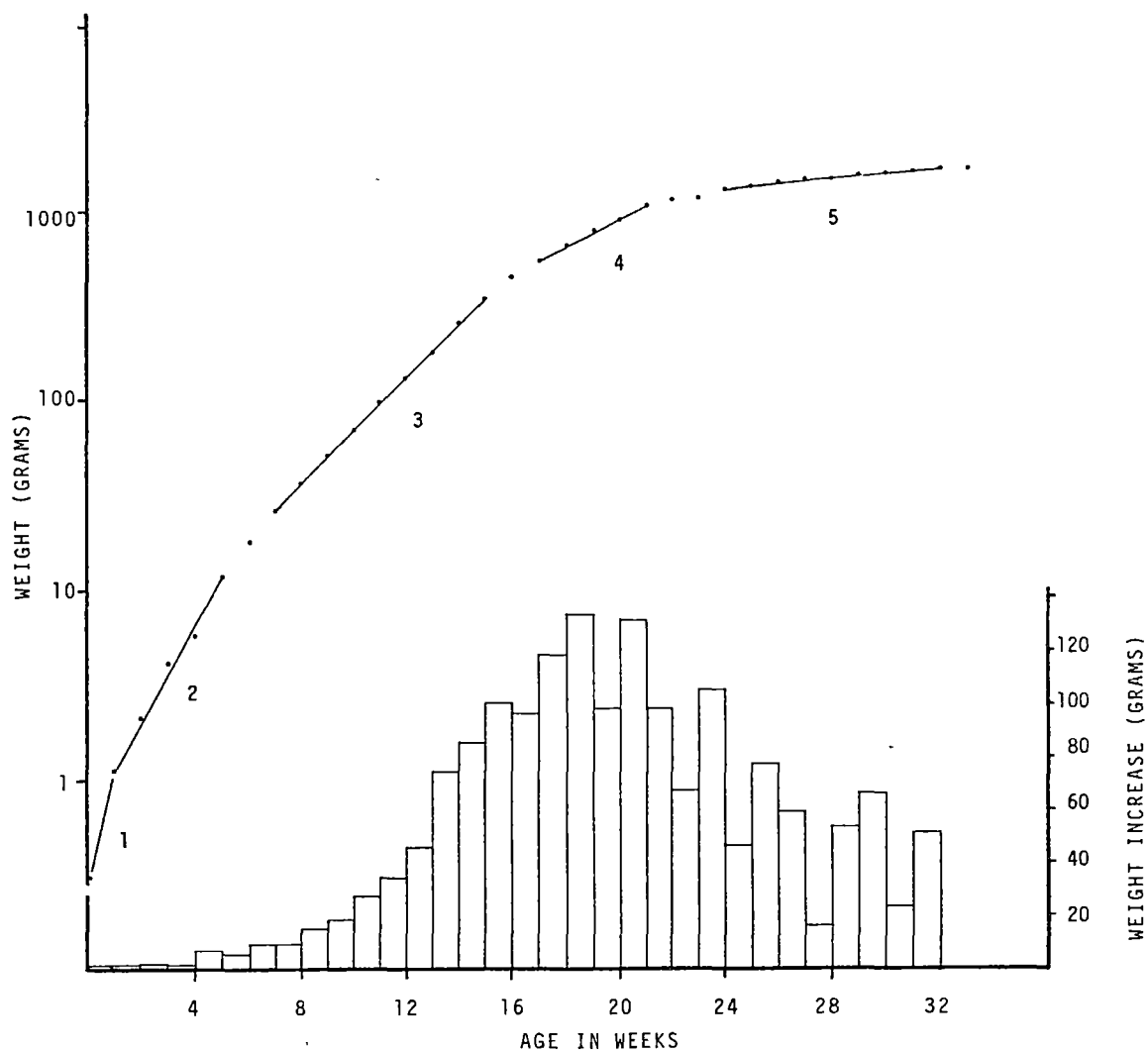


Figure 3.11 Logarithmic plot of mean weight against age. Five linear segments (1-5) have been delineated. Histogram of mean weight increase per week.

TABLE 3.8 Mean instantaneous relative growth rate (k) during periods of constant growth (from Figure 3.11)

Growth Curve segment (ref. Figure 3.11)	Age Range (days)	k $\ln.g.day^{-1}$	Stage of Development
1	1-7	1.86×10^{-1}	New born young, first week of life
2	7-35	8.49×10^{-2}	Young firmly attached to teat
3	49-105	4.56×10^{-2}	Pouch life until final emergence, young able to release teat
4	119-147	2.27×10^{-2}	Young at foot, sucking
5	168-224	4.75×10^{-3}	Young no longer sucking, until near maturity

It can be seen in Table 3.8 that k is inversely related to age. The data from which k has been estimated are mean values (weight) from seventeen young; however, there is some individual variation. The range for segment 3 was $k = 4.11$ to $4.99 \times 10^{-2} g.day^{-1}$ ($4.56 \pm 0.23 \times 10^{-2}$, $\bar{X} \pm S.D.$). The mean value of k obtained from six different bettong pouch young during this period was $4.6 \times 10^{-2} g.day^{-1}$. The value of k for the similar period of pouch life of *B. lesueur* was $4.15 \times 10^{-2} g.day^{-1}$ (calculated from Tyndale-Biscoe 1968).

One useful measure of the state of growth is to compare the size of the animal at a particular phase of growth with the size of an adult. These data are presented in Table 3.9 for pouch vacation (week 15) and, the age at which regular measurements of body parameters ceased (34 weeks).

TABLE 3.9 Mean weight and size of the Tasmanian bettong expressed as a percentage of adult size

	Week 15 (N=17) (%)	Week 34 (N=10) (%)	Maturity (N=15) (%)
Weight	20	97	100
Head length	66	97	100
Foot length	82	98	100
Tail length	61	97	100

It can be seen that by week 34 the young has virtually attained the size of an adult. At pouch vacation (15 weeks) the ratio of the weight of pouch young to that of its mother is approximately 1:5 or 20%, this is similar to that found in most other macropodids (Maynes 1972).

Another index of growth rate sometimes employed is that of the time that is required for a particular parameter to increase from 10% to 90% of the adult size (t10-90) (Maynes 1972). On this basis it is found that in the bettong foot length grows relatively more rapidly than the other structures measured. The times taken for various parameters to increase from 10% to 90% of adult size are as follows: head length 169 days; foot length 107 days; tail length 125 days; weight 116 days and linear equivalence 153 days. The use of weight in this context gives a somewhat distorted view of development as 10% of adult body weight (170 g) is not achieved until near the end of pouch life (91 days).

3.3.10 Comparison of the Morphology and Weight of Adults in the Field and Captivity

Table 3.10 compares the morphology of adult animals measured in the wild with adult animals reared in captivity. There are significant

differences in weight (captives are heavier) and foot length (field animals have longer feet). Head and tail lengths are similar in wild and captive populations.

TABLE 3.10 Weight and size of wild and captive animals $\bar{X} \pm \text{S.E.}(N)$

	Field Animals (N)	Captive Animals (N)	T-test Significance
Weight kg	$1.67 \pm 0.03(36)$	$1.78 \pm 0.04(19)$	$P \leq 0.05$
Head cm	$9.25 \pm 0.17(14)$	$9.31 \pm 0.06(23)$	n.s.
Foot cm	$11.96 \pm 0.09(20)$	$11.67 \pm 0.07(21)$	$P \leq 0.02$
Tail cm	$30.86 \pm 0.33(13)$	$31.67 \pm 0.29(24)$	n.s.

The mean weight of field animals is somewhat depressed as the data are based on adult animals and some animals in the wild appear to be mature at weights far lower than found in the captive colony (see Chapter 4).

3.4 DISCUSSION

At birth all marsupial young appear similar in that they are small and naked with relatively well developed forelimbs. A variable period of development in the pouch allows young to reach a semi-independent stage after which they leave the pouch, although they continue to suck for some time. After weaning, marsupials gradually reach their adult size and usually become completely independent of their mother.

The new-born bettong appear similar to many other macropodid young. It is extremely small in comparison to the size of its mother as indicated by the ratio of young to mother's weight of 1:5542. In terms of the linear equivalence the young measures 5.8% of the maternal body

size, not dissimilar to the value of 5.2% found by Maynes (1972) for the parma wallaby *Macropus parma* but lower than the corresponding value of 6.9% for the potoroo, *Potorous tridactylus* (Rose unpublished).

The relationship between the weight of the marsupial young and the log maternal weight has been demonstrated by Tyndale-Biscoe (1973);^{Rossel (1982b)} The values obtained for the bettong fit neatly into the established relationship which, put simply, implies that larger animals have larger young. Large eutherian mammals have longer pregnancies than small eutherians. This usually results in larger animals having larger young, although the litter size may decrease (Hogarth 1978). Within the Macropodidae the situation differs in that all but one produce a single young and that the variation in size is not necessarily due to variations in gestation length. The potoroo for example has the longest gestation length of all macropodids but has a relatively small young (Rose 1978). It seems that in the determination of the size of the macropodid neonate the rate of embryonic growth is just as important as the gestation length or the size of the mother.

The sex-ratio of 1:1 in the bettong is similar to that found in the majority of the macropodids. However, Caughley and Kean (1964) state that *Macropus giganteus* has a sex ratio favouring the male and more recently Johnson and Jarman (1983) present data illustrating a variation of sex-ratios (in the larger kangaroos) with differing ecological parameters; those animals living in more arid regions had more male pouch young. Furthermore Christensen (1980) has shown that although the overall sex-ratio in *Bettongia penicillata* was 1:1 there may be a seasonal factor involved as significantly more males than females were born in the summer months. Unfortunately the data base from wild caught Tasmanian bettongs is insufficient for analysis.

Sexual dimorphism does not occur in the pouch young of the Tasmanian bettong. It does occur in most kangaroos (Maynes 1976) but does not develop until some time after the young have left the pouch. However, sexual dimorphism is also absent in adult bettongs of all three extant species (this study; Maynes 1976).

Darwinian theory suggests that the larger size of males in those species with sexual dimorphism, is the result of competition among males for mates. Thus the more polygamous the species (males who mate with several females) the larger the males should be relative to the females (Ralls 1978). Bettong males are certainly aggressive towards each other and there is no evidence to suggest that bettongs are monogamous. Bigger mothers may be better mothers particularly in the latter stages of pouch life when increased metabolic demands must be met so that the large pouch young may be nourished and transported. However, it would be more than coincidental if females and males reached a similar size but for evolutionary reasons that differed widely.

The pouch environment is a stable one within which growth and development proceeds in an ordered manner. This results in narrow confidence limits being associated with all parameters measured during the studies. The confidence limits slowly increase after the young leaves the pouch. This commonly observed phenomenon occurs because young have now to cope with a changing environment and must expend energy in feeding and temperature regulation over a variety of ambient temperatures. In the wild, juveniles need to be able to travel in order to avoid predators, collect food and eventually to construct a nest. All of these activities divert energy that previously was available almost exclusively for growth. As the growth curves level off, the confidence intervals also increase when converting measured parameters into estimations of age. This makes age estimations based on

morphological measurements less reliable.

Wood, Carpenter and Poole (1981) state that, in general, fitted growth curves for individual animals of the one species will differ significantly from each other and that this between animal variation will contribute to the increasing width of the confidence limits. They propose a method that allows for between animal variation in the estimation of age and illustrate its use with data from *Macropus giganteus*. They state that the method 'is applicable to many situations where measurements have been taken sequentially on animals of known age'. It is unlikely, however, that this method will gain widespread use by field workers as the method for obtaining the confidence limits is 'extremely hard to apply by non-mathematicians, as it is both tedious and involves fairly difficult computing' (Carpenter, *in litt.*).

The advantages and disadvantages of the use of mathematically fitted growth curves in the estimation of age have been discussed by Shield and Woolley (1961). The widespread availability of computers now allows one to obtain a large number of fitted growth curves and provides the means to test for the equation of best fit. Recently Poole (1982) has shown that mathematically fitted growth curves can be used to accurately estimate the age of macropodids.

In any event, growth curves and their attendant equations are only of use if they provide a degree of accuracy not approached by simpler methods. In this study on the bettong it has been shown that better results could be obtained by the simple method of interpolation from the growth curves than by the use of mathematically fitted regression equations.

The preferred methods for age estimation after pouch vacation are those based on dentition.

The data obtained in this study on molar eruption are based on smaller samples than are the data on pouch young growth. Molar eruption in the Tasmanian bettong does not proceed at a regular pace; each eruption takes longer to occur than its predecessor. This results in a widening of the confidence limits and a decrease in reliability of age estimation at later stages of eruption. The applicability of this method to field populations has not been tested and it is reasonable to assume that the range of values will be larger in wild populations.

Nevertheless, the data as provided are likely to be of use to those involved in ecological studies on the Tasmanian bettong.

Dudzinski *et al.* (1977) showed that in *M. agilis* a linear relationship exists between the natural log of the kangaroo's age and the stage of molar eruption. By way of contrast this study has shown that the relationship in the bettong is curvilinear and is better expressed by a quadratic equation. The significance of this difference is obscure; but implies that the eruption of the last molars takes substantially longer than the earlier molars in *B. gaimardi*.

The term 'linear equivalence' was introduced into the literature on marsupial growth by Lyne and Verhagen (1957) and has been applied by Maynes (1972) in his study of the growth of the parma wallaby. The use of the cube root of weight as a generalised measure of linear growth was known to Huxley (1932) from the earlier work of Schmalhausen (1930). It is perhaps unfortunate that the term has not been applied more widely as it is a useful parameter that allows one to compare measurements of the head, foot and tail etc. against a standard. When these values, including the linear equivalence, are expressed in terms of adult size, additional information is obtained which can be examined on a functional, ecological or interspecific basis. Lyne and

Verhagen (1957) used the linear equivalence to compare growth rates of *Trichosurus vulpecula* with non-marsupial species (man, mouse, cow and sheep) and within different (Tasmanian and mainland) possum populations. In the bettong the fact that the value of the head length expressed as a percentage of the adult head size remains above that of the linear equivalence for the whole of pouch life no doubt reflects the overall importance of the head and its associated sense organs and brain in allowing the young initially to reach the pouch and then to suck. Foot length on the same basis remains below the linear equivalence until after the mid-point of pouch life when rapid growth of this parameter occurs, so that by the time they emerge from the pouch, the young have the characteristically large feet of macropodids. The functional adaptation of this mode of development is towards swift and agile movements when the young has to 'keep up' with the pace of its mother and to evade predators; this is not a requirement of early pouch life and consequently the hindlimbs can remain relatively small during that period.

For much of the pouch life the tail remains smaller than the corresponding linear equivalence. The functional significance of the bettong tail is to assist in locomotion and balance and in the construction of a nest. Maynes (1976) showed that in the parma wallaby, tail length was above the linear equivalence only during the period when the young was leaving the pouch. After this period it fell and remained below the linear equivalence. The situation is somewhat different in the bettong; once the tail length exceeds the linear equivalence (approximately one week before final pouch vacation) it remains so until maturity, prior to which time the young will have separated from its mother and constructed a nest of its own.

It is important to distinguish between changes in the relative size of various parameters as has been done so far and the actual rate of growth. Differential equations may be used to describe changes in the growth rate (Huxley 1932) and Kaufmann (1981) suggests that this is a better way of describing changes in size than the integrated forms of the growth equations, presented here in Table 3.5 which merely describe cumulative changes in size. The use of differential equations (e.g. 'k' is obtained by differential equation, Table 3.8) allows one to easily compare the growth rate between species, regardless of the actual size of the two species. The differences between changes in size and the growth rate in the bettong are clearly demonstrated in Figures 3.8, 3.9 and 3.10. Although the actual amount of weight increase per week rises for a considerable period, there is a constant series of decreases in both the mean percentage increase in weight and the instantaneous relative growth rate. The relative growth rate usually decreases with increasing size (Bertalanffy 1960) and Kaufmann (1981) states that the parameters describing the growth rate (e.g. 'k' in this study) constantly decrease as size increases. However, in the bettong and the parma wallaby (Maynes 1976) there are periods during and after pouch life when the growth rate remains constant, although each subsequent change in rate involves a decrease. It seems likely that these periods of constant growth will also be found to occur in other marsupials. The growth rate in marsupials then is not likely to be described by a single smooth curve or a straight line (with the differential equation) but by a graded series of lines each one corresponding to a particular stage of life. It would be profitable to further test Kaufmann's suggestions using eutherian species.

The concept of relative growth rate, 'k', and its description as the change in log₁₀ weight per unit time was developed by Huxley (1932). This concept was refined by Brody (1945) into the 'instantaneous relative growth rate' which is determined from linear sections of the graph when growth is occurring at a constant rate and involves the use of natural logarithms. The value of k (4.56×10^{-2} (Table 3.8)) obtained during the major portion of pouch life in the bettong when the young is growing at a constant rate is quite high when compared with other macropodids. Table 3.11 lists the k values (calculated from data provided in the sources) for a similar period of pouch development in a number of kangaroo species. Although there are some anomalies it can be seen that k values of $2.2 - 2.5 \times 10^{-2}$ predominate within the Macropodinae but that the Potoroinae have elevated k values. *B. gaimardi* has a larger k value than that found in *B. lesueur* and this accounts for the fact that the Tasmanian bettong is considerably larger than the boodie at pouch vacation even though the duration of pouch life is similar. Similarly, within the macropodines differences in k values to some extent reflect the duration of pouch life, e.g. *T. billardieri* and *M. eugenii* are similar sized animals with similar sized young at pouch vacation (Rose and McCartney 1982b) but the pouch life of *M. eugenii* is 50 days longer than for the Tasmanian pademelon.

Although the maternal weight of the macropodine *S. brachyurus* (2.6 kg) is not greatly different from that of the Tasmanian bettong (1.7 kg) the k value of the quokka is very similar to that of the other macropodines. It seems likely therefore that additional data may reveal sub-family differences in the general value of k.

The fact that many kangaroos of widely different size have similar k values (i.e. growth rates) is interesting and may indicate similarities in both the quantity and quality of the milk during comparable

TABLE 3.11 Relative growth rates (k) during pouch life in the Macropodidae. (k values calculated from data in sources.)

Species	Maternal Weight (approx. kg)	$k \times 10^{-2}$ (g.day ⁻¹)	Source
<i>Bettongia gaimardi</i>	1.7	4.56	This study
<i>Bettongia lesueur</i>	1.15	4.15	Tyndale-Biscoe 1968
<i>Potorous tridactylus</i>	1.11	3.7	Bryant and Rose (unpub.)
<i>Thylogale billardieri</i>	4.0	2.37	Rose & McCartney 1982a
<i>Wallabia bicolor</i>	10.1	2.24	Merchant & Sharman 1966
<i>Macropus parma</i>	3.3	2.35	Maynes 1972
<i>Macropus eugenii</i>	4.5	1.98	Murphy & Smith 1970
<i>Macropus rufus</i>	26.0	2.45	Sharman <i>et al.</i> 1964
<i>Macropus robustus</i>	15.0	2.29	Sadleir 1963; Ealey 1967
<i>Macropus fuliginosus</i>	28.0	1.84	Poole 1982a
<i>Macropus giganteus</i>	32.0	1.91	Poole <i>et al.</i> 1982b
<i>Setonix brachyurus</i>	2.6	2.52	Shield & Woolley 1961

phases of pouch life as well as similar patterns of sucking. Green *et al.* (1981) in their study of lactation in the tammar wallaby show how the constituents of milk change throughout pouch life and they suggest that similar changes are likely to occur in other macropodids. They also consider that variations in body weight, growth rate and duration of lactation may be more closely correlated with changing milk compositions than with the actual age of the young.

The comparison between field and captive animals (Table 3.10) revealed small, but statistically significant differences in weight and foot length. Animals in captivity expend less energy than those in the field, they do not have to carry and construct their shelter and food is provided. It is not surprising then that captive animals are somewhat heavier than wild animals. Field animals presumably cover greater distances over a much wider variety of terrain; this may result in looser foot ligaments and this in turn may lead to larger feet. The smaller feet of captive bettongs may be a consequence of inevitable in-breeding.

Parker (1977) found considerable differences in growth rate and the duration of pouch life in a captive population of *B. penicillata* and believes that this may be due to in-breeding (Parker pers. comm.).

Shield and Woolley (1961) discussed the growth of field and captive quokkas and concluded that growth rates were probably similar in both groups of pouch young. Sharman *et al.* (1964) suggested that in the stable environment of the pouch, the growth of young approximated an all-or-none phenomenon. It is true that growth of some macropodid pouch young in severe field conditions may differ from those in captivity (Ealey 1967). However, the very limited data that was collected on pouch young growth in the field and which indicated that some bettong pouch young grew at a slower rate than those in captivity (Appendix A), requires further information.

CHAPTER 4

REPRODUCTION

CHAPTER 4 REPRODUCTION

4.1 INTRODUCTION

The vast literature on the comparative biology of reproduction attests to the interest in this topic. On a broad scale, the reproductive differences between various vertebrate classes are reasonably clear-cut and are virtually sufficient by themselves to assign an animal to a particular class. The reproductive differences between the two major groups of therian mammals, the marsupials and the eutherians, were sufficiently great for Sharman (1970, 1976) to suggest an independent origin of viviparity for both groups. Martin (1969), partly on the basis of his studies of tree-shrew reproduction, showed that reproductive mechanisms could be used to indicate both taxonomic and phylogenetic relationships within the Class Mammalia. However he stated that it was necessary to make "quite broad comparisons in order to arrive at coherent phylogenetic conclusions".

When one examines reproduction within more closely related taxonomic groupings, it is often extremely difficult to use such information in order to draw evolutionary and phylogenetic implications because of the wide variety of reproductive patterns that is found. In their discussion on the evolution of ovarian function, Cumming and Findlay (1977) examined the wide variations in oestrous cycle lengths that exist between various eutherian species and even within species, e.g. breeds and strains of sheep and cattle. They hypothesized that there is a common underlying endocrine mechanism and that variations arose through changes in sensitivity to ovarian steroids of the hypothalamo-pituitary axis,

modulated by environmental effects. This hypothesis may possibly account for reproductive differences between closely related marsupials although no-one has yet examined this possibility.

Maynes (1973b), in showing that two related kangaroo species (*Macropus parma* and *M. dorsalis*) had extremely different patterns of reproduction, suggested that although detailed examination of the reproductive patterns of the Macropodidae are unlikely to be of any taxonomic significance, they may provide an insight into some of the evolutionary changes that have occurred in the reproductive cycles of Macropodids. Some other closely related macropodid species also differ considerably in their reproductive patterns, e.g. the two grey kangaroos (*M. giganteus* and *M. fuliginosus*, Poole and Catling 1974) as well as the red-necked wallaby, *M. rufogriseus banksianus* and its Tasmanian sub-species *M. r. rufogriseus* (Merchant and Calaby 1981). These observations emphasise the difficulties involved in arriving at coherent phylogenies based on reproductive parameters.

4.1.1 Evolutionary Advances in Reproduction within the Macropodidae

During the evolution of the Macropodidae a number of characteristic features has developed. Within marsupials as a group there has been a trend towards an increased median vaginal apparatus (vaginal cul-de-sac) in females as well as a decrease in the length of the birth canal (Sharman 1965). The evolution of these structures has proceeded farthest in the Macropodidae and some members of the sub-family Macropodinae possess a birth canal that is permanently open and becomes lined with cuboidal epithelium in parous females (Tyndale-Biscoe 1966).

These morphological developments in the macropodid mother have been accompanied by the production of larger young (at birth and pouch

vacation) than in other marsupial groups together with an extended pouch life. To some extent the production of larger neonates has been made possible by the development of a longer gestation period than is usual in many marsupials. The increased gestation length may be the result of either a longer luteal phase within the uterus or, more usually, by the extension of gestation into the post-luteal phase by what has been termed a feto-placental effect (Tyndale-Biscoe *et al.* 1974). These changes possibly constitute some of the major evolutionary advances in marsupial reproduction. When gestation extends into the post-luteal phase, it overlaps the follicular changes preceding ovulation (all Macropodids are polyoestrous) with the result that parturition is closely followed (or, on rare occasions, preceded) by oestrus. The egg normally produced and fertilised at this post-partum 'heat' develops to the unilaminar blastocyst stage before its development is arrested by complex neuro-endocrine events resulting from the sucking of the pouch young. Surrounded by a shell membrane, the blastocyst remains in the uterus either until the latter stages of lactation, when sucking becomes intermittent, or until the premature loss of the pouch young. This embryonic diapause (although found in both sub-families of the Macropodidae) is not a universal character, so that it is necessary to postulate that either it was present in the common ancestor and has been secondarily lost by some species or that it has evolved independently in both sub-families.

Embryonic diapause functions in a manner that prevents the occupation of the pouch by more than one young (twins are rarely produced, macropodids being monovular with the exception of *Hypsiprymnodon moschatus* (Johnson and Strahan 1983)). It also results in the rapid replacement of young that have left the pouch, although in some wallabies (e.g.

M. eugenii, the Tasmanian sub-species of *M. rufogriseus* and *Setonix brachyurus*) the resumption of embryonic development is also under environmental control. Concurrent secretion of milk of two different compositions by separate mammary glands has evolved so that both the small young in the pouch and the older young at foot can be nourished.

Within some members of the Macropodidae, there is evidence of a fetal influence upon the mother; for example, Kirsch and Poole (1972) have demonstrated that the genetic composition of hybrid fetuses can influence the duration of gestation in the grey kangaroos. Merchant (1976, 1979) and Merchant and Calaby (1981) provide evidence that pregnancy (i.e. the presence of a fetus) shortens the length of the oestrous cycle in *M. agilis*, *M. eugenii* and *M. rufogriseus*. It is likely that this effect will be found in other macropodids.

4.1.2 Reproduction in the Order Diprotodonta

The order Diprotodonta is comprised of three super-families, the Vombatoidea (koala and wombats), the Tarsipedoidea (*Tarsipes*) and the Phalangeroidea contains in addition to the Macropodidae the Burramyidae (pigmy possums), Phalangeridae (possums) and Petauridae (ringtails and gliders). Little is known about the reproduction of many of the species that comprise these taxa. All are polyoestrous and many are polyovular, although the brush possum, wombats and koala produce only one young. The Burramyidae are unusual, in that in contrast to most other marsupials, some are able to maintain blastocysts (presumably as a result of post-partum mating) within their uterus during lactation. This situation resembles the embryonic diapause found in the Macropodidae but differs in that there is growth of the blastocyst during lactation (Clark 1967). Little is known of the situation in this regard in the koala and wombats. The oestrous cycle length of the common wombat is similar to some

macropodids: 33 days (Peters and Rose 1979), and the koala has a long gestation length of 35 days (M. Smith pers. comm.). Both species have extended post-natal relationships between mother and young and may only breed twice in every three years. *Tarsipes* (Super-family Tarsipedoidea) is known to possess a permanently open birth canal (De Bavay 1950) and Renfree (1980) has demonstrated the occurrence of embryonic diapause in this animal.

Hence within other families of Diprotodonta we find a number of characteristics also present in the Macropodidae: a tendency to the monovular, polyoestrous condition; the potential for embryonic diapause (Burramyidae, *Tarsipes*); increased gestation length (koala); a lengthened period of maternal care for the young (koala and wombat), and a permanently open birth canal (*Tarsipes*).

4.1.3 Sub-family Potoroinae

There is some information available on the reproduction of the extant members of the Potoroinae: *Potorous tridactylus* (Hughes 1962; Shaw and Rose 1979); *P. longipes* (Seebeck 1981); *Bettongia lesueur* (Tyndale-Biscoe 1968); *B. penicillata* (Parker 1977 and pers. comm.; Christensen 1980); *B. gaimardi* (Flynn 1930); *Aepyprymnus rufescens* (Moors 1975; Johnson 1979) and *Hypsiprymnodon moschatus* (Johnson and Strahan 1983). With the exception of *H. moschatus* which regularly produces two young, reproduction in this sub-family differs little from that found in the Macropodinae.

The following section presents quantitative data on the duration of the oestrous cycle and gestation, embryonic diapause and pouch life in *B. gaimardi*. Births in captivity and the extrapolation to the birth dates of pouch young in the wild has allowed the determination of breeding seasons. Age at sexual maturity is documented as is the variation in a testicular index with age and weight. Aspects of mating are

described for the first time.

4.2 METHODS AND MATERIALS

4.2.1 Animals

The majority of the data were obtained from ten captive female bettongs. Six were wild caught and four reared in captivity. Some additional data were gained from other captive females.

4.2.2 Oestrous Cycles and Vaginal Smears

The method for obtaining a vaginal smear was the same as that used previously by Peters and Rose (1978) and Shaw and Rose (1979). This involved the insertion of a small glass tube into the urogenital sinus up to the posterior vaginal sinus. A thin tube with a 'cotton wool brush' on the proximal end was pushed through the glass tube and rotated several times. Removal of this thin tube was followed by the smearing of the 'cotton wool brush' onto the surface of a clean slide and immediate fixation of the smear with absolute alcohol and ether (50:50). The smears were stained with Shorr's (1941) stain.

Vaginal smears were obtained every second day from three females for approximately six months except near the periods of oestrus when they were obtained daily. These smears were evaluated quantitatively by the calculation of two indices; the Karyopycnotic Index (KI) and the Leucocytic Index (LI) (Hughes and Dodds 1968; Peters and Rose 1978). KI is the percentage of epithelial cells with pycnotic (shrunk and wrinkled) nuclei whereas LI is the percentage of all cells in the smear that are leucocytes. One hundred cells were counted each time. Vaginal smears were obtained less regularly from all other females although near

oestrus, animals were smeared daily. These smears were qualitatively evaluated, particular notice being taken of the rise in number of cornified cells and the presence or sudden absence of leucocytes. In one case, smearing continued throughout lactation.

The length of an oestrous cycle was taken to be the duration between successive periods of oestrus as measured by changes in the cellular constituents of the vaginal smear and/or the presence of sperm in the smear. The presence of a copulatory 'plug' in the urogenital sinus was accepted as sufficient evidence of oestrus, hence the successive appearance of 'plugs' could also determine the length of an oestrous cycle.

4.2.3 Mating

Mating was observed on one occasion only and the associated behaviour is described in the results section.

4.2.4 Gestation

The duration between the presence of a copulatory 'plug' or oestrus smear with sperm and the appearance of a new neonate in the pouch was taken as the gestation length. Vaginal smears were obtained for several days after a birth. Parturition was not observed.

4.2.5 Delayed Gestation

Unfurred pouch young were removed from the pouch (or were lost) and in the absence of males the period until the next birth was noted.

4.2.6 Pouch Life

The duration of pouch life from the first noted presence of a neonate in the pouch until final vacation was noted. At pouch vacation the mother was examined for the presence of a new young and for oestrus.

4.2.7 Monthly Breeding

The monthly occurrence of births over a number of years is presented in Figure 4.5. These data contain both field and captive observations and were obtained either by knowledge of the actual birth date or by extrapolation after determination of the age of pouch young using linear interpolation based on head lengths (see Chapter 3).

Breeding seasons in the wild were not investigated *per se* but data were obtained coincidentally with the capture of adult female bettongs for the University colony. Although trapping took place in every month of the year over a long period, no monthly data were obtained for any consecutive period of twelve months nor were all samples obtained from the same location. The captive population was sampled weekly throughout the study.

4.2.8 Experimental Removal of the Corpus Luteum

The quiescent corpora lutea were surgically excised from four females after anaesthesia was induced by Ketalar, and maintained by a Halothane/O₂ mixture. Simultaneously the small pouch young were removed. The corpora lutea were fixed in buffered formalin and in one case sectioned for histological examination and stained with haematoxylin and eosin.

4.2.9 Reproduction in Males

The change in a testicular index (TI) with increasing age (N=6) and weight (N=18) is presented. TI was measured (using vernier calipers) as the length x width of the scrotum. Urine was obtained from young males weekly near maturity and examined on a slide with a light microscope for the presence of sperm.

4.2.10 Maturity

Maturity was judged to have been reached when a female bettong produced a young or when a male achieved either a fertile mating or had copious sperm in the urine.

4.3 RESULTS

4.3.1 Vaginal Smears

Cyclic changes in the proportions of the cellular constituents of the vaginal smears were observed in all non-lactating female bettongs. The sequence and nature of the smear changes resembled those described by Hughes (1962) and Tyndale-Biscoe (1968) for *P. tridactylus* and *B. lesueur* respectively. Figures 4.1, 4.2, 4.3 and 4.4 illustrate the oestrous cycle of *B. gaimardi* in terms of fluctuations in the smear indices, KI and LI. For much of the cycle there appears to be an inverse relationship between the smear indices. The disappearance of leucocytes and mucous prior to oestrus was the most abrupt of the changes observed in the smear cycle and this criterion was subsequently used in conjunction with the onset of cornification to designate Day 0 of the oestrous cycle; i.e. oestrus. Although values of the smear indices

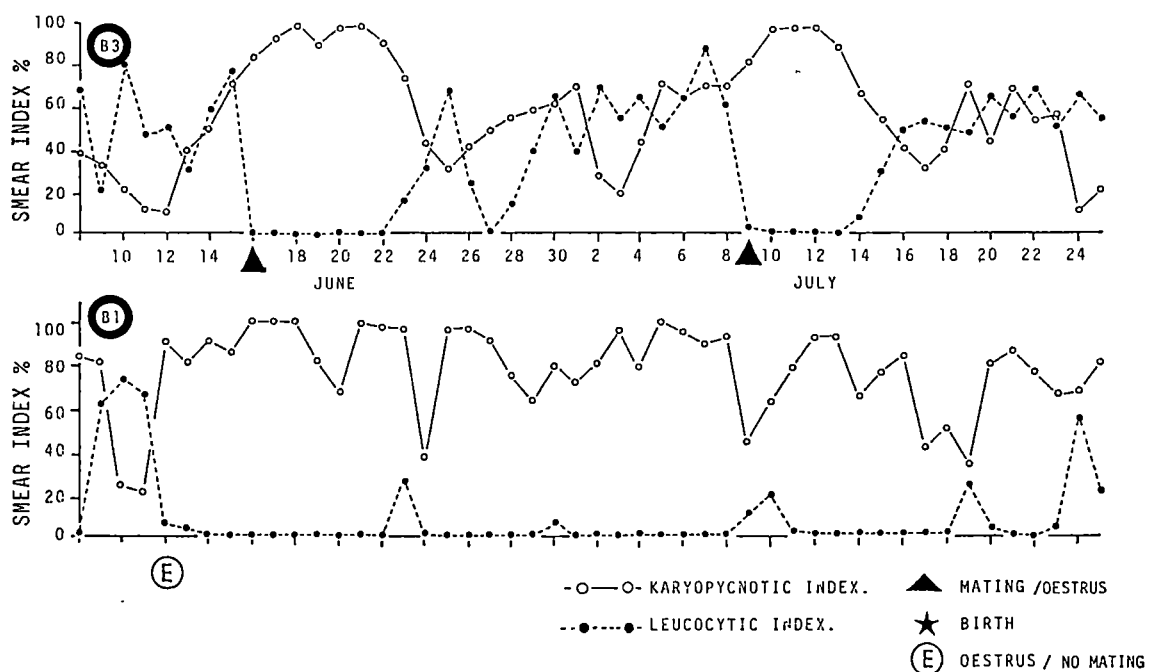


Figure 4.1 Vaginal smear indices of two bettongs (June-July)

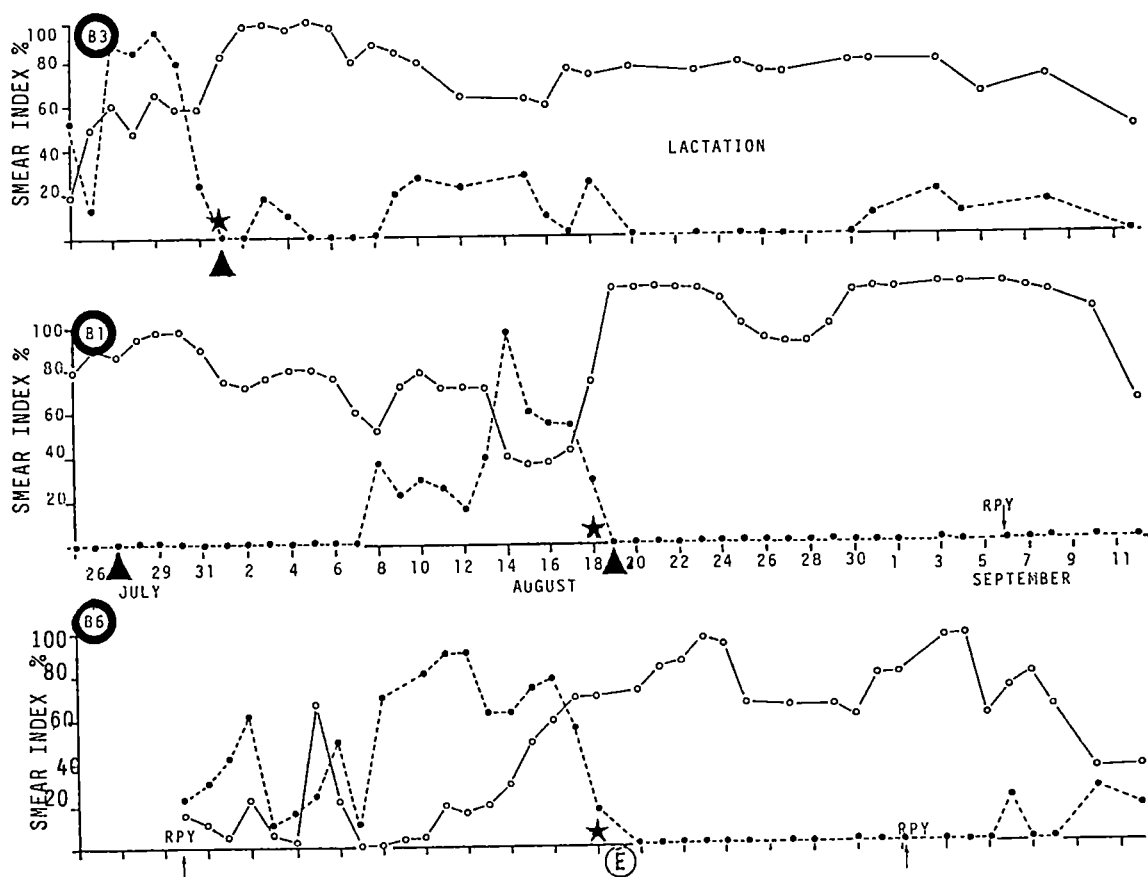


Figure 4.2 Vaginal smear indices of three bettongs (July-September).
Legend as for Figure 4.1.

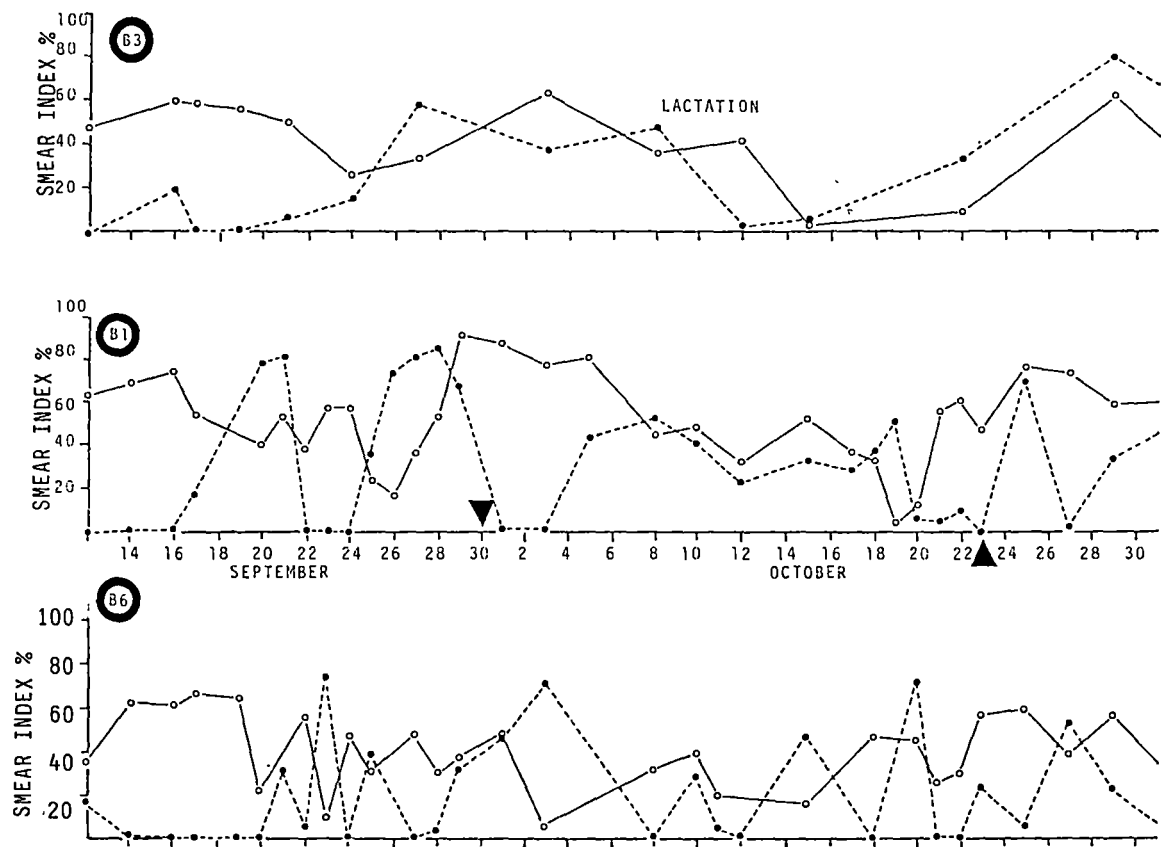


Figure 4.3 Vaginal smear indices of three bettongs (September-October).
Legend as for Figure 4.1.

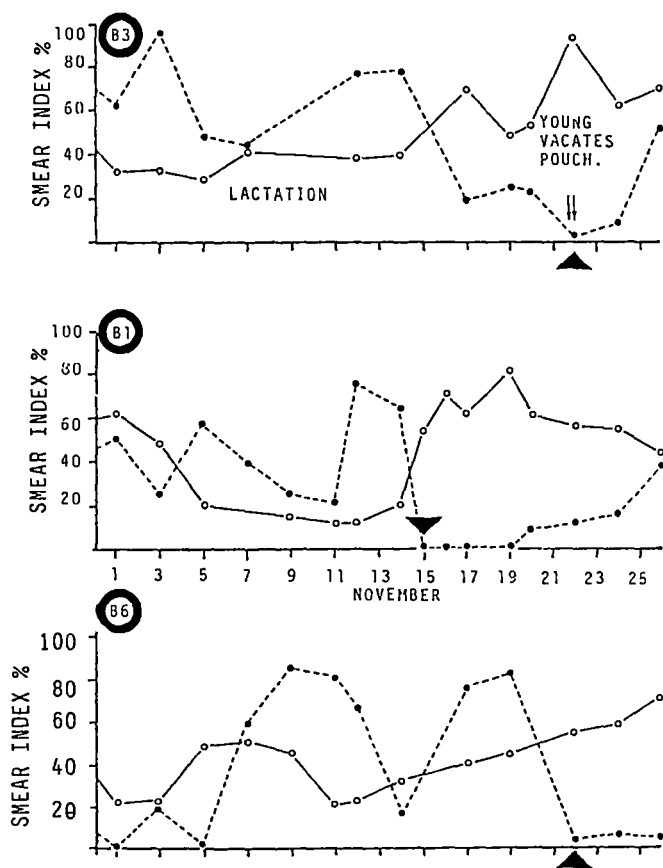


Figure 4.4 Vaginal smear indices of three bettongs (November).
Legend as for Figure 4.1.

varied between individuals and between cycles, the relationship between the two indices remained constant. Prolonged post-oestrous cornification was the most frequently observed deviation from the average pattern. In one instance this persisted for a period equivalent to two oestrous cycles.

During lactation, the two smear indices were positively correlated in complete contrast to their relationship during oestrous cycles.

The precise identification of the day of oestrus was made easier by the presence (or absence) of sperm in the smear; in addition, very large numbers of cornified cells in the smear indicated that oestrus had passed. There was an irregular pattern in the appearance of the strands of mucous that were usually present in the smears but they were consistently absent on the day of oestrus.

Sperm were only found in the smear on one day of the oestrous cycle though small round bodies (probably prostatic material) were sometimes present on the day after the finding of sperm in a smear. It seems likely, therefore, that the period of female sexual receptivity lasts for less than 24 hours.

A summary of data on reproductive cycles is provided in Table 4.1.

TABLE 4.1 Cyclic reproductive phenomena in female *B. gaimardi*

Parameter	N	Mean \pm S.D. (days)	Range	Mode
Oestrous cycle	46	22.6 \pm 3.5	17-37	22
Gestation length	15	21.3 \pm 0.8	20-22	22
RPY* - Birth	13	17.6 \pm 0.9	16-19	18
RPY - Oestrus	16	18.9 \pm 1.3	17-22	17-18
Birth - oestrus	35	0.5 \pm 0.5	0-2	0**

* RPY - removal of pouch young

** i.e. birth and oestrus occurring on the same night

4.3.2 Oestrous Cycles

Two rather long oestrous cycles of 38 and 46 days duration have been excluded from the calculations in Table 4.1. It seems very likely that the smear regime had somehow missed a period of oestrus somewhere in the middle of the 'cycle'. This so-called 'silent' oestrus has been commented upon by Moors (1975).

There is far greater variation about the mean oestrous cycle length ($22.6 \text{ days} \pm 3.5$) than there is for any of the other parameters recorded.

4.3.3 Anoestrus

Anoestrus, as determined by the absence of oestrous cycles and the presence of a dry and scaley pouch, was a rare occurrence because oestrous cycles occurred throughout the year in most females. Anoestrus usually occurred immediately after the final vacation of the pouch by a young bettong. Other animals also failed to produce young (one female failed to produce a young for over twelve months) but these animals always displayed a pink, moist pouch.

4.3.4 Mating

This was observed on one night only. Over a thirty minute period starting at 2100 h, a male bettong thrice attempted to copulate with a sexually receptive female. The male chased the female frequently for 45 minutes and the two moved around the cage in unison. Attempts by the male to grasp or examine the pouch were repelled by the female who would lie on her side and vigorously kick with her hind limbs. This type of behaviour is similar to behaviour exhibited by the female during non-oestrous periods. Eventually the male grasped the female from behind

and held her with both of his fore legs about her flank. His face was closely apposed to her neck region and it is probable that he bites (and salivates) into her fur, as this region is always as ruffled (and wet, see Plate 7.1a) on the morning after mating as the flank region. This disarrangement of the fur is a useful guide to the occurrence of mating in females whose cycles are not being monitored. Within one minute after the male grasped the female, his pelvis commenced thrusting movements for a period of approximately fifteen seconds. This type of behaviour was repeated twice during the ensuing thirty minutes. A vaginal smear obtained shortly after the copulatory period was over, failed to provide evidence that ejaculation had occurred, i.e. there were no sperm. However, a smear taken early the following morning had sperm present, so presumably a further intromission followed by ejaculation occurred later that night.

4.3.5 Gestation and Parturition

The mean gestation length was 21.3 days \pm 0.8; ($\bar{X} \pm$ SD) there was little variation in this value, the range being 20–22 days (Table 4.1). It seems unlikely that birth occurs during the day as young found in the early morning (0800 h) had not been present in the previous late afternoon (1800 h), so it may be assumed that birth occurs at night. Parturition was never observed nor were there any behavioural indications during the day as to the imminence of birth. It seems probable that birth occurs in the confines of the nest.

The distance between the pouch entrance and the opening of the urogenital sinus is short (approximately 10 cm) and this distance is even shorter when the bettong is hunched in its resting position with the tail between the legs. The crawl by the newborn young to the pouch would be a relatively brief activity.

It seems logical to assume that, as birth and oestrus/mating usually occur on the same night, birth would precede oestrus. Otherwise the persistent male and/or his ejaculate would be likely to interfere with the birth process.

4.3.6 Delayed Gestation

The mean duration in time from the removal of a pouch young (RPY) to birth of the previously quiescent blastocyst (delayed gestation) is 17.6 ± 0.8 days. This is approximately 4 days shorter than the normal gestation length. A very low value for a delayed gestation (11 days) was obtained from one female bettong (B14). The previous young (42 days old) of this mother suffered a loss in weight from the previous week (i.e. 18 days before the birth) until day 11 before birth when it was lost. Perhaps the loss of weight was associated with a decreased sucking stimulus which was sufficient to initiate blastocyst development during pouch occupancy.

4.3.7 Embryonic Diapause

Birth is usually associated with a post-partum oestrus. Post-partum oestrus occurs usually on the same night as birth (Table 4.1). The fertilised egg produced remains dormant in the uterus for most of the period during which there is a young in the pouch. Towards the end of pouch life the dormant blastocyst begins to develop with the result that birth coincides closely with final vacation of the pouch by the larger young.

Should the pouch young be lost or experimentally removed, a similar process is initiated and a new young may be born in the absence of a male.

Thirty-three pouch young were experimentally removed or lost during the study. In 21 cases a new young was later found in the pouch during the absence of a male (males were sometimes introduced near the time of an expected birth in order to obtain a post-partum mating). Females returned to oestrus 18.9 ± 1.3 days ($\bar{X} \pm SD$) after RPY.

4.3.8 Post-partum Oestrus

A post-partum oestrus occurs within two days after birth in females. Oestrus usually (N=19) occurs on the night of birth; it occurred one day later on fifteen occasions and on one occasion two days later than the birth.

Additional data on oestrus and post-partum oestrus and their association with pouch vacation are presented in Chapter 7.

4.3.9 The Relationship Between Oestrous Cycles and Gestation

There appear to be no significant differences ($t = 0.7$, $P < 0.5$) between the period of time that elapses from one oestrus to the next, whether or not a gestation and birth intervenes (Tables 4.2 and 4.3). However there is a significant difference between the variance (Variance Ratio = 54.5, $P < 0.01$). The fact that the variances between the two groups are unequal (Table 4.3) disallows (in this instance) the use of the student 't' test as a method for comparing means as the t-test assumes an equal variance. However, a one-way analysis of variance (Table 4.3b) indicates that the major variance is within the two samples and not between them. These two samples of oestrous cycle lengths, one with an intervening gestation and one without a gestation comprise all the available data from this study and included estimations of oestrus

based solely on changes in the vaginal smear without the corroborating evidence of a mating (i.e. sperm in smear and/or fur ruffling)

When these estimations are excluded there is a non-significant difference between the variance which allows now the use of the t-test. There is a significant difference in time when one compares (Table 4.2) the period between a mating and a post-partum mating (i.e. a fertile cycle) with a mating to the next mating (no gestation, i.e. an infertile mating). The fertile cycle is about one and a half days shorter than the infertile cycle. Similarly when one compares two consecutive oestrous cycles (matings at oestrus), one cycle of which is fertile and the other infertile the difference is also significant ($t = 3.28$, Table 4.4). The fertile cycle was the first cycle in four cases and the neonate was removed or lost on the day of birth.

When one compares the time to oestrus after RPY there is no significant difference whether or not there is an intervening oestrus, (though the numbers involved are less than other groups).

TABLE 4.2 Results of t-test analysis of the effect of gestation upon the duration of reproduction cycles

Parameter	N	$\bar{X} \pm \text{S.D.}$	Significance (t-test)
Oestrus to post-partum oestrus	18	22.2 ± 0.8)	N.S.*
Oestrus to oestrus (without birth)	28	22.8 ± 4.2)	
Mating to post-partum mating	16	21.9 ± 0.9)	P<0.01
Mating to mating (without birth)	18	23.4 ± 1.3)	
RPY to post-partum oestrus	11	18.5 ± 1.2)	N.S.
RPY to oestrus (without birth)	7	19.1 ± 1.6)	

RPY = removal of pouch young; N.S. = not significant (t-test);
* significant differences between variance (P<0.01)

TABLE 4.3 Statistical treatment of all oestrous cycle data (with and without gestation)
(a)

Oestrous cycle length (days)	$\bar{X} \pm \text{S.D.}$	N
With gestation	22.2 ± 0.8	18
Without gestation	22.8 ± 4.2	28

Variance ratio = 59.5 ($P < 0.001$)

i.e. there is apparently a significant difference between the variance of the two samples.

(b) One way Analysis of Variance Table

	Sum of Squares	Degrees of Freedom	M.S.
Between samples	5.73	1	5.73
Within samples	484.3	41	11.8

F statistic = $\frac{5.73}{11.8} = 0.48$. Not significant

TABLE 4.4 Oestrous cycles (matings only). Paired data.

Animal Number	Mating to post-partum mating (days)	Mating to Mating (days)
B1	23	23
B2	21	24
B2	23	27
B3	22	23
B6	22	24
B6	23	23
B11	23	23
B11	21	22
B15	21	22
B26	21	23
$\bar{X} = \text{days} \pm \text{S.D.}$	22.0 ± 1.0	23.5 ± 1.4

Paired t-test = 3.28, D.F. = 9, $P < 0.01$

4.3.10 Monthly Distribution of Births

Bettongs appear to be continuous breeders, both in captivity and in the wild (Figures 4.5 and 4.6). With one exception, all wild-caught adult females (molar index at least III.2, either had a pouch young or showed evidence of recently having had one (e.g. an enlarged teat). The one wild caught adult female (B14) that had neither a young nor an enlarged teat showed an oestrous type smear on the day after capture. Either this animal was undergoing her very first oestrus or she had recently lost a small young.

The monthly distribution of actual births in captivity (i.e. where birth dates were known) and the crude birth data from the wild (i.e. birth dates extrapolated from the age of the pouch young) are presented for individual years (Figure 4.5) and compounded (Figure 4.6). There are too few births in any particular year to suggest a seasonal aspect although there appears to be a trend for fewer births in spring and summer (Figure 4.6 and Table 4.5).

A chi-square test of the number of births in the four seasons was not significant ($\chi^2 = 4.71$, D.F. = 3) but when the seasons were amalgamated into the warmer, spring and summer months and the cooler, autumn and winter months the χ^2 value was 3.9 which is just significant $P < 0.05$. More births occur in the cooler months but this conclusion may be biased by collection variations.

TABLE 4.5 Births in the various seasons in Tasmania. Total of wild and captive over a 5 year period

Season	Months	No. of Births
Summer	December - February	25
Autumn	March - May	33
Winter	June - August	40
Spring	September - November	26

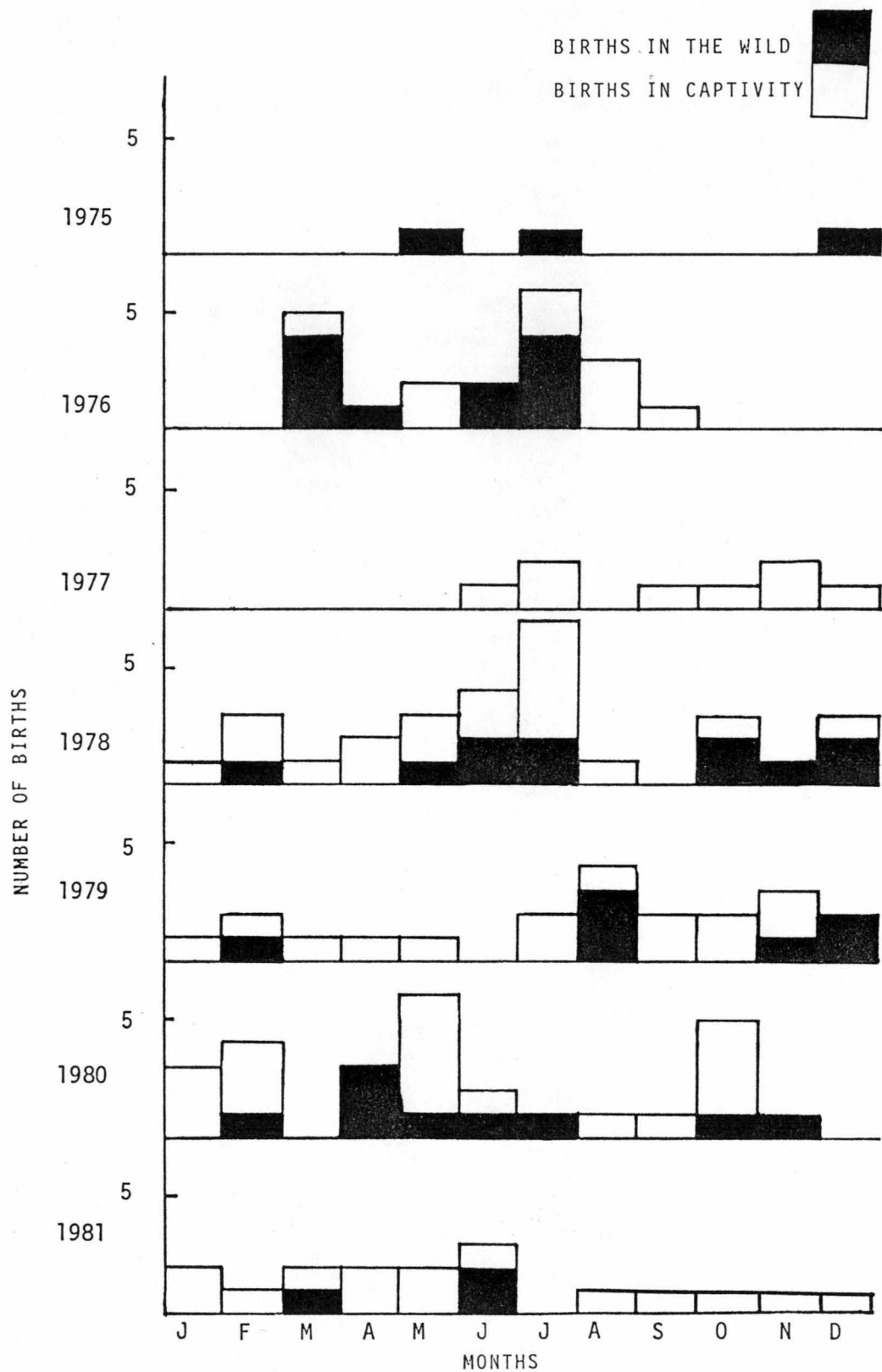


Figure 4.5 Monthly distribution of Bettong births 1975-81

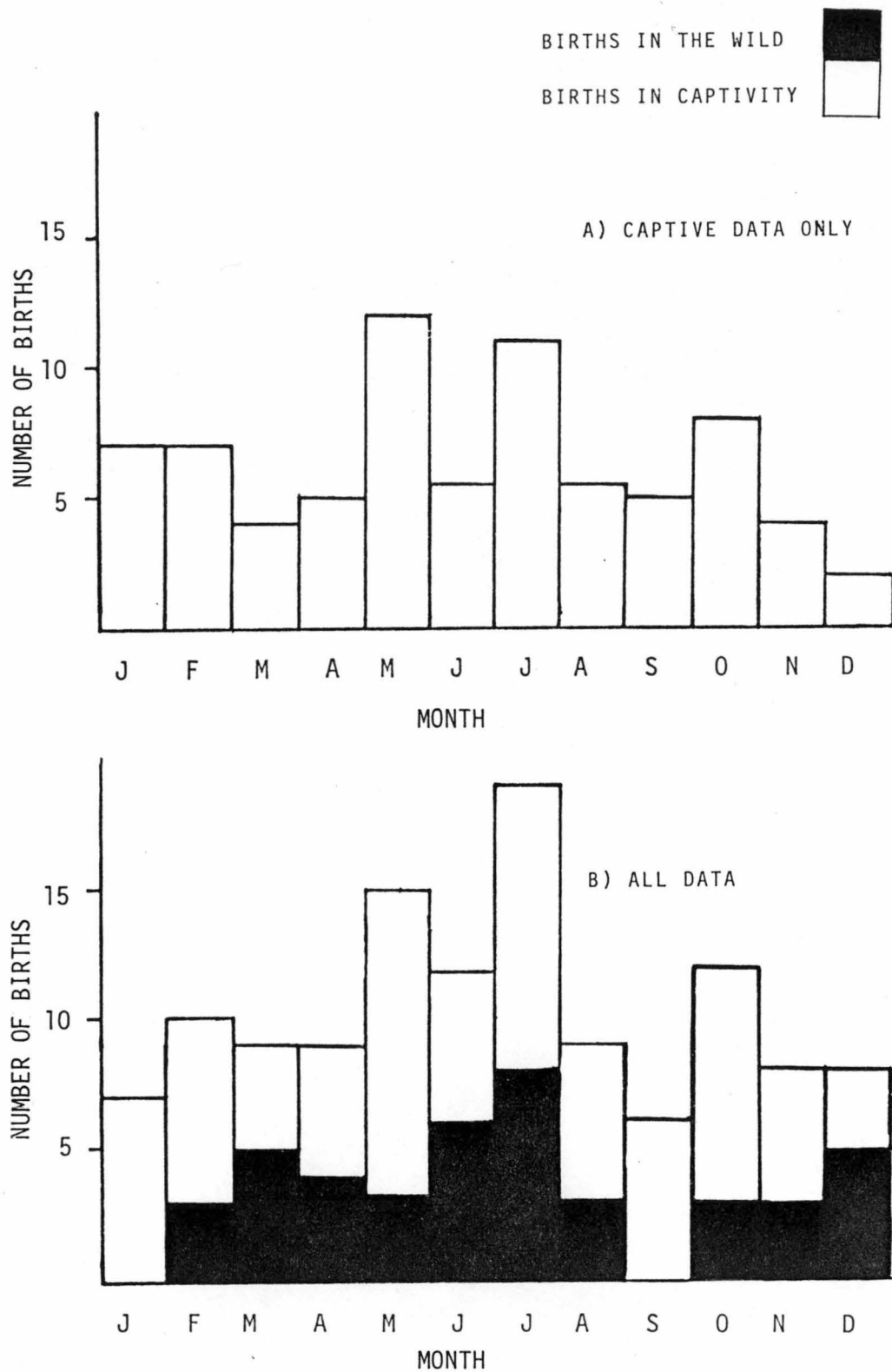


Figure 4.6 Composite histograms of bettong births per month (1975-81).

Pouch life is approximately four months (106 days) with the result that all young born during the 'peak' breeding months of May, June and July (Figure 4.6B) would leave the pouch in the spring and early summer.

4.3.11 Pouch Life

The duration of pouch life, i.e. the period from birth until permanent vacation of the pouch was found to be 106 days (Chapter 7). As pouch emergence coincides with a new birth and/or oestrus, a female bettong could, under ideal conditions, always have a pouch young. This certainly appears to be the situation in the wild. The pouch life duration of 106 days suggests that a female could rear three young in one year (in fact seven young in two years). Only one female in captivity ever produced and reared to pouch vacation three young in a twelve months period and it is likely that loss of young, stress, and other environmental factors in captivity and presumably in the wild also would ensure that less than the maximum number of young is reared each year.

4.3.12 Removal of the Corpus Luteum and RPY

Removal of the corpus luteum (CLX) from four quiescent females was followed by oestrous vaginal smears on day 6 after CLX, day 7 (twice) and day 18. In no case did parturition occur. The corpus luteum from the animal that came into oestrus 18 days after the operation did not have the histological appearance of a quiescent corpus luteum (see Chapter 5) but rather of an atretic corpus luteum. It seems likely that this animal may have acted as a control i.e. the quiescent C.L. was not removed. Using the assumptions of Tyndale-Biscoe *et al.* (1974) one can estimate the follicular phase of the oestrous cycle in *B. gaimardi* as 7 days. As the total length of an oestrous cycle is approximately twenty-two days, then the 'luteal' phase will be about fifteen days in

duration (i.e. 22 minus 7 days). Gestation (21 days) continues for a further six days (21 minus 15 days) over the length of this estimated luteal phase.

4.3.13 Male Reproduction

The 'Testicular Index' (TI) obtained in this study estimates the change in size of the testes (Figure 4.7). This index appears to increase linearly until about week 24 (day 168) after birth, after which its rate of change increases. This increased rate subsides by week 42 (day 294); subsequently there is little change in the index. Halfway between these two periods of change (week 32-34) the testicular index rises above a value of 6. Urine samples taken from all males with a TI above 6 contained copious sperm. This age also corresponds with a male's first fertile mating (see Section 4.3.14).

After pouch life there is a linear relationship between TI and weight, until males attain a weight of approximately 1500 g (Figure 4.7), beyond which there is a rapid rise in TI, in the face of small increases in weight. There are few differences in the relationship between TI and weight between wild and captive bettongs, although one wild male appeared to be mature at a lighter weight (1400 g) than any of the captive males. The values of TI from an immature male that had put on a large amount of weight (apparently because it sucked from its mother's teat while out of the pouch for an abnormally long period of eighteen weeks) are noted by the open star.

4.3.14 Sexual Maturity

Table 4.6 presents the age at sexual maturity for thirteen captive bettongs. The mean age of maturity was 284 days or approximately 40 weeks (9.5 months). The youngest male to mature was 180 days (26 weeks) of age and the youngest female was 224 days (32 weeks) of age. All animals were mature by age 52 weeks.

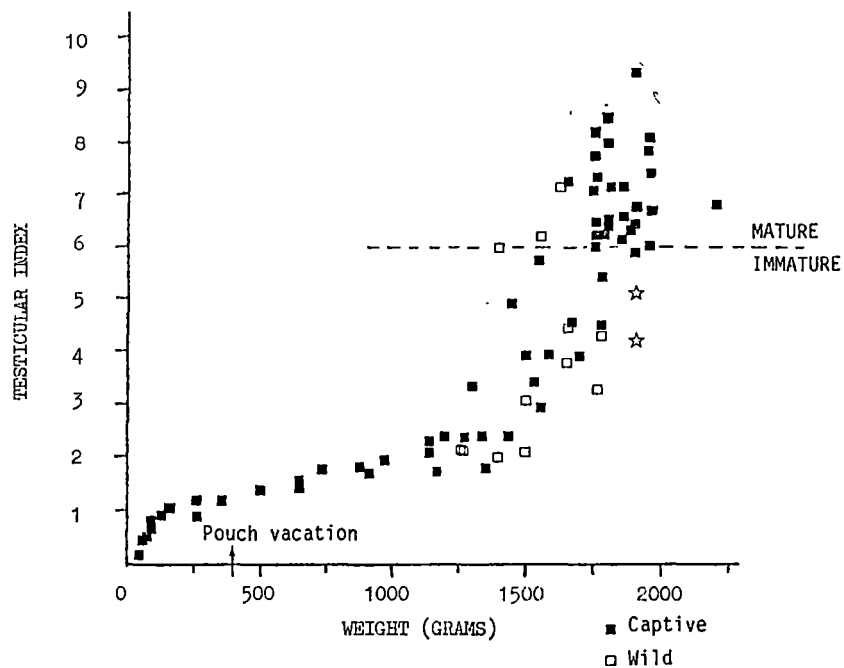


Figure 4.7 The association of testicular index with weight in wild and captive bettongs (N = 18). Star indicates immature but heavy individual.

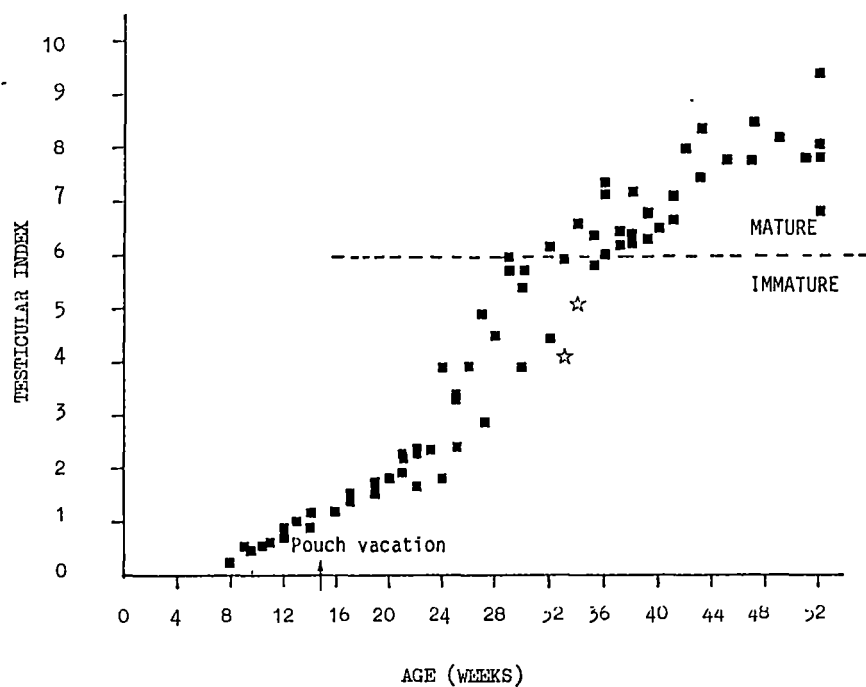


Figure 4.8 The change in testicular index with age in the captive population (N = 7).

TABLE 4.6- Age at maturity in thirteen captive bettongs

Sex	Date of Birth	Date of Maturity	Indications	Age at maturity (days)
F	29/6/77	11/7/78	Pouch young present	370
M	24/11/77	30/8/78	Sired young	277
M	24/7/78	22/3/79	Sperm in urine	180
F	30/8/78	26/7/79	Produced young	320
F	26/10/78	30/8/79	"	300
F	13/1/79	19/7/79	"	270
F	9/2/79	18/10/79	"	248
F	5/4/79	7/2/80	"	320
F	22/5/79	29/5/80	"	360
M	21/1/81	7/10/81	Sired young	259
F	-	7/10/81	Produced young	224*
F	-	18/11/81	"	289*
F	-	27/11/81	"	259*
				Mean = 284 days

* Collected as pouch young and age estimated from head length

The age of maturity in females was 296 ± 48 days ($\bar{X} \pm \text{S.D.}$) and in males it was 239 ± 51 . A student t-test value of 1.8 ($P < 0.1$) indicates that this is not a significant difference, possibly because the male sample size is very small.

4.4 DISCUSSION

Reproduction in the Tasmanian bettong conforms to the predominant macropodid pattern (Sharman *et al.* (1966); Sharman (1970); Tyndale-Biscoe (1973); Tyndale-Biscoe *et al.* (1974); Renfree (1981) and Tyndale-Biscoe and Hinds (1981). The lengths of the oestrous cycle and gestation are similar and a post-partum oestrus occurs shortly after birth. The single blastocyst formed after this post-partum mating remains dormant ('embryonic diapause') until near the end of pouch life when suckling becomes intermittent. Final emergence from the pouch is closely followed by birth and oestrus, with the result that both young (one in and one out of the pouch) suck concurrently from separate teats.

It has been convenient in the past to group the patterns of reproduction of diprotodont marsupials into four groups as suggested by Sharman *et al.* (1966). In the light of more recent information the previous groupings have been modified (e.g. Renfree 1981) and reproductive patterns of the kangaroos may be described by two groupings; Group 1 containing those macropodids (and some other diprotodonts, e.g. most of the Phalangerioidea) which are polyoestrous species in which the gestation period is considerably shorter than the oestrous cycle. Macropods in this group are *M. fuliginosus*, *M. giganteus*, *M. parma* and *M. parryi*. The majority of macropodid species including *B. gaimardi*, are accommodated within Group 2. These species are monotocous, polyoestrous species in which the gestation period is similar in length to the oestrous cycle. This usually results in diapause of the embryo formed at post-partum mating.

Flynn made records over a period of ten years of the reproduction of the Tasmanian bettong; this work culminated in his paper (Flynn 1930) on the uterine cycle during pregnancy and pseudo-pregnancy. In addition to a histological study of the reproductive organs (Chapter 5 provides

more details) he also described some aspects of the duration of the oestrous cycle, gestation, pouch life and the breeding season. In retrospect Flynn's studies suffer from a lack of knowledge of embryonic diapause.

The results from the present study show that reproduction in *B. gaimardi* is very similar to that of *B. lesueur*. Tyndale-Biscoe (1968) commented on the unique position that *B. lesueur* and *B. penicillata* hold in having the shortest gestation period of any species of Macropodidae yet examined. He further commented that the six week gestation period proposed by Flynn (1930) for *B. cuniculus* (= *gaimardi*) 'remains in doubt'. The present data justify this suspicion and further show that the gestation period in *B. gaimardi* (21 days) is the same as that in *B. lesueur* and *B. penicillata*. Although Flynn (1930) was unable either to breed bettongs or maintain pouch young in captivity, he suggested that on the basis of his limited observations, pouch life would be very short. He came to this conclusion because, being unaware of embryonic diapause in the bettong, he had to assume that blastocysts found in the uterus of animals with pouch young were stages of normal pregnancy. Furthermore as he believed that gestation was 6 weeks and that only one young occupied the pouch at a time, he concluded that pouch life must approximate six weeks also. Sharman (1965) pointed out that six weeks would be a very short pouch life and would conflict with observations of other macropodids. The correct estimate of pouch life in *B. gaimardi* (106 days) is in fact similar to that in *B. lesueur* (116 days, Tyndale-Biscoe 1968) and *B. penicillata* (100 days, Sampson 1971).

Within the Macropodidae, extant members of the genus *Bettongia* (*B. lesueur*, *B. penicillata* and *B. gaimardi*) have the shortest gestation and oestrous lengths. This distinction is emphasized by the fact that these parameters of reproduction (and several others) are extremely

similar in the three species (Table 4.6). This relationship is unlikely to be coincidental and indicates that within this genus, reproductive patterns are conservative and have presumably evolved to suit the various species to somewhat similar environments.

Zuckerman (1953) showed that *B. gaimardi* and *B. penicillata* could produce hybrids in captivity, though the fertility of these young is unknown. All three species of the genus *Bettongia* are now (but not in the recent past) geographically isolated, and it may be that speciation has occurred relatively recently.

Among the four extant genera of the sub-family Potoroinae, there are both similarities and differences in reproduction (Table 4.7). Although *Potorous tridactylus* and *Bettongia* spp. are similar in that they all exhibit embryonic diapause, their intra-uterine development differs (Chapter 5) as does their age of sexual maturity. Bettongs require at least eight to twelve months after birth until they mature whereas some *P. tridactylus* females are apparently able to mate shortly after leaving the pouch (Flynn 1923; Buchmann, pers. comm.). The potoroo is unusual in that it has a delayed gestation length considerably shorter than the length of the oestrous cycle (Shaw and Rose 1979).

The reproduction of *Aepyprymnus rufescens* as described by Moors (1975) appears to be similar to that of macropodids with a Group 1 reproductive pattern. However, Johnson (1978) presented a more detailed account of reproduction in this species that differs fundamentally from that by Moors (1975). Johnson (1978) showed that *A. rufescens* conforms to the more typical macropodid pattern (Group 2). The papers by Moors (1975) and Johnson (1978) are difficult to reconcile, though both suggest similar gestation lengths. The species investigated came from different regions and Moors had limited numbers.

TABLE 4.7 Some parameters of reproduction in the sub-family Potoroinae

	<i>B. gaimardi</i>	<i>B. lesueur</i>	<i>B. penicillata</i>	<i>A. rufescens</i>	<i>P. tridactylus</i>
Oestrous cycle length (days)	22.6	22.2	22 - 23	21 - 25	42
Gestation length (days)	21.3	21.3	21	21 - 23.6	38
Delayed gestation length (days)	18	20	21	18.7	29
Average pouch life (days)	106	116	100	114	120
Mean neonate weight (g)	0.300	0.320	0.290	-	0.377
Approximate maternal weight (kg)	1.78	1.15	1.35	3.0	1.02
Diapause	yes	yes	yes	no/yes?	yes

Data from this study, Tyndale-Biscoe (1968); Parker (1977 and pers. comm.); Hughes (1962); Shaw and Rose (1979); Moors (1975) and Johnson (1979).

Little is known of the reproduction of *Hypsiprymnodon*, but it appears to be unique amongst the Macropodidae in the regular production of two young at a time (Ride 1970; Johnson and Strahan 1983).

In a previous paper, Rose (1978) demonstrated that, with the possible exception of the genus *Bettongia*, oestrous cycle lengths within the Macropodidae are of little taxonomic value as several closely related species have patterns of reproduction that are quite different. However, phylogenetically, the lengths of oestrous cycles and gestations are important as they and their component parts indicate the way in which the evolution of an extended gestation period has been accomplished within a single oestrous cycle.

In many marsupials, parturition coincides with the end of the luteal phase in the uterus. In those macropodids with a post-partum oestrus, gestation is extended past the luteal phase into the follicular phase by what has been termed a feto-placental influence (Tyndale-Biscoe *et al.* (1974). In the few macropodids in which pregnancy does not occupy most of the oestrous cycle length we find that gestation is accommodated in an oestrous cycle that has been significantly lengthened by an increase in the luteal phase which now approximately corresponds to the gestation length (Table 4.8).

Although the evolution of an increased gestation length in the macropodids has been accomplished by one of two different processes, there is no significant difference in the gestation lengths between the two groups (Table 4.9).

Presumably the variations in oestrous cycle and gestation lengths within the macropodids have evolved to suit particular species to their environment. The external environment may influence reproduction in a number of ways, one pathway operating via the hypothalamus. Changes in the responses of the hypothalamus to the environment may be due to

TABLE 4.8 Duration (in days) of some components of the oestrous cycle and gestation period in the Macropodidae

		Gestation length	Luteal [*] phase	Oestrous cycle	Delayed gestation	Post-partum oestrus
Group 1	<i>Macropus fuliginosus</i>	30.5	26.5	34.9	—	—
	<i>Macropus giganteus</i>	36.4	34.7	45.6	28–32	±
	<i>Macropus parma</i>	34.5	31.8	41.8	—	±
	<i>Macropus parryi</i>	36.3	36.2	42.2	—	—
Group 2	<i>Aepyprymnus rufescens</i>	21–23	—	21–25	18.7	±
	<i>Setonix brachyurus</i>	27	16	28	26	+
	<i>Macropus eugenii</i>	28.3	20	28.4	27	+
	<i>Macropus rufogriseus</i>	29	22	32	27	+
	<i>Macropus rufus</i>	33	23	34.6	31	+
	<i>Bettongia lesueur</i>	21.3	**	22.2	20	+
	<i>Bettongia gaimardi</i>	21.3	16	22.8	18	+
	<i>Bettongia penicillata</i>	21	**	22–23	21	+
	<i>Potorous tridactylus</i>	38	27	42	29	+
	<i>Macropus agilis</i>	29.4	**	30.6	26.5	+
	<i>Wallabia bicolor</i>	37	**	31	30	+
	<i>Thylogale billardieri</i>	29.6	19	30	28.5	+
	<i>Macropus robustus</i>	32	**	33	30.8	+
	<i>Petrogale xanthopus</i>	31.5	**	32	**	+

* calculated as in Tyndale-Biscoe *et al.* (1974)

** not investigated

Data mainly from Tyndale-Biscoe *et al.* (1974), other data from Calaby and Poole (1971), Parker (1977 and pers. comm.), Shaw and Rose (1979), Rose and McCartney (1982) and Rose (unpublished observations).

TABLE 4.9 A comparison of the oestrous cycle and gestation lengths ($\bar{X} \pm \text{S.D.}$) in the two groups of macropodids

	Group 1	Group 2	t-test
Gestation length	34.4 ± 4.7	29.0 ± 5.6	not significant
Oestrous cycle length	39.6 ± 4.6	30.4 ± 5.4	$0.05 > P$

changes in the ability of peripheral receptors to detect environmental change or to changes in the threshold to which the hypothalamus responds. These in turn could be influenced by circulating pituitary hormones or ovarian steroids, as suggested by Cumming and Findlay (1977). For example, if the hypothalamus were to become less sensitive to oestrogenic feedback, the follicular phase might be extended, resulting in a lengthened oestrous cycle.

The possibility that the feto-placental unit can have an effect upon the duration of pregnancy and the oestrous cycle is one of the most interesting of the recent discoveries about marsupial reproduction. Some studies have tended to discount this possibility, e.g. Sharman and Calaby (1969); Sharman (1970); Walker and Hughes (1981). Tyndale-Biscoe *et al.* (1974), however, were amongst the first to explain the disparate length in gestation and the luteal phase in Group 2 macropodids by use of the term 'feto-placental' influence. This term had also been used in regard to marsupial reproduction by Moors (1974) but in a somewhat different context.

As the placenta is fetal tissue, it follows that its role is determined by the fetal genotype (Tyndale-Biscoe *et al.* 1974). Kirsch and Poole (1972) have shown that there is a variation in the usual gestation length when interspecific hybrids of *M. giganteus* and *M. fuliginosus* are produced. As the only difference in this situation was the presence in the fetal genotype of genes from a male of a different species it

seems again likely that the fetus/placenta has had some effect on the gestation length. When the female hybrids reached sexual maturity they had oestrous cycles intermediate in length between females from the two parent species.

The duration of that feto-placental influence which extends pregnancy beyond the 'luteal phase' varies within the Macropodidae. In many species it may last between 8 to 11 days, e.g. *Setonix brachyurus*, *Macropus eugenii* and *Thylogale billardierii*, but in *Wallabia bicolor* it continues for 16 days. The duration of the feto-placental influence in *B. gaimardi* may be considered as equivalent to the 6 days by which gestation surpasses the luteal phase.

Macropodids may have evolved an extension of pregnancy by a feto-placental influence, but this has not evolved along the path found in eutherians in that it does not allow (with one exception, *W. bicolor*) pregnancy to continue past the length of the oestrous cycle. This may be an evolutionary handicap that marsupials have been unable to overcome. However, an attractive viewpoint is that marsupials (and the macropodids in particular), have solved the fundamental problem of producing well-developed young (in this case, at the end of pouch life) in a different but no more primitive way than that of eutherians.

That the presence of the fetus/placenta might affect the length of the oestrous cycle was first demonstrated by Merchant (1976) in the agile wallaby *M. agilis*. He has subsequently also demonstrated this phenomenon in *M. eugenii* (Merchant 1979) and both sub-species of *M. rufogriseus* (Merchant and Calaby 1981). In each case, the period from oestrus to post-partum oestrus was significantly shorter than the period from oestrus to oestrus (i.e. without an intervening gestation). Similarly he showed that RPY to post-partum oestrus was significantly shorter than RPY to oestrus in all species except *M. rufogriseus*.

banksianus.

Merchant and Calaby (1981) suggest that this effect (perhaps it should be called the 'Merchant effect') may be more widespread than reported to date. This study has shown that the 'Merchant effect' does apply to the Tasmanian bettong in regard to the effect on the oestrous cycle. Possibly the 'Merchant effect' acts to synchronise birth and post-partum oestrus.

Nevertheless it has not yet been universally accepted that there is an influence (other than morphogenetic) by the fetus on the reproduction of the mother, and Walker and Hughes (1981) urge caution in the interpretation of data on this topic.

External factors often influence the reproduction of mammals. It is logical to assume that mammals will tend to produce their young at a period of the year when environmental conditions are optimal for the survival of both mother and young (Sadleir 1969). Applying this logic Sharman *et al.* (1966) and Tyndale-Biscoe (1973) have suggested that those marsupials living in regions where spring is a time of abundant food (predominantly south-east Australia) will breed so that young leave the pouch during the spring when there is an increase in the growth of vegetation. This 'rule' generally holds true, though factors other than purely environmental ones can affect the onset and duration of the breeding season, e.g. interspecific competition (Dickman 1982).

The breeding season of all five Tasmanian macropodids is illustrated in Figure 4.9. The breeding season is presented as the monthly distribution of births. Sadleir (1969) defines a breeding season as the period of the year between the first and last conception date, but this definition would not allow for the inclusion of some birth dates delayed due to embryonic diapause as their conceptions may have been many months prior to parturition.

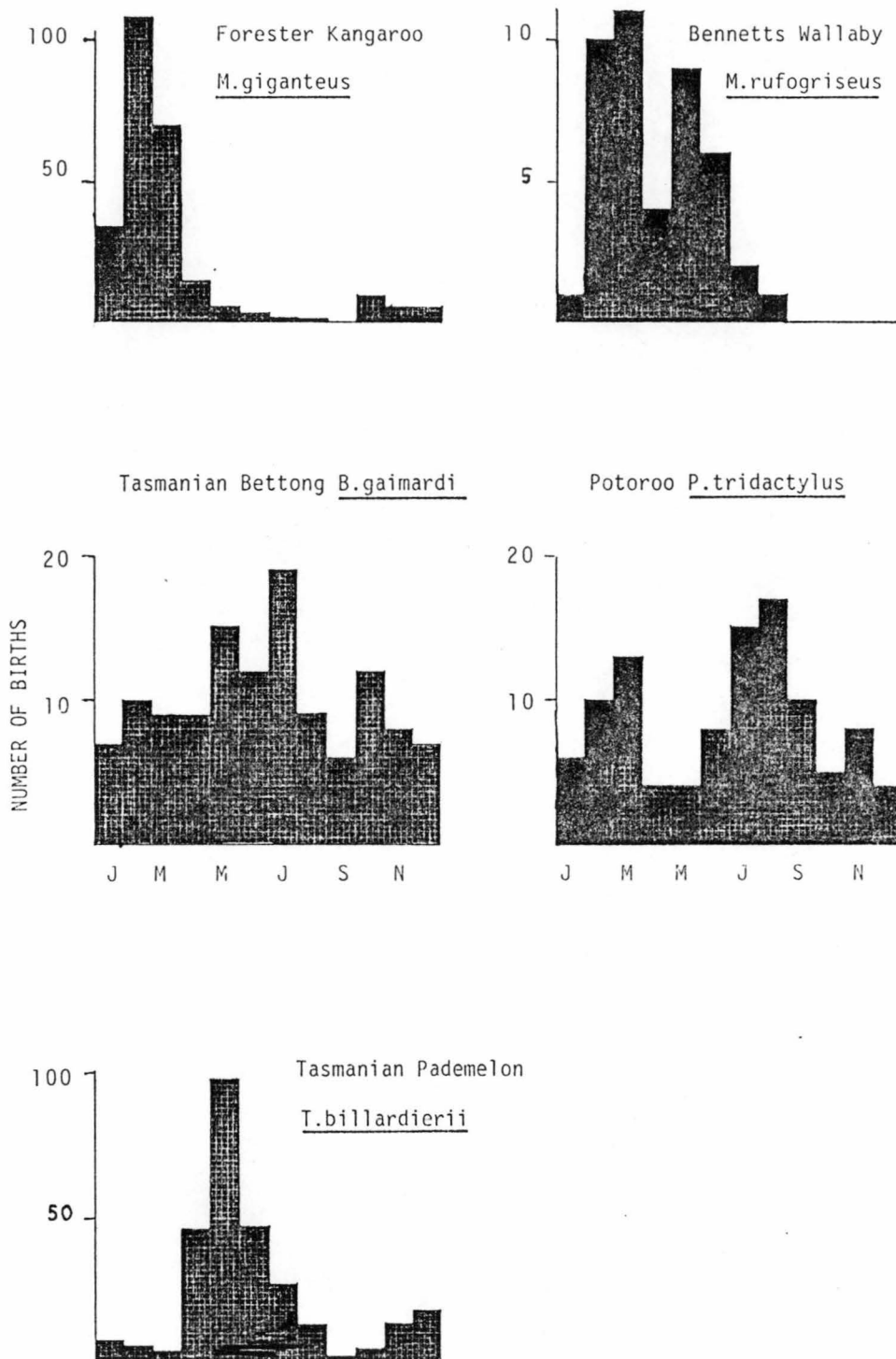


Figure 4.9 Breeding season of the Tasmanian Macropodidae. The sources of the data are provided in the text.

It can be seen that all three Tasmanian macropodines are seasonal breeders. *M. giganteus* (Pearse 1981), *M. rufogriseus* (Merchant and Calaby 1981) and *T. billardieri* (Rose and McCartney 1982a) breed in a way that ensures that the majority of young leave the pouch during the spring and early summer. By way of contrast, *P. tridactylus* (Buchmann pers. comm.) and *B. gaimardi* (this study) are basically continuous breeders, though there may be minor peaks and troughs in the monthly distribution. Guiler (1960) and Hughes (1962) also failed to demonstrate a distinct breeding season for the potoroo and similarly Flynn (1930) and Zuckerman (1953) suggested that the Tasmanian bettong probably bred for most of the year.

There are important dietary variations between the two Tasmanian rat-kangaroos, which are both mainly fungivorous, and the larger herbivorous kangaroos. Possibly there is less seasonal variation in the food eaten by the potoroinines or perhaps more likely they are able to vary their diet opportunistically throughout the year. As the two rat-kangaroos have different habitat requirements there will be little direct competition between them.

The relatively short pouch life and the early maturity (within a year) suggest that the bettong could have a relatively high breeding potential. However, it is unlikely to fully realise its maximum breeding potential in the wild. If one assumes the production of two young per year, one male and one female, bettongs would replace themselves (i.e. 'reproduce') once a year. A breeding life of approximately four years (one year until maturity and few bettongs live longer than five years in captivity) would allow each female to produce on average four females and four males in a life-time. Various factors including predation would lower this figure so that it appears that bettongs would be lucky to reproduce themselves more than twice during a life-time.

This could account in part for an inability to replenish animal numbers in the wild after the heavy predation and environmental change that has occurred on mainland Australia (King 1981) and may partly explain the endangered status of all mainland bettongs.

From the brief investigation carried out here into male reproduction one can conclude that, most likely, males are able to produce sperm and breed throughout the year, although this conclusion is based partly on indirect evidence from the females. Sampson (1971) found little variation in the size of the testes of *B. penicillata* throughout a twelve month period. It may be that there are slight changes in size and activity of the male accessory glands of the bettong during the warmer months of the year that may in part explain the slight decline in breeding. The use of the testicular index as a measure of maturity in the field appears to be justified, although in captivity the sampling of sperm in the urine would be an easier method. Additional work on male reproduction could involve obtaining more information on the age at maturity and effect of attaining maturity, which is accompanied by aggression between father and son, on dispersion in the field.

CHAPTER 5

INTRA-UTERINE DEVELOPMENT AND THE CHANGES IN THE REPRODUCTIVE TRACT DURING THE REPRODUCTIVE CYCLE

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5.1 INTRODUCTION

The anatomy of the reproductive tract of most members of the sub-family Potoroinae has been described by Pearson (1944, 1945). As in all marsupials, there are paired ovaries, uteri and lateral vaginae. The Potoroinae differ from the Macropodinae by the presence of an anterior vaginal expansion or caecum, but the function of the reproductive tract differs little between these two groups.

It seems likely that In the bettong ovulation is spontaneous and usually occurs alternately in left and right ovaries. At ovulation a single egg is shed and a corpus luteum develops, also spontaneously. Most natural matings appear to be fertile and the single egg produces only one embryo. After ovulation there is an initial luteal phase in both uteri associated with the developing corpus luteum. The pregnant ^(gravid) and non-pregnant ^(non-gravid) uteri within the one animal appear similar during this early phase. Also it is difficult to distinguish between pregnant and non-pregnant animals. The latter situation (i.e. in non-pregnant animals) has been termed 'pseudo-pregnancy' by Hill and Donoghue (1913). By the time the corpus luteum has attained its maximum size there is a considerable size difference between the pregnant and non-pregnant uteri of the one animal.

The regression of the corpus luteum is associated with the growth of new follicles in the contra-lateral ovary. Although the corpus luteum shows signs of regression, pregnancy continues in most macropodids

and in fact progesterone levels may remain elevated for some time (Lemon 1972; Hinds and Tyndale-Biscoe 1982). A feto-placental influence has been postulated (Tyndale-Biscoe *et al.* 1974) as the factor controlling the continuation of pregnancy during late gestation.

The marsupial egg is surrounded at ovulation by a thin ~~membrane~~ zona pellucida and subsequently acquires a mucoid coat ^{g shell membrane} (Hughes 1974).

After cleavage The blastocyst then expands and the three primary germ layers are established. In most marsupials embryonic development proceeds beyond the somite stage and up to the formation of the fetal rudiments before the shell membrane ruptures (Hughes 1974). This rupture corresponds with a rapid growth phase and organogenesis. The neonate in all marsupials is born via a median vagina (Sharman 1970; Shaw and Rose 1979). This structure is transient in most species of marsupial, including the potor-
oines, but in some macropodines the median vagina remains open (patent) and lined with epithelium after the first birth.

Birth in many macropodids is accompanied by a post-partum oestrus and ovulation. In this case, if the egg is fertilised, the embryo only develops to a unilaminar blastocyst stage of approximately eighty to one hundred cells (Clark 1966; Smith 1981).

The corpus luteum from the post-partum ovulation then becomes 'quiescent' and its secretions are insufficient to induce a secretory (luteal) phase in the uterus. The blastocyst so formed at the post-partum mating remains in a dormant state termed embryonic diapause (Tyndale-Biscoe 1963). Secretions from the corpus luteum increase in response to changes in sucking by the pouch young, i.e. when sucking becomes intermittent or ceases altogether, and this in turn allows gestation to continue. Since the embryo is at the 80-100 cell stage the time to parturition after RPY is usually of a shorter duration (2-3 days) than a normal pregnancy.

Some of the histological changes that occur in the reproductive tract of a pregnant and non-pregnant macropodid were first described by Flynn (1930). The present work and that of Flynn involved the study of *B. cuniculus* (= *gaimardi*). Flynn (1930) and Kerr (1934, 1936) detail some information on embryonic size in relation to the histological changes occurring in the reproductive tract. However, in their studies the ages of the embryos were unknown because they were unable to breed the bettong in captivity; consequently Flynn was unable to ascribe a time scale to the histological changes in the reproductive tract of the bettong.

The aim of this study is to extend and in some cases to reassess previous results by examining the histology of the reproductive tract of *B. gaimardi* at known stages of pregnancy and pseudo-pregnancy.

5.2 METHODS AND MATERIALS

Reproductive material was obtained from eleven adult female bettongs. Reproductive tracts and embryonic material (if present) were obtained during anoestrus, embryonic diapause (lactating female), and stages of the reproductive cycle and delayed gestation as indicated in Table 5.1. Reproductive cycles were initiated by removing the pouch young. The day of RPY was designated day 0.

Females were anaesthetised with Ketalar (10 mg kg^{-1}), followed by Halothane and oxygen administered via a closed circuit anaesthetic machine (Komesaroff, Model AVS-31). The reproductive system of the female was removed (while the heart was still beating) and placed into 10% buffered formalin. Shortly after, both uteri (or the pregnant uterus if identifiable) were opened and examined for embryonic material. The greater diameter of the blastocyst or the yolk sac was measured as

TABLE 5.1 Histological material: animals used and their reproductive status

Animal No.	Reproductive State	Pregnancy State
B20	Anoestrus, no pouch young	not pregnant
B30	Lactating, small pouch young	pregnant
B28	2 days after RPY	not pregnant
B22	4 days after RPY	not pregnant
B19	7 days after RPY	pregnant
B25	9 days after RPY	not pregnant
B36	11 days after RPY	pregnant
B11	11 days after RPY	not pregnant
B2	14 days after RPY	pregnant
B24	14 days after RPY	not pregnant
B14	17 days after RPY	pregnant

was the length of the primitive streak or the crown-rump length of the fetus.

The ovaries, uteri and vaginae were embedded in paraffin wax and sectioned at 8 μ m approximately. The sections were stained with haematoxylin and eosin.

Measurements were obtained of the diameters of the uterine glands (the basal glands were measured for consistency) and of the nuclear diameter of the cells of the corpus luteum. These measurements (30 in each case) were made using a graduated eye-piece calibrated against a micrometer slide. Measurements of the blastocyst were also obtained in a similar manner but later embryos were measured with vernier calipers. The Wilcoxon ranked sums non-parametric statistical test was applied to test for significant differences between the uterine gland diameters within animals.

5.3 RESULTS

5.3.1 Female Reproductive Tract

The gross anatomy has been described by Owen (1834) and Pearson (1945) and is similar to that found in *B. lesueur* (Tyndale-Biscoe 1968).

5.3.1.1 The Ovary

The ovaries are ovoid bodies, which are composed mainly of corpora albicantia and to a lesser extent connective tissue. Follicles are restricted to the outer cortical region. The active C.L. was identified by a combination of its increased size, deeper staining and larger cells with more rounded nuclei, and its greater vascularisation. There usually appear to be from 3 to 5 corpora albicantia in each ovary, most of which are smaller and their cells more vacuolated than the single (when present) active corpus luteum (C.L.). The distinction between corpora albicans and active corpus luteum was not as clear in *B. gaimardi* as that described by Tyndale-Biscoe (1968) in *B. lesueur*.

The corpus luteum reaches a maximum size of 4.6–4.7 mm (N=2) on day 14 after RPY and is much larger than that found in *B. lesueur* (2.1 mm Tyndale-Biscoe 1968).

5.3.1.2 The Uterus

The quiescent and anoestrous uterine bodies each measure approximately 7 by 5 mm (N=2). By day 7 after RPY a pregnant uterus (i.e. one uterine body with an embryo) measured 16 by 10 mm and the contralateral non-pregnant uterus measured 13 by 9 mm. Flynn (1930) described a pregnant uterus containing a 5.5 mm embryo (estimated at approximately 9 days after RPY based on embryonic growth data obtained in the present study) that measured 17 by 9 mm and the non-pregnant uterus 12 by 5.5 mm.

On day RPY+11, the pregnant uterus measured 24 by 13.5 mm, the non-pregnant uterus measured 15 by 8 mm. Near term (day RPY+17), the pregnant uterus expanded to 25 by 19 mm.

The uteri are lined by a single layer of columnar epithelium that is usually ciliated. The endometrium comprises an underlying stroma which contains coiled uterine glands; the appearance of these glands varies throughout the reproductive cycle and pregnancy. Typically the uterine glands comprise a single layer of ciliated epithelial cells with basal nuclei; the luminal diameter of these glands also varies throughout the cycle. The terms basal and luminal are used to distinguish the region of gland near the myometrium from that near the uterine lumen.

5.3.2 Reproductive Phases

Several stages of reproductive condition may be recognised by both functional and histological criteria. These include the following phases:- anoestrus and lactational quiescence; the luteal phase, and pro-oestrus or the follicular phase. It is possible to further distinguish a post-luteal phase, which merges with the ensuing pro-oestrus phase.

The following results contain a description of the corpora lutea, uteri, vaginae and embryos during major phases of the reproductive cycle and pregnancy. Where appropriate some of Flynn's (1930) and Kerr's (1934, 1936) data will be incorporated, with acknowledgement, into the description.

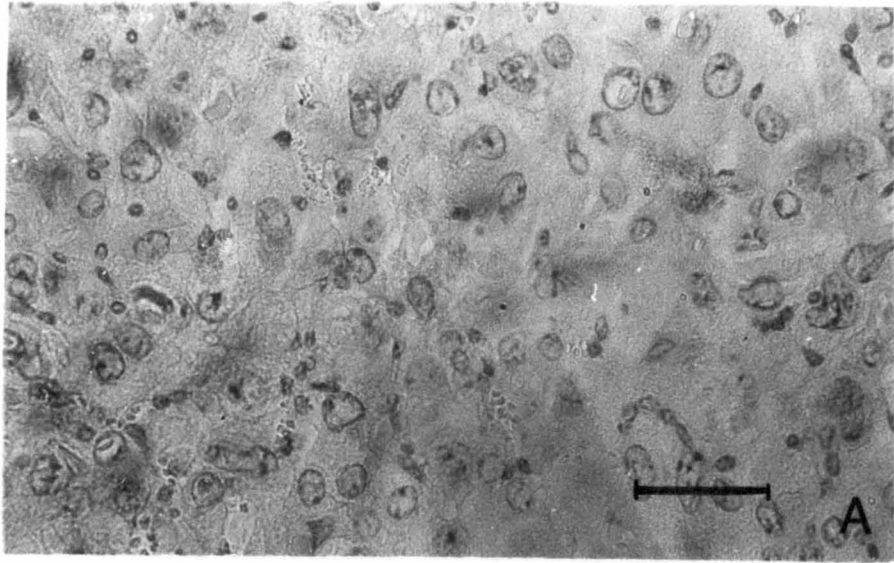
5.3.2.1 Anoestrus and Quiescence (B20, B30)

During anoestrus (animal B20), active corpora lutea (C.L.) could

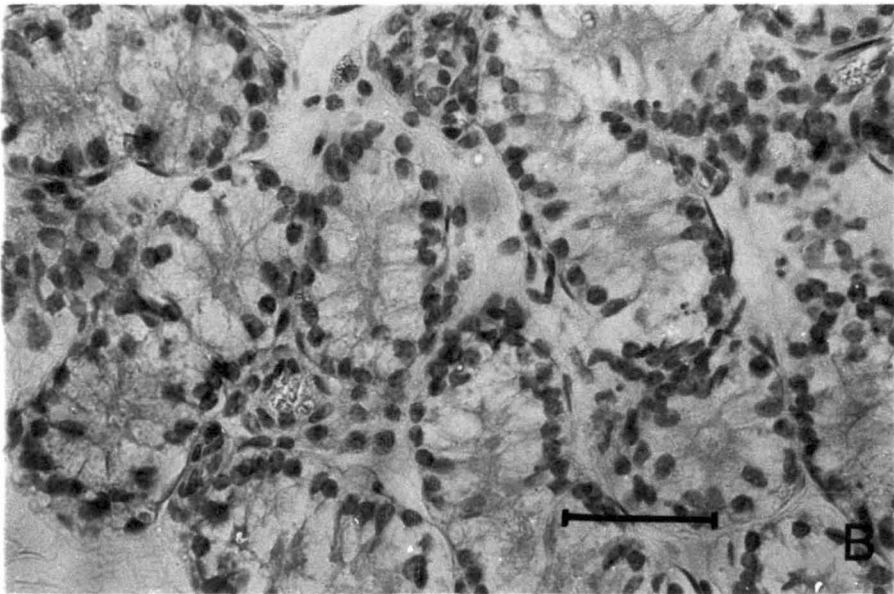
not be found though a number (4) of degenerate structures were present with many cytoplasmic vacuoles. A quiescent corpus luteum was present on the right ovary of a bettong (B30, Plate 5.1a) which had a five week old pouch young. This C.L. contained numerous round cells with round to oval nuclei and without cytoplasmic vacuoles. In both cases (B20, B30), the uteri were small although their appearance was different in histological section. Both lumina were narrow and the luminal epithelia in each case ranged from 30-34 μm (Flynn, 1930, recorded 19-23 μm) and appeared ciliated, though the epithelium of the quiescent uterus was more folded. The uterine glands during anoestrus consisted of ciliated columnar cells with lightly stained basal nuclei. Gland diameters varied as indicated by Flynn (46-58 μm) and had a mean of 54 μm (Table 5.2). By way of contrast, the uterine glands of the lactating female were coiled and crowded (Plate 5.1b), though there was little evidence of secretion or much vascularisation.

The epithelial lining of the lateral vaginae was complete and non-cornified and all cells had round uniform nuclei; the width of this layer measured approximately 100 μm .

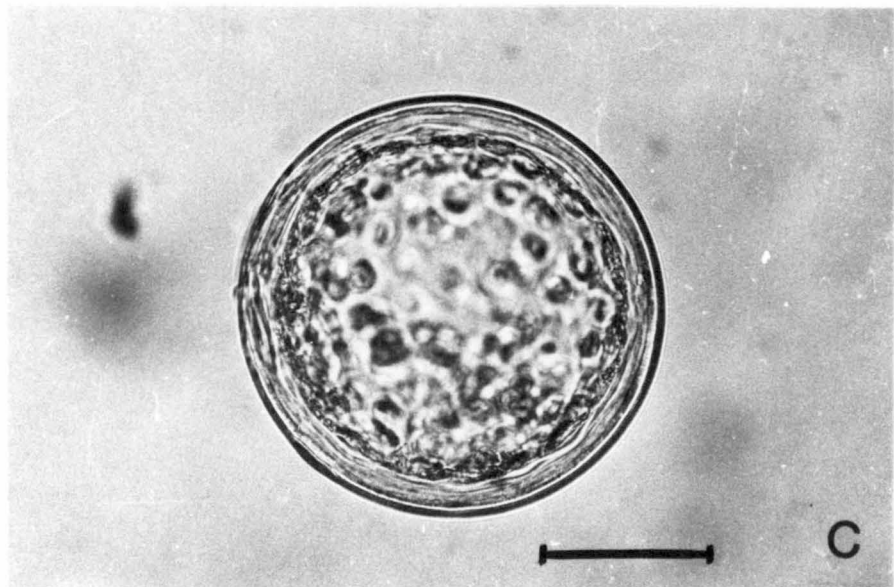
A blastocyst was recovered from the right uterus of B30 (lactating female). This was a unilaminar blastocyst measuring 260 μm in diameter (Plate 5.1c). The protoderm was encased in a shell membrane, mucoid coat and the zona pellucida. Kerr (1934) describes a similar sized embryo of approximately 80 cells which had a shell membrane 4.8 μm wide. The shell membrane initially increases in thickness as the embryo grows and is at its maximum when the embryo measures 308-364 μm in diameter (Kerr 1934). It then decreases in thickness and Kerr (1934) describes a bilaminar blastocyst of 700 μm with a shell thickness of 3.1 μm .



A. Corpus luteum, scale = 100 μ



B. Uterine glands, scale = 50 μ



C. Quiescent blastocyst, scale = 100 μ

TABLE 5.2 Diameter of basal glands (N=30)

Stage	Uterus ipsilateral to corpus luteum \pm S.E. (μ)	Uterus contralateral to corpus luteum \pm S.E. (μ)	Significance
Anoestrus*	53.6 \pm 1.4	54.3 \pm 1.6	N.S.
Quiescent**	64.1 \pm 2.2	56.9 \pm 2.3	N.S.
2 days after RPY	67.8 \pm 1.9	67.1 \pm 1.8	N.S.
4 days after RPY	96.5 \pm 1.9	95.1 \pm 2.7	N.S.
7 days** after RPY	96.0 \pm 1.7	89.1 \pm 2.1	P<0.01
9 days after RPY	106.8 \pm 2.6	98.7 \pm 2.3	P<0.05
11 days after RPY	84.8 \pm 4.3	73.9 \pm 4.0	N.S.
11 days** after RPY	106.8 \pm 3.8	85.8 \pm 2.8	P<0.001
14 days** after RPY	107.1 \pm 3.8	80.3 \pm 1.8	P<0.001
14 days after RPY	65.4 \pm 1.1	61.3 \pm 1.2	N.S.
17 days** after RPY	92.3 \pm 3.6	74.3 \pm 2.1	P<0.001

* No corpus luteum present, N.S. = not significant, Wilcoxon ranked sums

** Pregnant animal

5.3.2.2 The Luteal Phase (Day 4 to Day 11 After RPY)

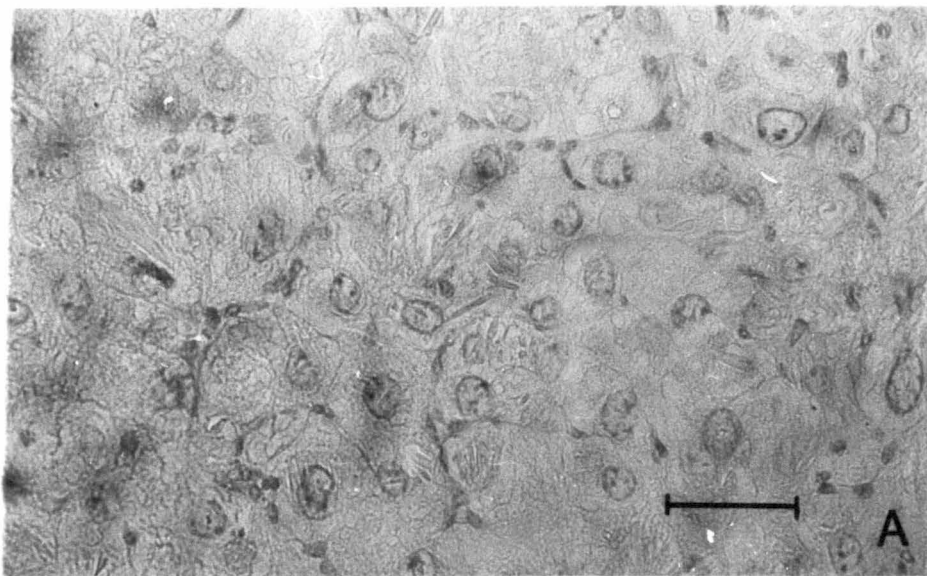
This is initiated experimentally by removal of the pouch young and is characterised by an increase in size of the corpus luteum, the initiation of a secretory uterus with resultant changes in the uterine glands and epithelia, the development of a sub-epithelial network of capillaries and slow growth of the embryo.

Two days after RPY (B28), the corpus luteum was similar in appearance to non-active corpora lutea but by day 4 (B22), although mitotic figures were not apparent in the now vascularised C.L., the gland

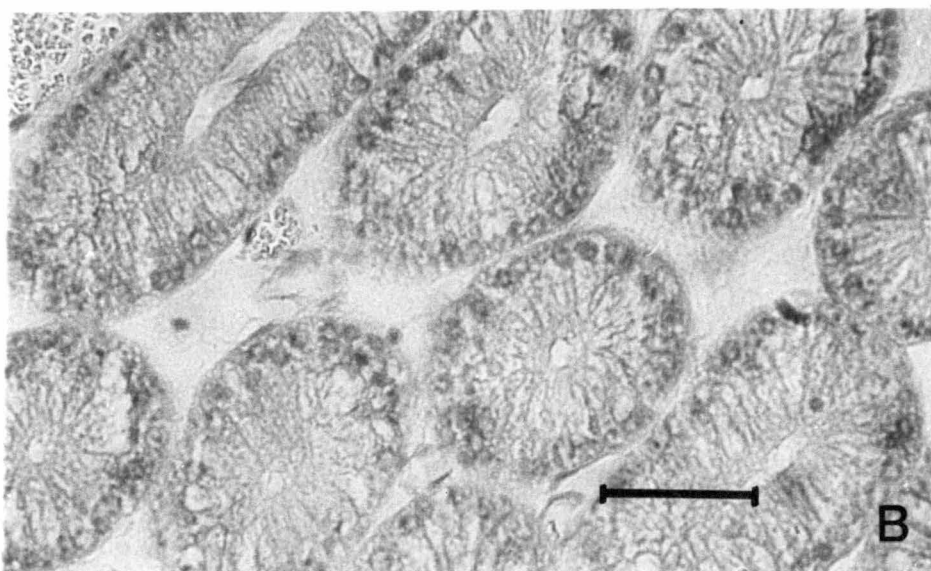
consisted of larger nucleated rounded cells with prominent nucleoli and a darker staining cytoplasm; blood vessels were obvious but localised (Table 5.3, Plate 5.2b).

The epithelium of the uterus ipsilateral to the corpus luteum (B22, day 4 RPY) had thinned to 16–20 μm (Flynn recorded 10–20 μm) and the cytoplasm was more basophilic. The sub-epithelial capillary bed which was first noticed on day 4 after RPY thereafter developed rapidly. It was prominent on day 7 (B11) after RPY.

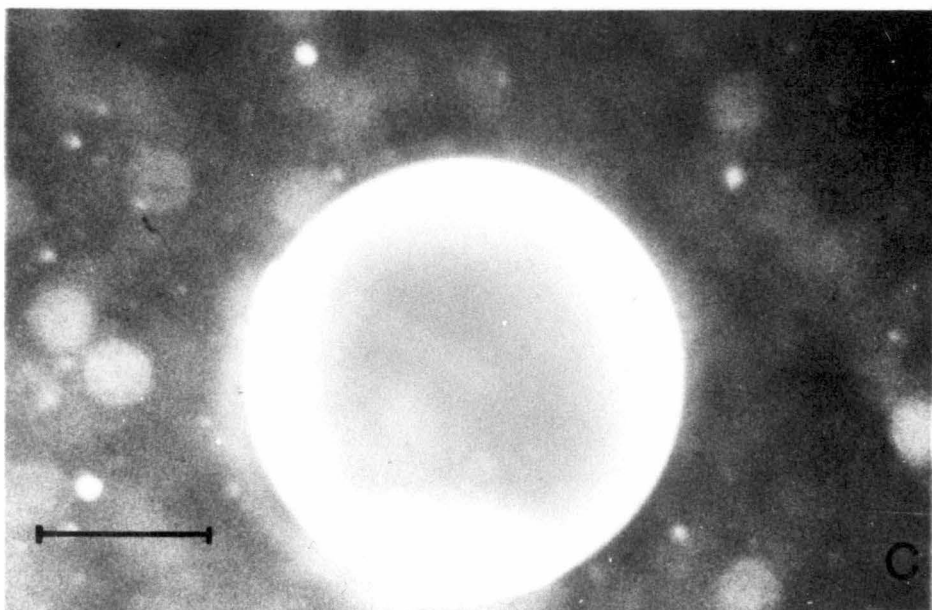
The basal uterine glands increased in size during the luteal phase and their nuclei remained in a basal position though a few nuclei could be found more luminally. By day 2 the glands were much more coiled with darker staining nuclei, though secretory products were not noted in the gland lumen until day 4. The basal gland lumina remained small though the luminal diameter of many of the apical glands increased dramatically. A similar situation occurs during the luteal phases in *Trichosurus vulpecula* (Shorey and Hughes 1972). Shorey and Hughes suggest that, in the possum, this is due to a high secretory activity which results in a substantial decrease in cell height and loss of cytoplasm, and which in turn causes dilation of the glands. By day 7 there was a distinct difference in the staining intensity between the basal and apical epithelia of the uterine glands, the basal glands appearing darker. There was a significant difference ($P < 0.01$) between the mean diameter of the basal glands of the two uterine horns at day 7, the glands being somewhat larger on the pregnant side (Table 5.2, Plate 5.2b). The embryo (maximum diameter = 2.55 mm, Plate 5.2c) obtained from the pregnant uterus was at an advanced primitive streak stage. Flynn (1930) also found that there were obvious differences between the two uteri by the time a primitive streak embryo was formed.



A. Corpus luteum, scale = 100 μ



B. Uterine glands, scale = 50 μ

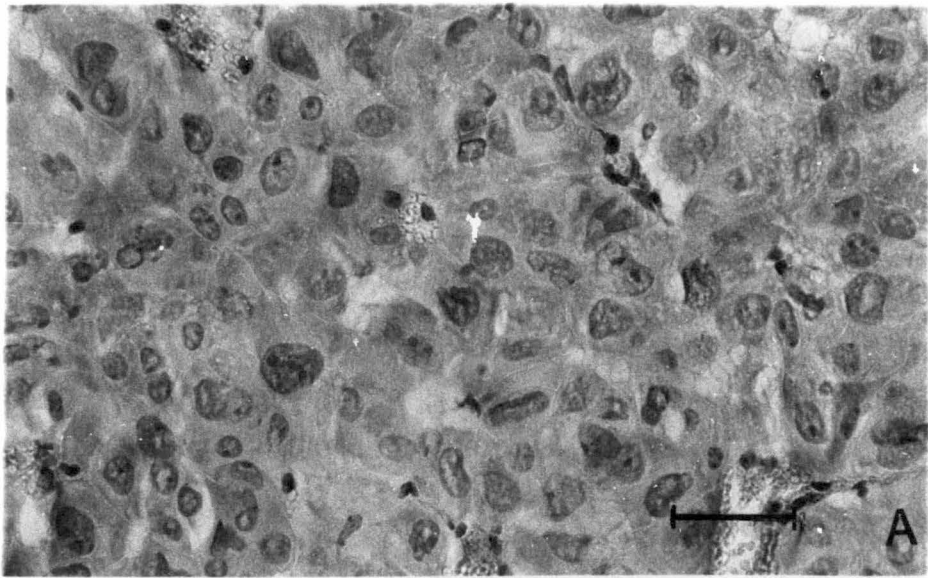


C. Primitive streak stage, scale = 1 mm.

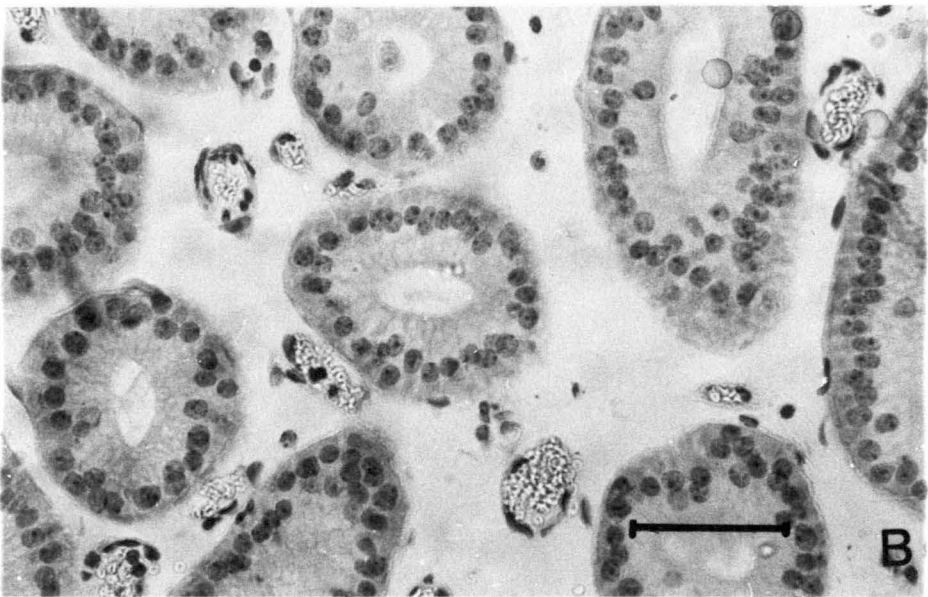
5.3.2.3 Days 9 to 11 After RPY (B25, B36, Plate 5.3, B11)

During this period the richly vascularised corpus luteum developed to a maximum size in both diameter of the gland (4.7 mm) and nuclear diameter (26 μm , Table 5.3). However, by day 11 there was slight nuclear shrinkage and the first appearance of cytoplasmic vacuoles (Plate 5.3a).

At day 9 after RPY (B25) the basal glands of the uterus closest to the C.L. appeared closely packed and full of secretory product; the apical glands were smaller and darker staining. The opposite uterus displayed smaller glands that were less packed. According to Flynn, the uterine epithelium of the non-pregnant uterus at this time degenerates and then is restored, the cell height increasing from 12 to 20 μm (17 to 23 μm in this study). There were significant differences ($P < 0.05$) between the diameter of the uterine glands of the uteri ipsilateral and contralateral to the C.L. in the non-pregnant animal B25, 9 days after RPY. There were both morphological and histological differences between the pregnant (Plate 5.3b) and non-pregnant uteri of animal B36 (11 days after RPY) and the uterine gland diameters were significantly different ($P < 0.001$, Table 5.2). The pregnant uterus ^{appeared.} far more vascularised than earlier stages. Although animal B11 (RPY+11, non-pregnant) demonstrated larger glands in the uterus ipsilateral to the C.L. when compared to the contralateral uterus, these were not statistically significant, in part due to the greater variation about the means. The uterine epithelium of the pregnant side was relatively thin (16.5 μm this study; 18 μm in a similar stage obtained by Flynn 1930). The epithelium of the pregnant uterus (B36, day RPY+11) was significantly thinner (16.5 μm) than that of the non-pregnant side (23.3 μm , $t = 3.3$, $P < 0.01$). The epithelial cells were flat with



A. Corpus luteum, scale = 100 μ



B. Uterine glands, scale = 50 μ



C. Early fetus with embryonic membranes, scale = 2 mm.

widely separated nuclei, allowing some capillaries to pass quite close to the uterine lumen and hence the embryonic membranes (Flynn describes a similar phenomenon). The subepithelial capillary network was well developed and blood vessels were obvious elsewhere in the stroma which had become expanded by the presence of fluid.

Embryonic growth is rapid during this phase and although the embryonic membranes were not sectioned for histological examination it seems likely that the shell membrane thins and perhaps starts to rupture soon after day 11 of the delayed gestation. An embryo at day 11 after RPY (B36, Figure 5.3c) had a well developed head region, paddle-like forelimbs and a crown-rump length of 7.5 mm.

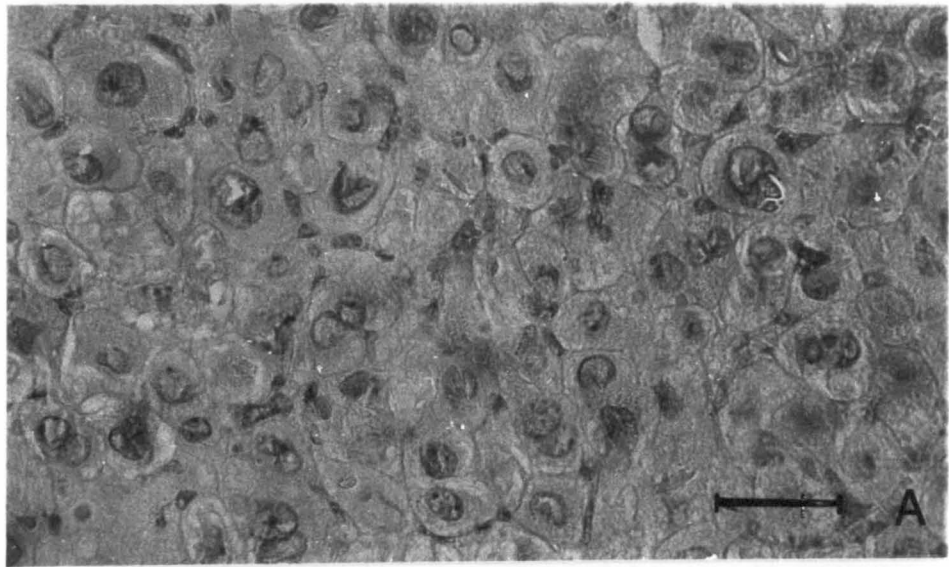
5.3.2.4 Post Luteal/Pro-oestrous Phase (Day 14 to 18 after RPY), (B2, Plate 5.4; B24 and B14, Plate 5.5)

The degenerative changes that occurred in the corpus luteum (e.g. cytoplasmic vacuoles) heralded the post-luteal phase. This phase merged with the pro-oestrous phase, which was characterised by follicular development in the contralateral ovary. Major histological changes occurred between the pregnant and contralateral non-pregnant uteri, though in the non-pregnant animal (B24) these differences were no longer evident (Table 5.2). Towards the end of the pro-oestrous phase the vaginal complex must prepare itself for parturition while oestrus is foreshadowed by changes in the vaginal epithelium and the development of a Graafian follicle. Stored secretory material was released by the uterine glands and this secretion may be enhanced by cytoplasm from degenerating gland cells as in *Potorous tridaactylus* (Shaw and Rose 1979). The thin uterine epithelium and associated increase in the sub-epithelial vascular network would provide a suitable respiratory surface for the developing fetus.

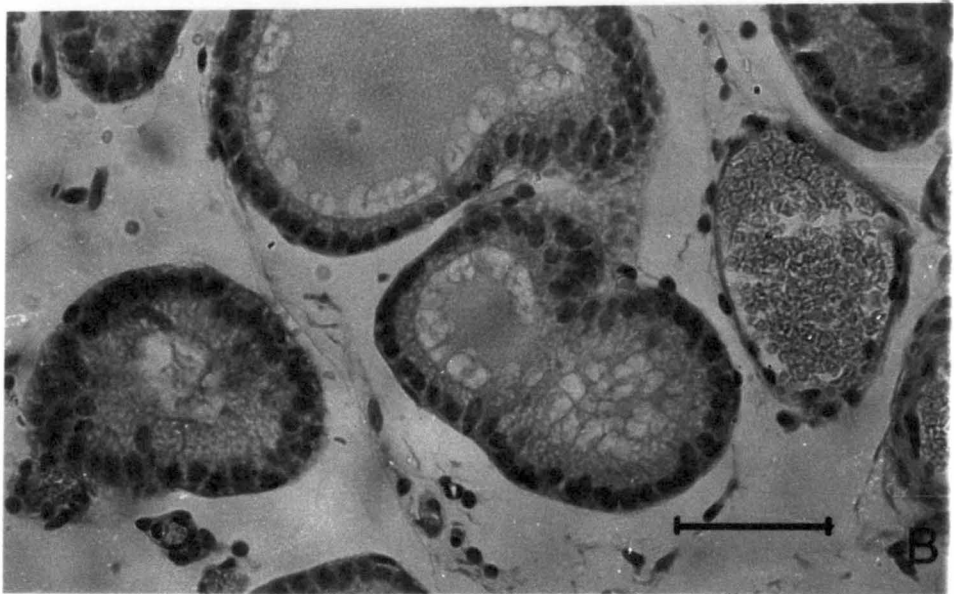
The corpus luteum exhibits increasing signs of degeneration (Plates 5.4a, 5.5a). There was a decrease in the mean diameter of the luteal nuclei (Table 5.3); the nuclear membrane appeared somewhat crinkled and vacuoles were prominent in the cytoplasm. Vascularisation was decreased and the gland stained with less intensity than during the luteal phase. The contralateral ovary contained three large (0.7 to 1.1 mm) follicles on day 14 (B24) after RPY; follicles of this size were not seen in the ovaries of animals 11 days after RPY. The margins of the lumen of the non-pregnant uterus in B2 and B14 (both were pregnant animals) were much more closely apposed than in the pregnant side. Some of the basal glands of the non-pregnant side still had secretory products though the apical cytoplasm stained more intensely.

The pregnant uteri were extremely vascularised (Plates 5.4b and 5.5b) and the epithelium was folded. There were significant differences in the measured diameter of the basal glands of the pregnant uteri compared with those in the contralateral uteri (Table 5.2). These differences were not apparent in a non-pregnant animal B24, fourteen days after RPY in which the glands of both uteri appeared small and similar in size to those found in the quiescent animal (B30 and B28; RPY+2 days). Animal B24 had a prominent corpus luteum in one ovary, so that it seems likely that this animal had ovulated but failed to conceive.

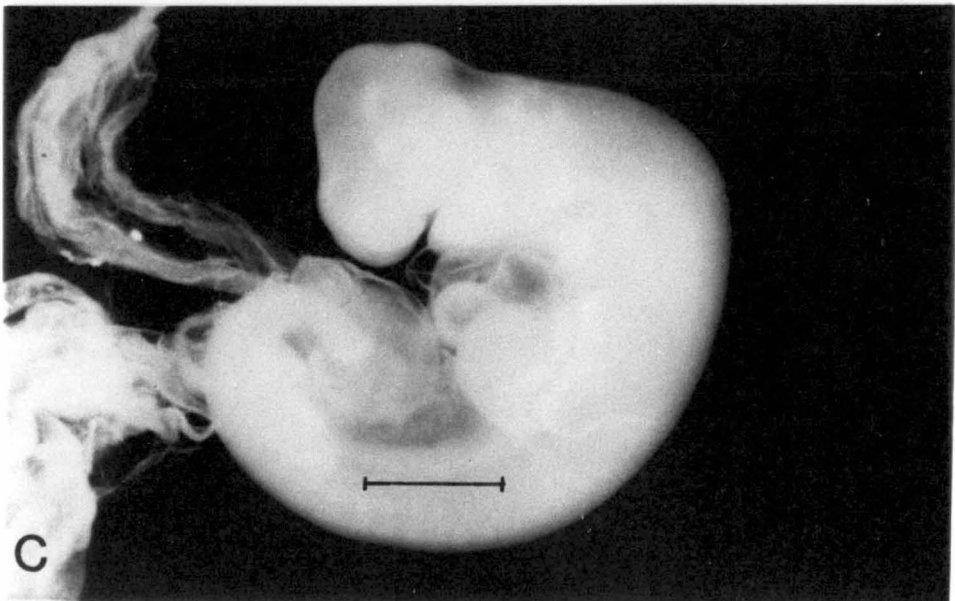
The pregnant uterus was stretched by the growing fetus at day 14 and more so at day 17 after RPY (one day pre-partum), so that the uterus became progressively thinner. At day 17, the uterine epithelium measured 15 μ m and there was a prolific capillary network beneath. Only a few uterine glands had secretory product present though some of these had greatly distended lumina. On the contralateral side the glands



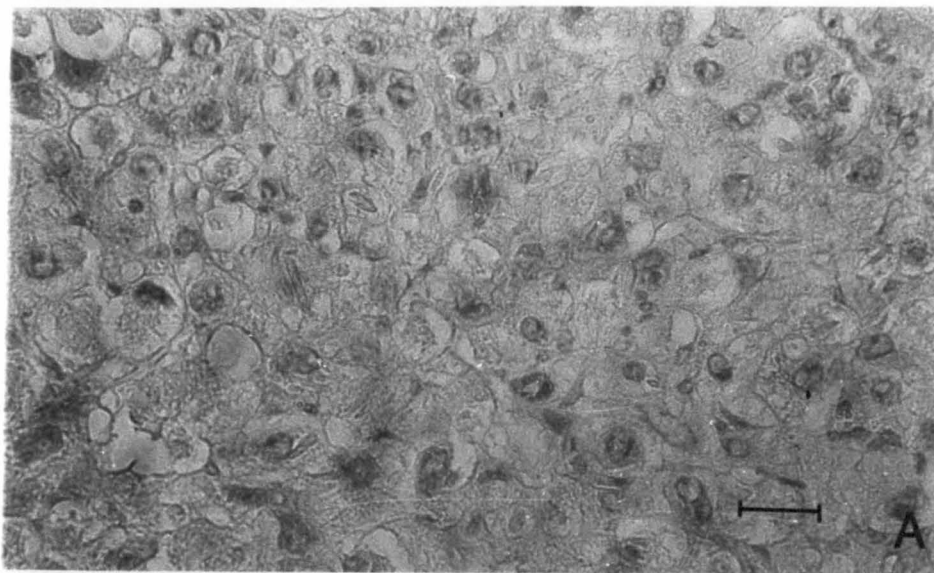
A. Corpus luteum, scale = 100 μ



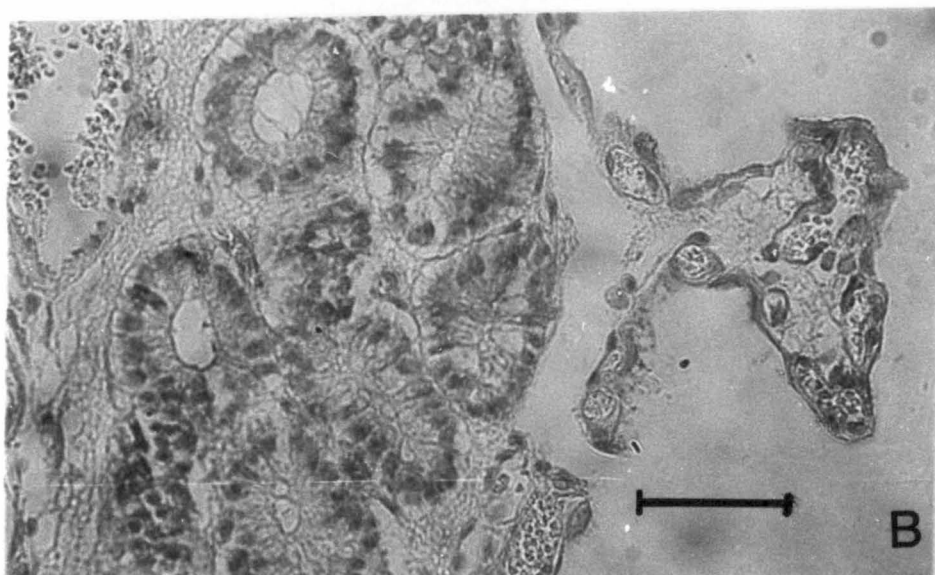
B. Uterine glands, scale = 50 μ



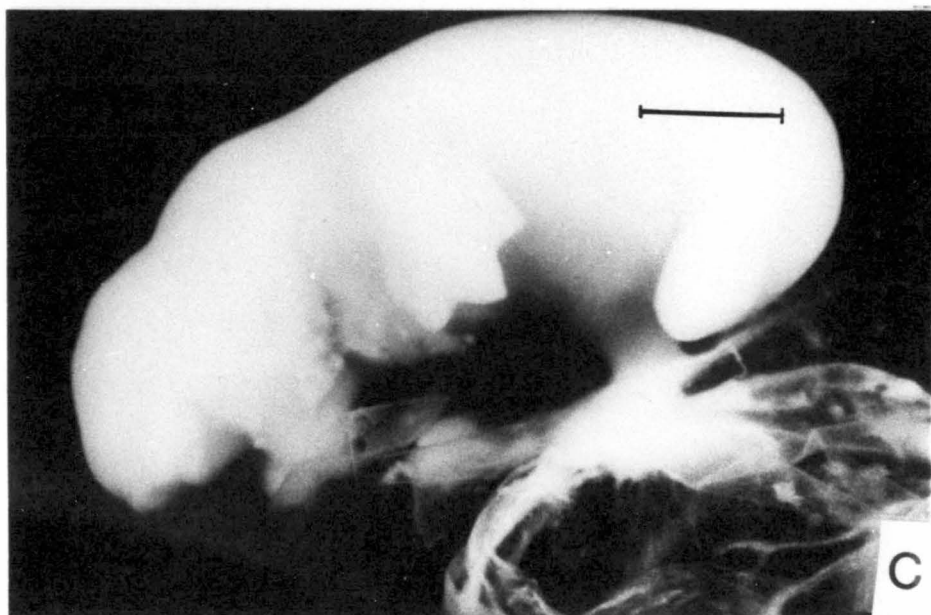
C. Fetus 4 days prior to birth, scale = 2 mm.



A. Corpus luteum, scale = 100 μ



B. Uterine glands and sub-epithelial capillaries,
scale = 100 μ



C. Near term fetus, scale = 2 mm.

were smaller but appeared more numerous as they were more closely packed; the epithelium appeared thicker and no capillary network was evident.

At day 14 after mating the lateral vaginae had a smooth lining of nucleated epithelial cells, and the vaginal lumina contained leucocytes. By day 17 after RPY the vaginal surface was rough, the nuclei flattened and becoming pycnotic; leucocytes were still present within the lumen. The luminal diameter of the median vagina had enlarged, but it was not possible to determine if it was completely open.

During the post-luteal/pro-oestrous phase of gestation the embryo grew at an exponential rate. At day RPY+14 an embryo (crown-rump length 8.2 mm, Plate 5.4c) had a more developed head and tail region and a much more prominent heart than the slightly earlier embryo (Plate 5.3c). A near full-term fetus (Plate 5.5c) developed from the primitive streak stage in about seven days; all prenatal organogenesis must occur during this period.

5.3.3 Summary of Results

The data base for this section is somewhat limited in that it is not possible to fully distinguish changes typically occurring over the time period from changes due to individual variation. Nevertheless there are several unmistakable trends in the regular changes in the corpus luteum, the uterine glands and embryonic growth.

Corpus Luteum

There is an increase in the mean nuclear diameter of the corpus luteum from the quiescent state, through the early luteal phase (Table 5.3) until plateau levels are achieved by day 7 after RPY. The nuclear

diameter decreases slightly after day 11 but not to the basal level of the quiescent corpus luteum; the actual diameter of the whole gland, however, reaches a maximum on day 14 RPY. From the histological data presented, it appears that the duration of the luteal phase after removal of the pouch young is approximately 14 days; this compares reasonably favourably with the calculation of 15 days for a normal oestrous cycle obtained in Chapter 4 (Section 4.3.12). Degenerative changes are widespread by day 14 after RPY.

Uterine Glands

There appear to be few changes in the mean diameter of the uterine glands over the first two days of delayed gestation, though there may be some increase in the degree of coiling. However, by day 4 RPY there was a dramatic increase in the diameter of the basal glands. Not until day 7 after RPY do significant differences appear between glands from the two uteri in the one animal, the larger glands appearing in the uterus ipsilateral to the C.L. The maximum gland size is attained between days 9-11 after RPY and this rapidly wanes in non-pregnant animals to near quiescent levels; in pregnant animals the uterine glands associated with the pregnant side remain enlarged throughout gestation.

Embryonic Growth

Five embryos were obtained during this study: a quiescent blastocyst; an advanced primitive streak stage; two early fetuses and a near term fetus that appeared deformed (Plate 5.5c). Embryonic growth is slow at first. From a 200 μm zygote after fertilisation (Kerr 1934), the embryo grows slightly until quiescence (260 μm this study, although this value includes the mucoid coat and shell membrane). Seven days

TABLE 5.3 The mean nuclear diameter of the corpus luteum during the reproductive cycle

Animal	Days After RPY	Mean Nuclear Diameter (N=30) (μm)	Standard Error
B30	0	14.2	0.9
B28	2	18.9	0.7
B22	4	25.1	0.9
B19	7	24.7	0.8
B25	9	24.3	0.7
B36	11	24.0	0.4
B11	11	26.2	1.1
B2	14	24.7	1.0
B24	14	20.5	0.8
B14	17	21.9	0.6

after RPY a primitive streak stage is well developed, and by days 11 and 14 after RPY a well-formed fetus is present, with paddle-like forelimbs and easily distinguishable heart. One day before parturition the embryonic vesicle was not quite so large as might be expected. This fetus appeared to be deformed, possibly anencephalic. Shaw and Rose (1979) found a similar embryo at a late stage of pregnancy in the potoroo, *P. tridactylus*.

5.4 DISCUSSION

5.4.1 The Reproductive Tract and Phylogeny

The reproductive tract of the Macropodidae is important from an evolutionary and phylogenetic viewpoint. In some Macropodinae (but not the Potoroinae) the median pseudo-vaginal canal remains patent in parous females (Pearson 1945); this situation is unlike that in other marsupial groups except *Tarsipes*. Generally, in marsupials, evolution has been accompanied by an increase in the size of the neonate and greater development of the median vaginal apparatus (Sharman 1965); this development is greatest in the Macropodidae which also produce the largest young. This is partly due to the fact that the size of the neonate is related to maternal size (Tyndale-Biscoe 1973) and most kangaroos are larger than other marsupials. However, the smaller potoroo and bettongs have relatively larger neonates (0.3 g) than other similar sized marsupials.

Pearson (1944, 1945, 1946, 1950) developed the view that the Potoroinae could be separated from the Macropodinae at the family level on the basis of certain structures present in their reproductive tracts. He listed a number of potoroine features including the presence of an anterior vaginal expansion (vaginal caecum). A similar structure was also found in *Setonix* by Waring *et al.* (1955), who were unsure whether it was homologous with the potoroine structure, and in *M. rufus* (Sharman 1964) and *M. giganteus* (Clark and Poole 1967). However, Tyndale-Biscoe (1965, 1968) has since found that in *Lagostrophus fasciatus* (Macropodinae) and *Bettongia lesueur* (Potoroinae) both the anterior vaginal expansion as described by Pearson (1945) and the structure found in *Setonix* (which he called the anterior vaginal cul-de-sac) occur together; therefore the two structures cannot be homologous. Tyndale-

Biscoe also showed that both the above species lack a permanent birth canal. Apparently the same condition applies in *Aepyprymnus* (Potoroinae) (Moors 1975) which has a vaginal caecum and cul-de-sac as well as lacking the permanent birth canal.

It appears then that the anterior vaginal expansion as described by Pearson may represent a valid taxonomic character of the Potoroinae, though also found in *Lagostrophus* sp.

Tyndale-Biscoe (1968), on the basis of reproductive tract anatomy, raised the possibility that *Lagostrophus* was related to the Potoroinae. The newly discovered potoroine (*P. longipes*) with a chromosome number of 24 (Johnstone pers. comm.) is also suggestive of closer relationship between *Lagostrophus* and the Potoroinae (the same number as in *L. fasciatus*).

It would indeed be unusual if, in some of the Potoroinae, the young were born via the lateral vagina, the supposedly ancestral condition. Yet this has been suggested for *P. tridactylus* by Flynn (1923) and Pearson (1946). The evidence for this was reviewed by Tyndale-Biscoe (1968); it consisted of one unborn fetus in a lateral vagina. This situation is highly unlikely, particularly in view of the fact that the potoroo gives birth to the largest young in relation to the maternal weight of any macropodid. Shaw and Rose (1979) found that birth in the potoroo is via the median vagina as in all marsupials so far studied and it seems most probable that the Tasmanian bettong also gives birth via the median vagina.

5.4.2 Changes During the Reproductive Cycle and Gestation

In general, the histological changes in the reproductive tract of female *B. gaimardi* appear to be similar to those found during the delayed

cycle in other macropodid marsupials, e.g. *S. brachyurus* (Sharman 1955; Tyndale-Biscoe 1963), *M. eugenii* (Renfree 1972; Renfree and Tyndale-Biscoe 1973) and *P. tridactylus* (Shaw and Rose 1979). The results presented also correspond to previous findings on *B. cuniculus* (= *gaimardi*) by Flynn (1930). Changes from the quiescent state are apparent in the uterine histology by day 4 RPY. There are significant differences between the basal gland diameter of the paired uteri (Table 5.2) from day 7 RPY which are initially associated with the local presence of the corpus luteum but which later in pregnancy (during the post-luteal/pro-oestrous phase) are correlated with the presence of the feto/placental unit. Similar differences have been described in *M. eugenii* (Renfree and Tyndale-Biscoe 1973) and in *P. tridactylus* (Shaw and Rose 1979).

The ovarian artery and vein supply the ovary, oviduct and uterus and have extensive ramifications contributing to the vascular plexus noted in this study and in other macropodid species (Lee and O'Shea 1977; Shaw and Rose 1979; Towers 1980). Progesterone concentrations can be considerably higher in the ovarian vein than in the peripheral plasma (Shorey and Hughes 1973). It is possible that the vascular plexus provides a local pathway for progesterone to pass between the ovary (with the active C.L.) and the ipsilateral uterus. A similar local pathway has been suggested for the local action of prostaglandin F₂ in the ewe (Liggins 1972). This could explain in part the unilateral stimulation of the uterus ipsilateral to the corpus luteum during the luteal phase of the delayed cycle. It is also possible that the number of progesterone receptors in the uterus varies during the cycle as found by Leavitt (1974) in the hamster. The characterisation and the properties of a progesterone receptor in the uterus of the quokka has recently been described by Owen, Cake and Bradshaw (1982).

These authors demonstrated experimental changes in the concentration of receptor sites under the influence of oestrogen. If the concentration of receptor sites varies between the two uteri during the reproductive cycle this could also explain the differences observed between the uterine glands.

The importance of the differences between uteri (during the luteal phase) is not at all clear as Renfree (1972) has shown that blastocyst transfer to the contralateral uterus can lead to a successful pregnancy in *M. eugenii*, i.e. the presence of a local corpus luteum is not essential for pregnancy.

Indeed, after an initial but varying period, the corpus luteum can be removed from macropodids without interrupting pregnancy (day 8 in *S. brachyurus*, Tyndale-Biscoe 1963^b; day 6⁶ in *M. eugenii*, Young and Renfree 1979; and day 6 in *P. tridactylus*, Bryant and Rose (unpublished data)). This has led ^{Tyndale-Biscoe et al (1974)} Walker and Hughes (1981) to suggest that the early functional capacity of the C.L. is such that it is able to induce sufficient proliferation and secretory activity in the uterine endometrium of macropods to maintain the entire period of gestation.

The luteal phase, as measured by significant increases in the diameter of the uterine glands, is already well established by day 4 after RPY. Hence the luteal phase in the bettong probably starts a day earlier on day 3 as it does in the potoroo (day 2-3, Shaw and Rose 1979). In contrast, in other macropodids, the luteal phase, based on histological changes observed under the light microscope, starts somewhat later (day 6-9, Rose 1978). More accurate timing of the initiation of the luteal phase can be obtained by electron microscopy, e.g. Tyndale-Biscoe (1979) and Walker and Hughes (1981). The early start of the luteal phase in the bettong presumably permits the early activation of the quiescent blastocyst, resumption of development and completion of the relatively short gestation. This rate of embryonic growth

(based on the limited data) is similar to that found in most other macropodids but is somewhat greater than in the potoroo.

Although Owen (1834) noted that the endometrium of the pregnant uterus of a grey kangaroo was thicker than the non-pregnant contralateral endometrium and Flynn (1930) detailed the histological differences between pregnant and non-pregnant uteri of *B. cuniculus* (= *gaimardi*), the implications of these early findings were unclear. More recently Renfree (1972) and Renfree and Tyndale-Biscoe (1973) demonstrated differences between the endometrium of the paired uteri of the pregnant tammar wallaby. The findings established renewed interest in the role of the feto/placental unit in macropodid reproduction.

In the mid- and final ^{third} trimester of pregnancy in the Tasmanian bettong, there were obvious differences between the pregnant and non-pregnant sides of the paired uteri. These differences were not apparent in non-pregnant animals at similar stages in the reproductive cycle (B11 and B24). This suggests that during the post-luteal period there may be a feto/placental influence similar to that suggested for *M. eugenii* by Renfree and Tyndale-Biscoe (1973) and for *P. tridactylus* by Shaw and Rose (1979). How such a feto/placental influence might be mediated is unknown. The pregnant uterus is stretched by the yolk-sac distension of the growing embryo and in part this must be responsible for the altered histological appearance, in particular the thinning of the uterine epithelium. Experiments could be designed to test whether a mechanical effect might be wholly responsible for the observed changes.

Although the corpus luteum does contain progesterone while it is regressing (Renfree, Green and Young 1979), as mentioned previously it can be dispensed with during the post-luteal phase. However, in the tammar (and the bettong, Appendix C), elevated levels of progesterone

are found in the peripheral plasma during the latter part of the delayed cycle (Lemon 1972; Hinds and Tyndale-Biscoe 1982). It appears likely that these levels of progesterone are involved to some extent with the maintenance of pregnancy and preparations for parturition and lactation.

It is often stated (e.g. Sharman 1970) that one of the major differences between the reproduction of marsupials and eutherians is the inability of marsupials to extend their pregnancies by placental secretion of hormones. Although several workers have shown that the yolk sac placenta is able to synthesise progesterone *in vitro* (Bradshaw *et al.* 1975 in the quokka and Heap *et al.* 1980 in the tammar) it seems unlikely that the higher levels of progesterone in the latter part of the cycle are derived from the feto/placental unit. FINDLAY *et al.* (1983) demonstrated that in the tammar the peak levels of progesterone in late gestation were abolished by ovariectomy. Others have failed to find significant differences in progesterone levels between pregnant and non-pregnant macropodids in late pregnancy (Cake, Owen and Bradshaw 1980; Hinds and Tyndale-Biscoe 1982).

For the present it appears that the causes of the significant differences in histology between pregnant and non-pregnant macropodid uteri must remain obscure.

However, it seems certain that the fetus does play a number of roles in the reproductive cycle of macropodids. The 'Merchant effect' has been demonstrated in a number of species including *B. gaimardi* (this study) and recent work by Tyndale-Biscoe, Hinds, Horn and Jenkin (1983) has shown that the differences between pregnant and non-pregnant cycles in the tammar reflect real differences in the profiles of progesterone, prolactin, LH and prostaglandin F2d. The findings of Tyndale-Biscoe *et al.* (1983) led them to suggest that the conceptus

is not only involved in the onset of parturition but also in the timing of post-partum oestrus and ovulation! By way of contrast no-one has demonstrated or suggested a fetal role in the alteration of the female reproductive cycle in non-macropodids, although these marsupials have not been studied to the same extent.

5.4.3 Intrauterine Development

On the basis of the few studies of intrauterine development available Tyndale-Biscoe (1973) suggested that the variations in gestation length of marsupials are a result of variation in the early free vesicle stage. The period from appearance of the first somites and vascular yolk sac to birth was believed to be of short and uniform duration. Hughes (1974) also divided embryonic growth in marsupials into two phases. The first phase, occupying about two-thirds of pregnancy, is characterised by the continuing presence of the shell membrane. The second phase is initiated by the rupture of the shell membrane and it is during this period that most fetal differentiation occurs. Furthermore, Lyne and Hollis (1977) state that in marsupials the rate of development is very slow at the beginning and exceedingly rapid during the final stages of pregnancy. This is partly explained by the fact that much of marsupial embryonic growth is exponential with a characteristic J shaped curve. However, Lyne and Hollis (1977) have shown that there is no increase in vesicle diameter in bandicoots up to the 75 cell stage. This is probably also the case in the Macropodinae as Tyndale-Biscoe (1963) has shown that there are no significant differences in size between unfertilised ova and delayed blastocysts in the uterus of *M. rufus*, *M. robustus* and *S. brachyurus*. The situation in the Tasmanian bettong (and potoroo) differs in that there is some growth during the period up to diapause (Kerr 1934, 1936). Smith (1981) gives

details of one potoroo blastocyst obtained from a lactating female that consisted of 143 cells, more cells than have been reported for any other macropodid.

Figure 5.1 illustrates intrauterine growth by the delayed blastocyst in a number of species after removal of pouch young (RPY). It can be seen that there is a varying period of slow growth followed by a period of rapid exponential growth in each case. The rate of exponential growth does not increase after rupture of the shell membrane (day 18 in *P. tridactylus*, 15-18 in *M. rufogriseus*, day 20 in *M. eugenii* and day 12(?) in *B. gaimardi*). Hughes and Shorey (1973) have shown that the shell membrane would not inhibit the passage of nutrients in the brush possum and Renfree (1973) provides evidence for the role of nutrient transport across the shell membrane in *M. eugenii*. The rate of rapid growth is remarkably similar (although use of a log scale reduces differences) in all species except the potoroo, and consequently a difference of only one or two days in gestation length could account for the differences in size between the smallest and largest macropodid neonates (0.290-0.828 g). The variation in the length of the initial slow phase is probably related to the time for the luteal phase to be initiated in the uterus.

Although the growth phase is initiated earlier in both the potoroo and bettong than in other macropodids, the embryonic growth rate of the potoroo is slower but gestation much longer (29-58) thus accounting for the fact that at birth the neonate is of a similar size to that of the bettong, whose embryonic growth rate is similar to that of other macropodids but in which the delayed gestation length is only 18 days. The slower growth of the potoroo may be due to its longer cell cycle as compared to eutherians and other marsupials (Bick and Brown 1969; Thrasher 1970) as suggested by Rose (1978) and Sharman (1981).

INTRAUTERINE GROWTH IN SOME MACROPODIDS

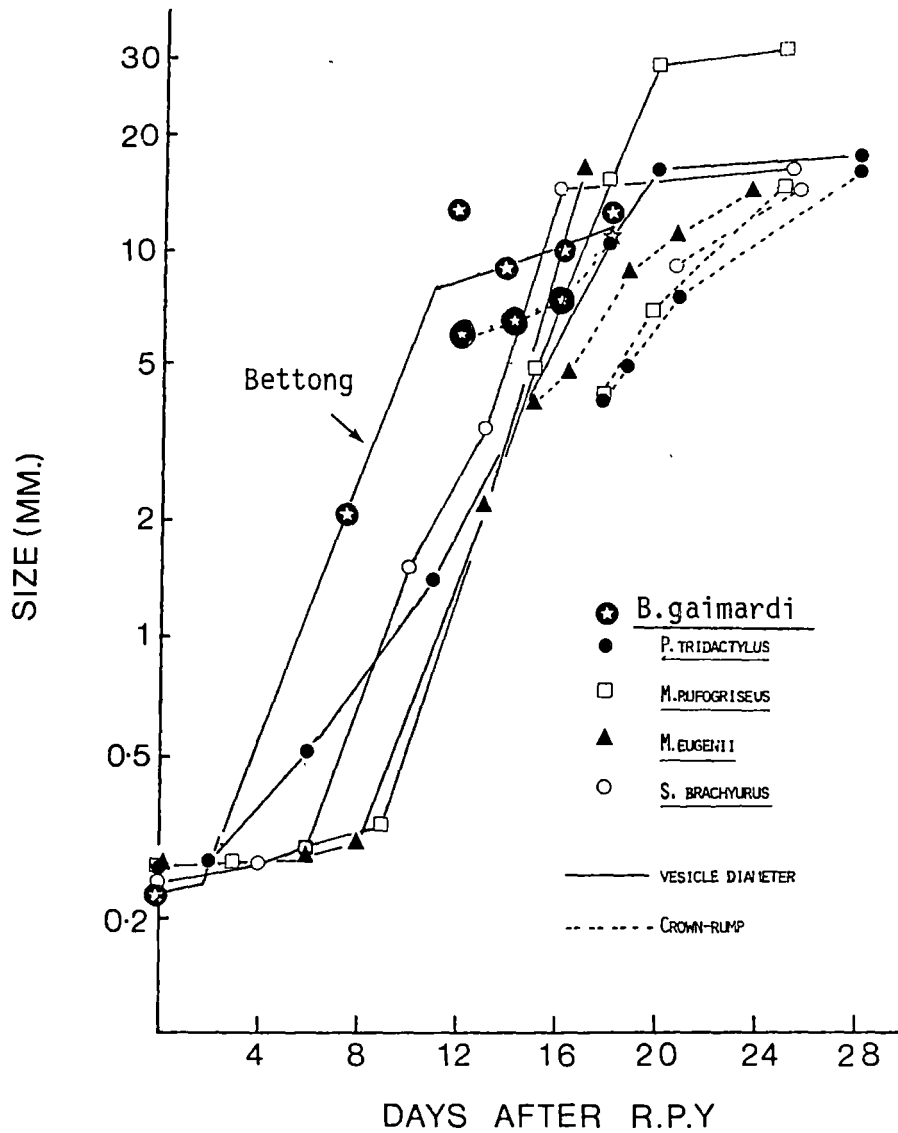


Figure 5.1 A comparison of intrauterine growth of the Tasmanian bettong with other macropodids. The figure is modified from Rose (1978) and presents new data from this thesis & Flynn (1980)

Bettong data - line of best fit.

Within the Macropodinae, gestation lengths by themselves are unlikely to provide much phylogenetic information as embryonic growth is so uniform and variations in the gestation length are mainly a result of differences in the length of the initial slow phase.

It is uncertain what advantage has been gained by the Macropodidae in evolving an extended gestation length and the associated production of larger young. As all macropodids (except *Hypsiprymnodon*) produce a single young (unlike many other marsupials) it may be that the extra size confers some advantage in the journey to the pouch and subsequent attachment to the teat. One other important aspect is the greater maturity of endocrine organs such as the adrenal cortex and the more advanced physiological function of important organs such as in the liver in macropodids (Janssens *et al.* 1978; M. Walker pers. comm.). The apparent fetal influence on the oestrous cycle length demonstrated in this study (the 'Merchant effect') could be mediated in kangaroos by the adrenal cortex. This is possible as the secretions from the fetal adrenal gland have been implicated in many functions including parturition in eutherian mammals (Liggins 1972).

CHAPTER 6

BASAL BODY TEMPERATURE AND THE OESTROUS CYCLE

CHAPTER 6 BASAL BODY TEMPERATURE AND THE OESTROUS CYCLE

6.1 INTRODUCTION

Although Squires (1868) showed that an alteration in temperature occurred in women during their menstrual cycle, this observation received little attention until it was discussed by Van de Velde (1904, 1928) as an indicator of ovulation and corpus luteum function. Subsequently, during the 1940's it was utilised as a means of detecting ovulation in women by numerous workers, e.g. Rubenstein (1937, 1940), Greulich *et al.* (1943), Tompkins (1945), Buxton and Atkinson (1948) and Buxton and Engle (1950). In general they found that as the menstrual cycle progressed, the body temperature reached a lowest point or 'nadir' which was immediately followed by a mid-cycle rise that was maintained throughout the remainder of the cycle. It was uncertain from this early work whether it was the nadir or the mid-cycle rise in temperature which acted as the indicator of ovulation. This uncertainty could not be resolved until reliable methods for the detection of ovulation were obtained. This technology involved the measurement of small changes in hormonal levels and their correlation with actual evidence of ovulation.

It was thought by early workers that the mid-cycle rise in progesterone (from the corpus luteum) was directly responsible for the rise in basal body temperature (Davis and Fugo 1948; Israel and Schneller 1950). This hypothesis was corroborated by Tausk and Visser (1971) and Moghissi *et al.* (1972) who showed that the mid-cycle temperature rise coincided with an increase in plasma progesterone and that these events occurred two days after a peak in LH. Progesterone

is known to have a thermogenic effect in other species, e.g. cattle (Wrenn *et al.* 1959) and rats (Freeman *et al.* (1970). Morris *et al.* (1976) have since demonstrated that in women the temperature nadir usually occurs on the day of the LH peak.

Falling temperatures during the follicular phase of the menstrual cycle have been attributed to a temperature depressing effect by oestrogens (Davis and Fugo 1948; Israel and Schneller 1950). More recently Graham *et al.* (1977) have found that falling temperatures were associated with increasing oestrone levels in the chimpanzee and that the temperature nadir occurred the day after the oestrone peak. There is some variability in the rate of temperature rise after the nadir in women (Siegler and Siegler 1951) and this led Graham *et al.* (1977) to conclude that the nadir is a better indicator of ovulation than is the temperature rise.

It should be remembered however that it is unlikely that any single measurement of a physiological parameter such as body temperature or hormonal levels can give a precise determination of ovulation which is after all a climatic morphological event.

There have been comparatively few studies on the endocrinological induction of these temperature changes. Freeman *et al.* (1970) have shown that the thyroid gland is not the mediator of the thermogenic response to progesterone in rats and that the pituitary gland is essential for the effect to be realised. Benoit *et al.* (1976) have argued that the hormone dependent temperature changes known to occur during the ovarian cycle of mammals are of two types which differ in cause, effect and mechanism. In the first case progesterone from the corpus luteum acts through a central, metabolic action to induce the post-ovulatory (mid-cycle) rise in basal body temperature now familiar because of its extensive utilisation as an aid to human contraception

and conception. Secondly, oestradiol secreted by the growing Graafian follicle results in increases in the blood flow to the reproductive tract and this induces local temperature changes in the surrounding tissues via a mechanism of perfusion and heat removal. However, the latter activity could not explain the falling basal body temperature prior to the mid-cycle rise.

Zuspan and Rao (1976) and Zuspan and Zuspan (1979) do not accept these hypotheses and argue instead that both the rise and fall in body temperature are only secondary to the secretion of ovarian hormones and that the primary cause is the change in production and secretion of norepinephrine (noradrenaline) which is a thermogenic neurohormone. This view was based on their observation of a mid-cycle plasma amine surge associated with a change in basal body temperature (Zuspan and Zuspan 1973). They suggested that the ovarian hormones, oestrogen and progesterone, alter the storage and secretion of norepinephrine and that this is the primary cause of fluctuating thermogenesis during the female reproductive cycle.

The rhythmical variation of body temperature throughout the reproductive cycle observed in humans is also found in other animals, for example: *Macaca nemestrina* (White *et al.* 1973; Steiner *et al.* 1977); *Macaca mulatta* (Herbert *et al.* 1970); *Pan troglodytes* (Graham *et al.* 1977); the rabbit (Kihlstrom and Lundberg 1971); the bitch (Christie and Bell 1971); the cow (Williamson *et al.* 1972; King, 1977); the ewe (Abrams *et al.* 1971) and numerous studies on the laboratory rat (e.g. Marrone *et al.* 1976). However, there appears to be no other published reports outside of domestic or laboratory animals apart from the work in captivity on the marsupial, the common wombat *Vombatus ursinus* by Peters and Rose (1979). These authors also found fluctuations in basal body temperature during the oestrous cycle which could be correlated

with certain reproductive events, e.g. oestrus.

The lack of information about wild animals is attributable to the difficulty and tediousness of data collection and analysis rather than a reflection on its value once obtained. However, modern technology makes the data collection and analysis process much easier and more reliable. It was considered that telemetric monitoring of body temperature in a wild native mammal (the Tasmanian bettong) might provide a useful way of obtaining extensive data relevant to reproductive cycles without the need to disturb animals (if captive) or to regularly capture animals (in the wild). Such data could be useful for the determination of periods of oestrus and perhaps the pin-pointing of the time of ovulation or as a reflection of hormone levels.

6.2 METHODS

Three mature females were used for these experiments in which radio transmitters (telemeters) were implanted, two of these had pouch young ^(initially, which were removed) and one was undergoing oestrous cycles.

The cages in which the animals were maintained were made of weld-mesh with angle iron sides and dimensions of 1 m x 1 m x 1 m. There was a false floor made of compressed wood ('Canite'), underneath which was a coiled aerial attached to a battery operated A.M. receiver. The cages (which were earthed) were housed in a windowless metal shed some distance from the main building of the Zoology Department. The light regime was 12 h dark and 12 h light and in the colder months a 1000 watt strip heater was switched on during the day when the animals were resting. Animals were placed in the cages one week prior to the implantation of the telemeters.

The telemeters were commercially available (Model M, Mini-Mitter Co. Indianapolis, In., U.S.A.) and their signal in the form of 'beeps' was received by standard A.M. radio receivers. These transmitters are temperature sensitive and the rate of 'beeping' is directly proportional to temperature. Prior to implantation, each telemeter was calibrated in a water bath over a range of temperatures from 32°C to 40°C. Throughout this range all telemeters showed a linear response. The telemeters were found to be accurate to at least 0.1°C as claimed by the manufacturer. After calibration they were enclosed in a plastic capsule, dipped into molten embedding wax ('Paraplast') and then tied into the shortened finger of a surgical glove. They were then stored at 4°C until required in order to maximise their operating life (3-6 months, depending on battery size).

One day prior to implantation, the telemeter package was sterilised in a solution of Zephiran (Benzalkonium with chlorhexidine, 10%). After an intra-muscular injection of Ketalar (Ketamine hydrochloride 10 mg kg⁻¹) anaesthesia was maintained with Halothane and oxygen. Animals were shaved and then swabbed before surgery which involved a small mid-line incision, anterior to the pouch; a telemeter was then pushed through the cut and attached to the internal body wall. Before suturing of the wound an A.M. receiver was used to ensure that the telemeter was operating. After a short recovery period the animals were returned to the recording cages and the experiment commenced.

6.2.1 Electrical Apparatus and Recording Device

A 'black box' was used to improve the recordings and to maintain a highly regular sequence. To reduce possible interference the A.M. radio output was 'optically isolated' from the rest of the circuit, that

is the received pulse after detection and amplification was translated into a light pulse which was detected by a photo-transistor. This pulse was then 'cleaned' by conversion to a fixed length pulse with a squared leading edge before being recorded. A cassette tape-recorder was adjusted to record for a period of 40 seconds every hour on the hour. The time-base was highly accurate and had a battery back-up in case of power failure. A multiplexer divided the 40 second time period into four periods of ten seconds, each containing a different initial 2 second recognition beep. This allowed up to four separate inputs to be recorded sequentially onto the one tape. These arrangements enabled approximately four days of continuous recordings to be obtained from a standard 90 minute cassette tape (Hitachi UD90).

At a later date the tape was played back using the same model tape recorder to that used for the recordings. The output from this recorder was entered into an electronic counter that measured the time duration between consecutive leading edges of the beeps within each of the ten second periods. This counter, which was also used during the calibration of the telemeters, produced a digital readout in milliseconds which was then tabulated manually against the time of day. Thus data could be obtained for each animal every hour of every day of the experiment.

The measurement reflecting the lowest body temperature (i.e. the measurement with the longest time interval between beeps) during each day of the oestrous cycle was obtained and converted to temperature by use of the calibration curves. All temperature data for one ten day period were used to determine the diurnal rhythm.

6.2.2 Reproductive Data

Animals were examined for the presence of new young and/or mating at appropriate times (if a male was present) and vaginal smears were obtained near the expected time of oestrus in the late afternoon so that basal body temperature would not be disturbed.

Basal body temperature measurements were obtained over a total of thirteen oestrous cycles in three bettongs. Animal B1 provided data during seven weeks of pouch life (lactation) after which the young died. This initiated a succession of cycles but only two cycles could be recorded before the battery of the telemeter required replacement. The animal unfortunately died during anaesthesia when recovery and subsequent replacement of the transmitter was attempted. A second animal (B2) provided a complete set of readings over three oestrous cycles (no pregnancies). Eight relatively complete sets of recordings were obtained from a third bettong, B3.

6.2.3 Pregnancy and Mating

Animal B1 gave birth to a new young 17 days after the loss of her pouch young. This birth was associated with a post-partum oestrus as determined by vaginal smear but no male was present and consequently no pregnancy resulted. B3 produced three young during the experiment, one from a normal gestation and two from delayed gestation. A male was placed with B3 twice near the expected time of oestrus and vaginal smears were taken daily. The cage size was far too small (a consequence of the low power output of the transmitter) to allow the housing of bettongs in pairs for more than a few days as the animals were likely to injure themselves.

6.2.4 Analyses

The temperature data were used either as 'raw' or 'standardised' data. The standardised data were obtained by averaging the daily minimum temperatures from each of the thirteen oestrous cycles so that a mean value was gained for each cycle. Subsequently, individual values were subtracted from their relevant mean value so that each oestrous cycle could be described in terms of the daily fluctuation in body temperature from the mean. The use of the standardised data had the great advantage of allowing direct comparisons to be made between days of different cycles as well as reducing temperature variations due to individual differences in set point temperature or stage of battery discharge within the same animal.

A one-way analysis of variance was performed on both sets of data (raw and standardised) to compare the variation within individual days of the oestrous cycles with the variation within individual cycles. A significant F-statistic would suggest that there were real differences between certain (unspecified) days of the oestrous cycle. A t-test matrix was constructed (using the standardised data) to compare the mean from each day of the oestrous cycle with the mean from every other day of the oestrous cycle. This form of analysis would determine whether there were significant changes in body temperature between specified days of the cycle. For the analyses only values 10 days on either side of oestrus were used as all cycles were not of the same length.

6.3.5 Periodic Regression

In the consideration of time-dependent repetitive patterns in biology, e.g. circadian rhythms, the standard analysis has involved some form of periodic regression (Bliss 1967). In theory this

involved the mathematical fitting of a sine wave of known frequency to the data and testing the hypothesis that the data and the sine curve are not significantly different. In practice, however, few biological data are as symmetrical as the sine wave and it becomes necessary to introduce some form of harmonic analysis. This makes the assumption that any series of rhythmic data can be described by the superposition of a number of sine waves. This task may be accomplished by use of the 'Fourier series' with which one can add harmonics which represent further cycles within the fundamental period. However, if extended indefinitely the computed expression will fit any data but may have little biological meaning. The objective should be to add only enough harmonics (perhaps two or three) to reduce the scatter about the fitted curve to a level that is essentially random, i.e. reaches the situation where all the non-random data have been explained by the regressions. Also it is possible to reduce non-sensical rhythms by 'digital filtering' with the result that cycles or periods over or below certain predetermined values are disregarded.

Using the Fourier transformation the data may be presented graphically (a 'power spectrum') and a series of frequencies generated that are inversely related to the period of the cycle and its harmonics, i.e. $\text{Period} = 1/\text{Frequency}$. The 'power' or height of each frequency on the graph is a reflection of the amplitude of the frequency.

An analysis of variance can be carried out to test whether the fundamental period and its various harmonics explain the majority of the variation. If this condition is met then the fundamental period could be specified as the actual length of say the bettongs' oestrous cycle. This type of analysis has been attempted recently by Treagust, Randall and Folk (1980) on the diurnal temperature fluctuations in the American opossum *Didelphis virginiana* but apparently has never before been applied.

to temperature rhythms related to reproductive events such as the oestrous cycle. For this analysis the data were entered into a computer (Burroughs 6800) as a continuous stream of daily basal body temperatures.

6.3 RESULTS

6.3.1 Diurnal Variation

All the bettongs monitored demonstrated a diurnal rhythm typical of that expected from a nocturnal animal. Temperatures reached a maximum during the night while the animal was active and feeding whilst minimum temperatures occurred during the day when the animal was at rest. Figure 6.1 illustrates the hourly variation in the recordings from bettong B2 over a ten day period. These data have been averaged to produce a curve of the mean hourly temperature variation over 24 hours (Figure 6.2). From Figure 6.2 it can be seen that the mean maximum temperature (37.5°C) occurred at midnight and the mean minimum temperature (36.2°C) occurred at midday, hence there was a diurnal variation of approximately 1.3°C . The daily minimum temperature is defined as the basal body temperature (BBT) and is the value used in the following section on temperature variation throughout the oestrous cycle (Section 6.3.4). The Fourier power spectrum (Figure 6.3), obtained from the analysis of the 10 days of hourly temperature recording, demonstrates only one major frequency peak, indicating a cycle with a period lying between 22.3 and 24.4 hours with no significant harmonics. Table 6.1 contains the statistical evaluation (Bliss 1970) of these data and it can be seen that the vast majority of the variation can be explained by the 24 hour fundamental period, i.e. by a sine wave with a period of 24 hours.

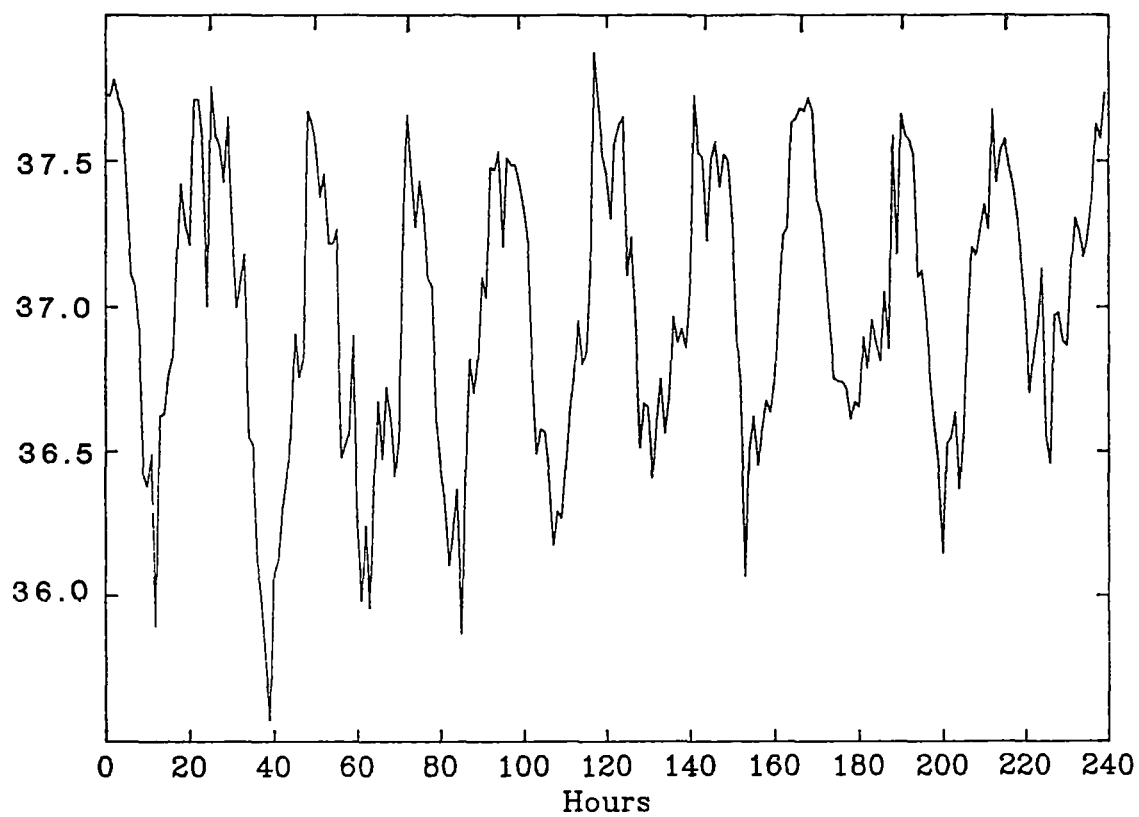


Figure 6.1 Diurnal variation in body temperature over a ten day period, Bettong No. 2.

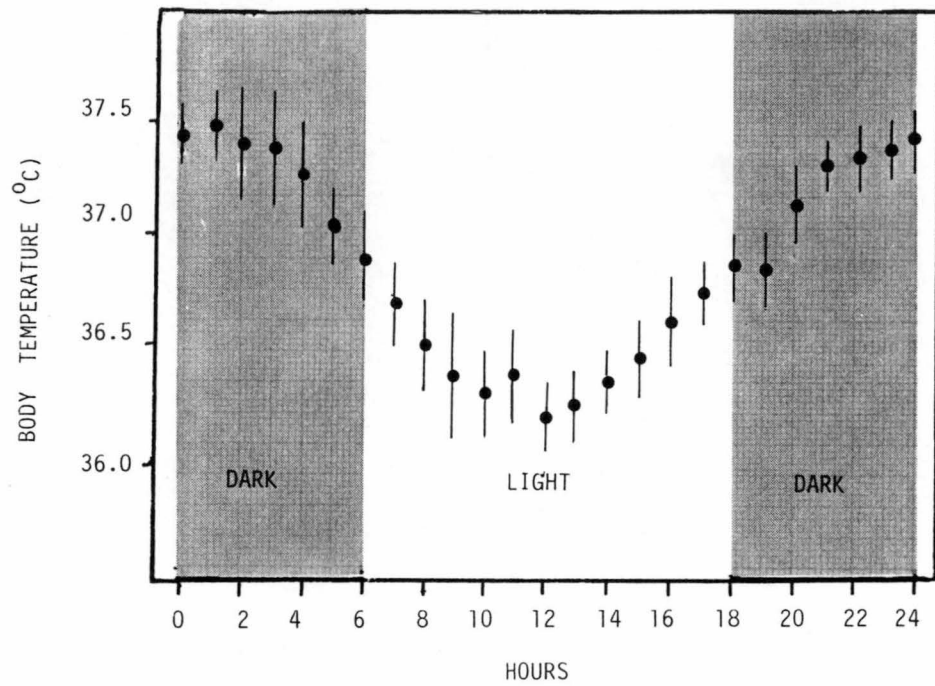


Figure 6.2 Mean body temperature (\pm S.E.) over one ten day period (B2). Data from Figure 6.1.

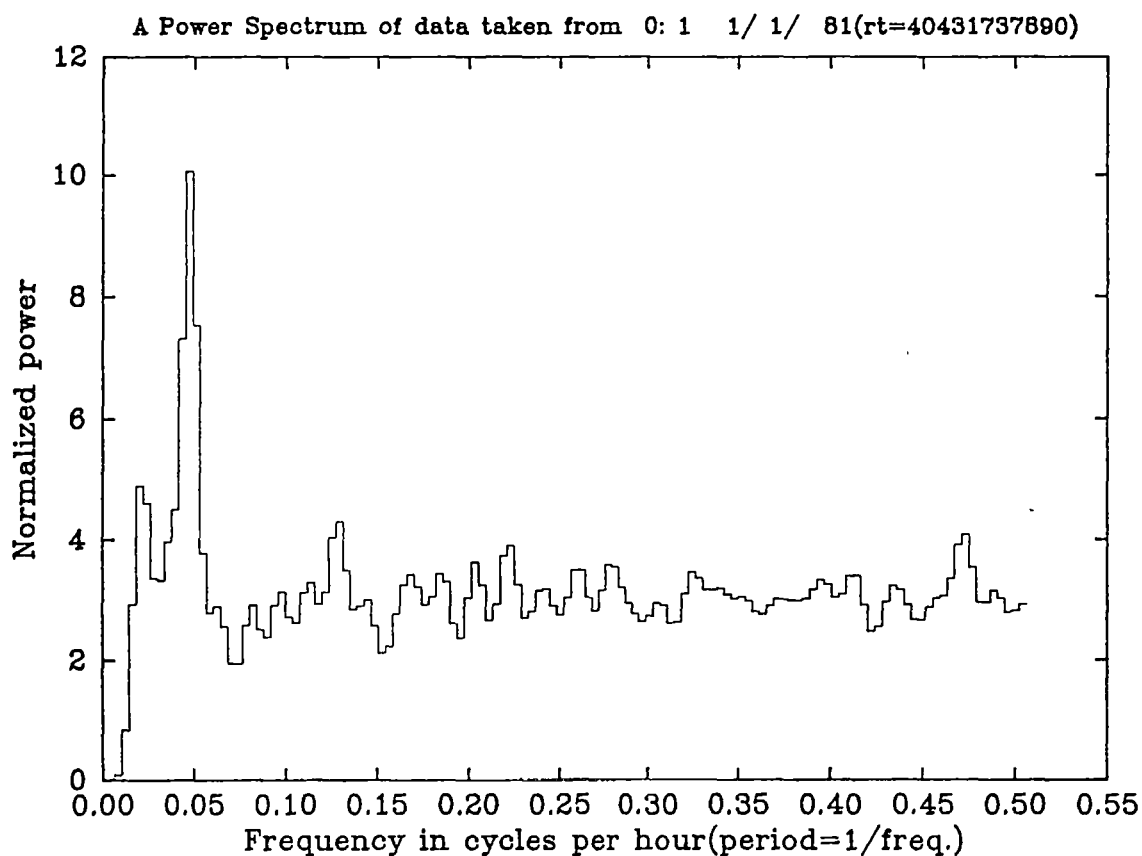


Figure 6.3 Fourier power spectrum of data in Figure 6.1.
Note only one peak has been drawn out with a
period of $\bar{X} = 23.3$ hrs.

TABLE 6.1 Periodic regression analysis of the diurnal rhythm (24 hours)

Term	DF	MS	F
Variation between days	9	16671.3	13.6*
24 hour fundamental period	2	694000.0	567.6*
1st harmonic	2	3946.5	3.2*
2nd harmonic	2	45.1	0.1
Scatter about curve	17	1163.6	0.9
Residual (random error)	153	1222.5	

* significant

It can be seen from Table 6.1 that the fundamental period (24 hour) and the 1st harmonic account for such a large amount of the variation that the scatter about the curve has been reduced to non-significant levels, i.e. those data not explained by the harmonics are random values.

6.3.2 T_{\max} and BBT

The mean daily maximum temperature (T_{\max}) and the daily BBT were obtained from 76 consecutive recordings from bettong B3 and the values obtained were

$$T_{\max} = 37.52 + 0.29 (\bar{X} \pm \text{S.D.}) ^\circ\text{C}$$

$$\text{BBT} = 36.17 + 0.50 ^\circ\text{C}$$

There is a highly significant difference between the variance of these two values (F-statistic = 3.52, $P < 0.001$), the variance about the BBT is almost twice that of T_{\max} .

6.3.3 Time of BBT

The hour ($\bar{X} \pm \text{S.D.}$) at which the BBT occurred was calculated for the week prior to and the week after oestrus using data from six cycles (two from each animal). The results were variable though there appeared to be a trend towards the BBT occurring earlier during the week before oestrus. The basal body temperature occurred at 11.38 ± 3.07 h. in the week before oestrus and at 12.86 ± 3.86 h in the week after oestrus. A t-test gave a non-significant result ($t = 1.82$, D.F. = 66, $P < 0.1$).

6.3.4 BBT and the Oestrous Cycle

Curves representing the average body temperatures for each of the 10 days before and after oestrus (thirteen oestrous cycles) are presented in Figure 6.4 (raw data) and Figure 6.5 (standardised data). From these two figures it can be seen that the BBT remains elevated but fairly stable from day -10 to day -4, after which there is a decrease in temperature until one or two days before oestrus. A small peak occurs at oestrus which is followed by a drop to the minimum value (day +2) obtained over the whole cycle. One day later, i.e. day three after oestrus, there is a sudden jump in BBT to the maximum value for the whole cycle. Body temperature then gradually decreases until day +8 or 9 post-oestrus when the basal body temperature rises again.

The analysis of variance for the standardised data is presented in Table 6.2. The F-statistic of 1.73 was significant at the 5% level and indicates that there is more variation between days of the oestrous cycle than within days. A t-test matrix was constructed (Appendix B) which showed that there were significant differences between certain days of the oestrous cycle. The minimum obtained on day +2 after oestrus

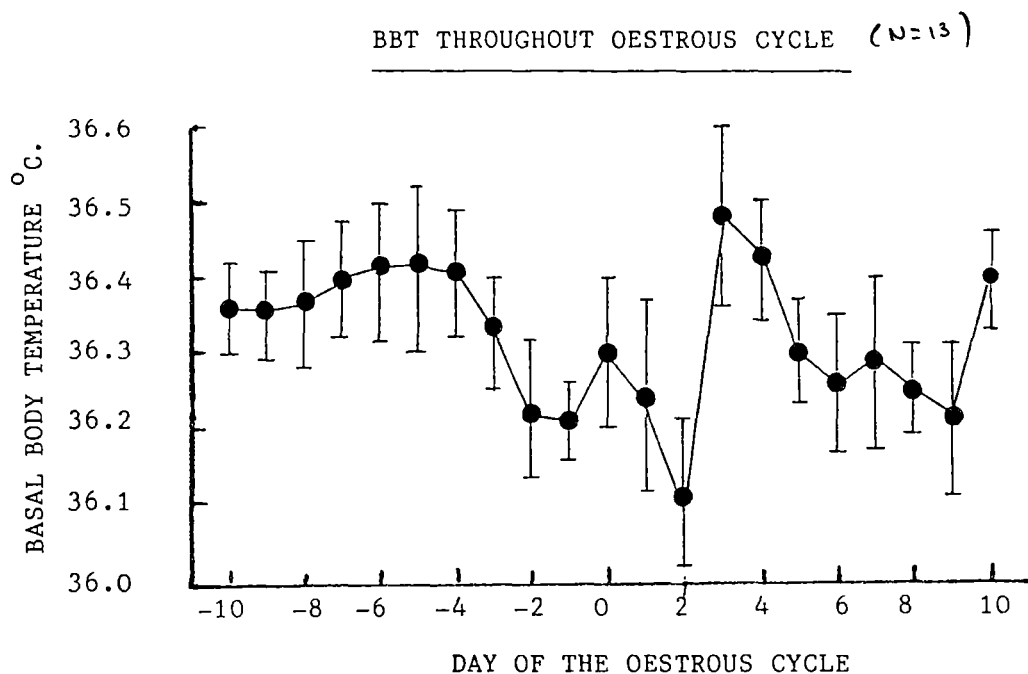


Figure 6.4 Changes in the mean basal body temperature (\pm S.E.) during the oestrous cycle (Raw data, N = 13). Day 0 = oestrus.

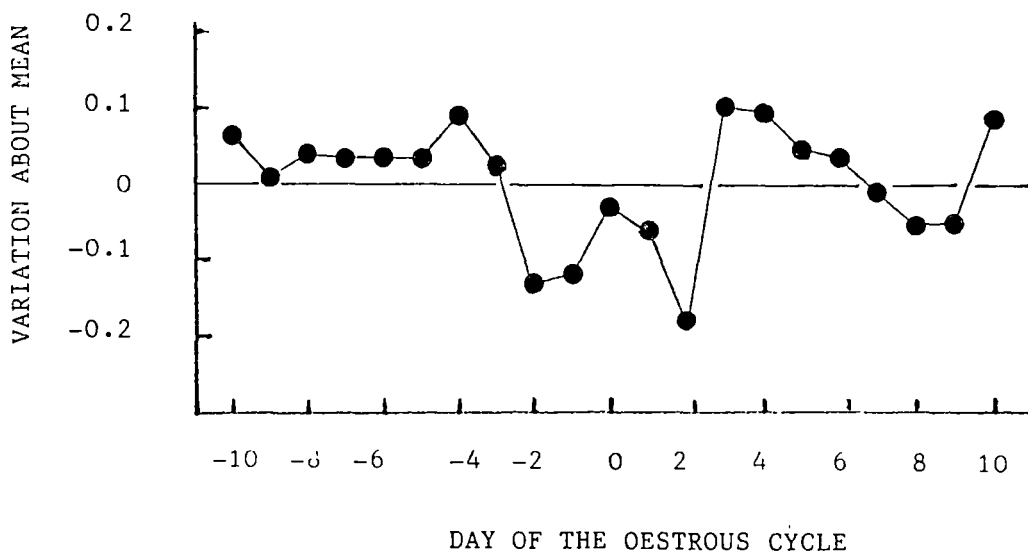


Figure 6.5 Daily variation from cycle mean during the oestrous cycle (Standardised data, N = 13). Day 0 = oestrus.

was significantly different ($P < 0.05$) from the mean values obtained from day -10 to day -3 prior to oestrus. The jump in temperature on day +3 is also statistically significant ($P < 0.01$) and remains so until day +5.

TABLE 6.2 One-way analysis of variation of the standardised data for each day (-10 to +10 after oestrus) for the thirteen oestrous cycles

Source of Variation	Sum of Squares	Degrees of Freedom	Variance
Between days	1.4536	20	0.0727
Within days	8.2140	196	0.0419
Total	9.6676	219	
<hr/>			
F-statistic = 1.7343	P < 0.05		

6.3.5 Individual Results

Figures 6.6 and 6.7 illustrate results from individual animals and demonstrate the changes in BBT that occur during pregnancy; in one case pregnancy was due to removal of the pouch young while the other was a natural pregnancy. Lactation had little apparent effect upon the BBT which remained stable during the early and middle phases of pouch life. It would be fascinating to know what effect a large young would have on the body temperature of the mother but the young died before it was furred.

6.3.6 Harmonic Analysis, the Fourier Regression and the Oestrous Cycle

Harmonic analysis (Bliss 1970; Table 6.3) demonstrated that a cycle (sine wave) of length 2 days and its first harmonic could explain

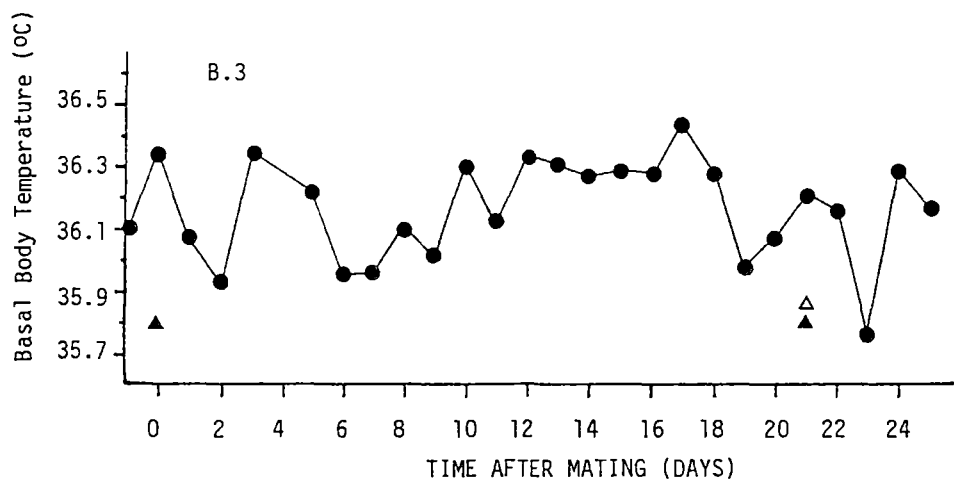


Figure 6.6 Basal body temperature during gestation in B3.
Solid triangle = mating, open triangle = birth.

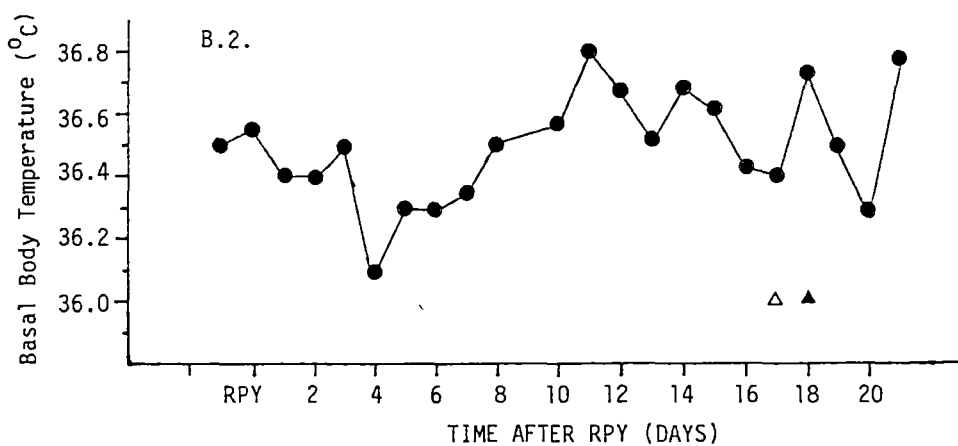


Figure 6.7 Basal body temperature during delayed gestation in B2, following removal of pouch young (RPY).
Solid triangle = oestrus, open triangle = birth.

the majority of the variation in the data and reduce the scatter about the curve to random values. Three cycles were excluded from this analysis (cycles 3, 8 and 13) as these each had some values missing and the analysis required complete cycles. The greater F-statistic found with the second harmonic is interesting as the Fourier series also drew out a band of similar width which similarly had a high amplitude (Figure 6.9). The zero variation between cycles is a consequence of the use of the 'standardised data' as the mean of each cycle was designated zero (Figure 6.5).

TABLE 6.3 Harmonic analysis of BBT throughout the oestrous cycle.
Fundamental period = 21 days (22 day cycle)

Term	DF	MS	F
Variation between cycles	9	0.00	0.00*
Fundamental period (oestrous cycle)	2	0.13	3.6**
1st harmonic	2	0.18	5.0**
Scatter about curve	16	0.06	1.6
Residual (random error)	144	0.04	-

* cycle means taken as zero (0)

** significant, excluded 3 curves that were depleted 3,8,13

When the Fourier series was applied to the raw data three major frequencies were drawn out that explained the majority of the variation (Figure 6.9). Peak 1 occurred with a band width that reflected a cycle length between 19.7 and 28.5 ($\bar{X} = 24.1$) days; peak 2 similarly occurs within a band width of 11.1 and 13.5 ($\bar{X} = 12.3$) days and peak 3 between 3.8 and 4.0 ($\bar{X} = 3.9$) days. It is tempting to suggest that peak 1 approximates the oestrous cycle and that peak 2, though of higher

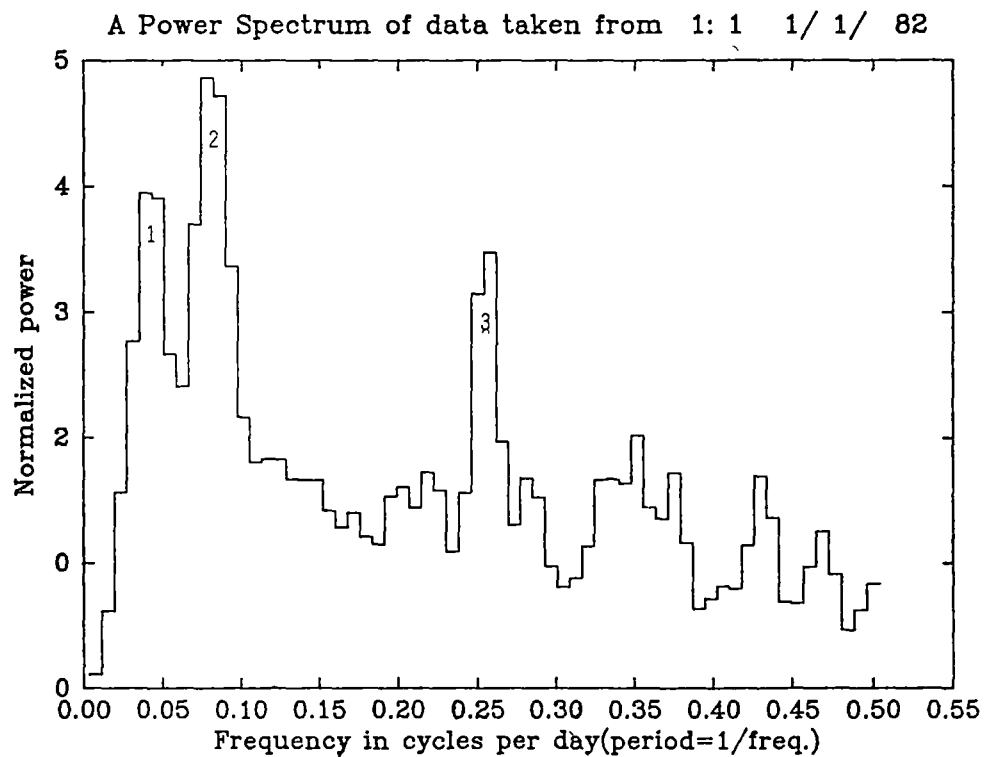


Figure 6.9 Power spectrum of oestrous cycle data.
 3 peaks are distinguished with mean periods
 of:- 1 = 24.07 days, 2 = 12.3 days and
 3 = 3.9 days.

amplitude, is merely a harmonic of peak 1 as its period is almost exactly half that of peak 1. Peak 3 may or may not be related to the reproductive cycle. As the minimum and maximum lengths for the oestrous cycle obtained during this study (Section 4.3.1) were 17 and 46 days it was decided to digitally filter the data to remove consideration of cycles outside of these limits by the Fourier series. The filtered power spectrum provides little additional information; although it appears that there are two closely related cycles operating with periods 24.4 to 27.0 days and 18.9 to 20.5 days respectively, this separation may be an artefact (Figure 6.10). The third peak at 13 days is a reflection of the fact that the filter does not cut 'cleanly'; hence a cycle below 16 days has not been completely filtered out.

6.4 DISCUSSION

This study has demonstrated, by the use of telemetry, a circadian rhythm in the body temperature of *B. gaimardi*. Much of the previously reported research into diurnal rhythms in body temperature has been obtained on restrained animals by the use of rectal probes. Poole and Stephenson (1977) have suggested shortcomings in this method but there are only a few studies on marsupials that have used unrestrained animals and telemetry. Guiler and Heddle (1974) were the first to use this method (with *Sarcophilus harrisi*) and Brown and Dawson (1977) worked in a similar way with kangaroos but only collected data from each animal over 2-3 days. Treagust *et al.* (1979) specifically studied the circadian rhythm of body temperature of unrestrained opossums over periods up to 11 days and Peters and Rose (1979) studied the common wombat over an extended period. More recently May (1982) has measured

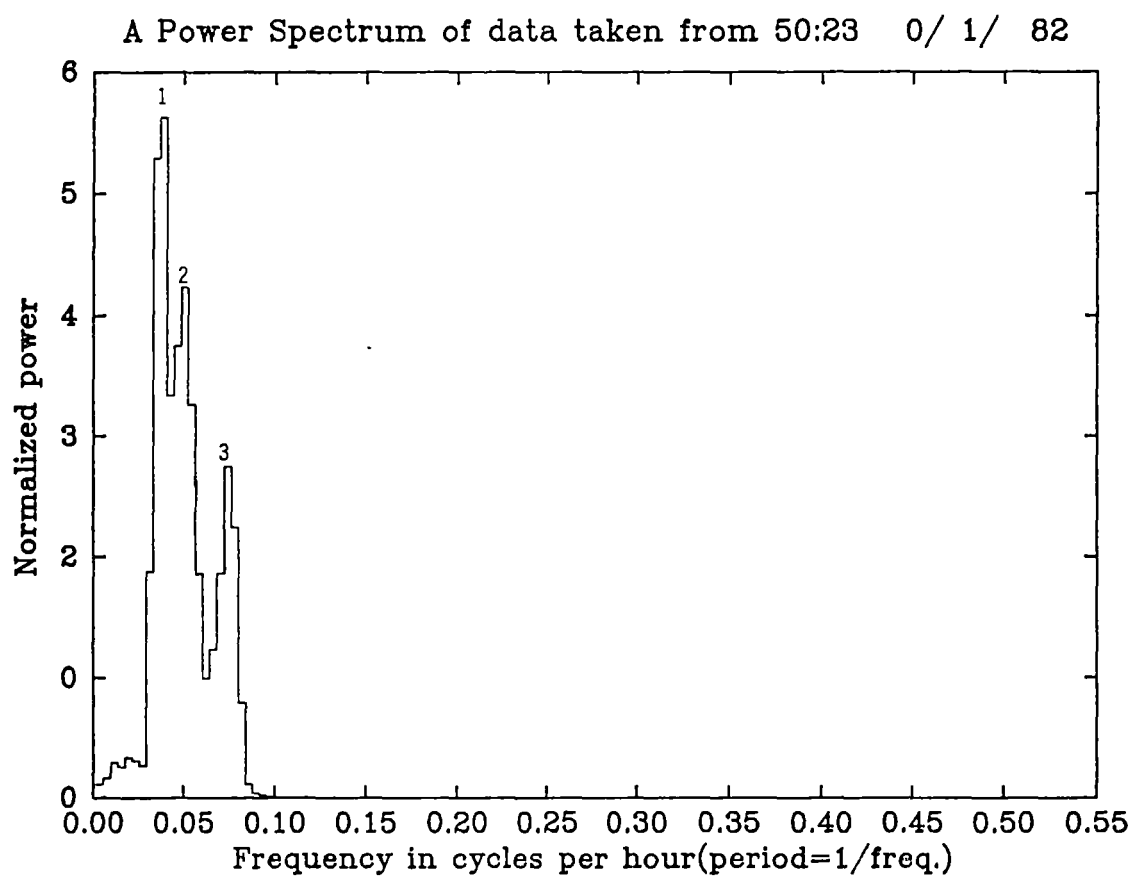


Figure 6.10 Filtered power spectrum detecting three peaks with a mean period of 1 = 25.6 days, 2 = 19.7 days and 3 = 13.5 days.

body temperature by radiotelemetry continuously over a period of several days in the sugar glider (*Petaurus breviceps*). All authors have found temperature rhythms typical of nocturnal animals, i.e. temperatures are lower during the day when animals are resting.

The mean daily variation in body temperature ('nycthemeral' variation) in the bettong was 1.3°C and is similar to that found in three species of unrestrained kangaroos (1.8°C) in outdoor yards by Brown and Dawson (1977) and 1.7°C in the unrestrained opossum (Treagust *et al.* 1979). However, as shown in Section 6.3.2 the variation in BBT was far greater than that in T_{max} which remains relatively constant; as a result the degree of diurnal fluctuation in body temperature will vary in female bettongs throughout the oestrous cycle. A similar finding was made by Peters and Rose (1979) with the common wombat. These observations suggest that there may be separate controls for the set-point of basal and maximum body temperatures as proposed by Hammel (1967). They also suggest that perhaps future research describing nycthemeral rhythms in body temperature should also detail the reproductive condition of the test animals.

The mean daily minimum temperature (BBT) in the bettong was 36.2°C and this is similar to the resting values (mean of 35.5°C) found in a range of diprotodont marsupials by Dawson and Hulbert (1970). However, resting values are not directly comparable with basal temperatures. The basal body temperature (the lowest temperature measured during a 24 h period when the animal is asleep or resting) only is found at the time of the circadian 'trough' and is due to the physiological setting of the animal (Treagust *et al.* 1979); resting body temperatures may be obtained at any time during the day when the animal is resting or asleep. Resting values taken at different times during the day are also not strictly comparable and may account for published

variation in the body temperature of the same species, e.g. Hudson and Dawson (1975) found the resting body temperature of the potoroo *Potorous tridactylus* to be $35.9 \pm 0.5^{\circ}\text{C}$ ($\bar{X} \pm \text{S.D.}$) over a range of ambient temperatures while Nicol (1978), working over similar ambient temperatures with the same animal, obtained mean values between 36.7 and 36.9°C .

It has been known for some time that the body temperatures and metabolic rates of marsupials are less than those obtained from similar sized eutherian mammals (Dawson and Hulbert 1970) although the significance of this finding is unclear. Nagy (1980) has recently shown that the metabolic rates of some marsupials in the field are quite similar to values obtained from eutherian mammals. Perhaps the body temperatures of active marsupials in the field may also be closer than suspected to eutherian body temperatures measured under comparable conditions; the use of radiotelemetry would allow this question to be answered. (One 'free ranging' bettong had a T_{max} of 38.2°C ($N=5$) Dick and Rose unpublished data.)

Radiotelemetric measurement of body temperature has the advantage of providing sufficient data for computer analysis of circadian and other rhythms. That there are significant changes in BBT that occur throughout oestrous cycles in a cyclic fashion requires statistical verification. The analysis of variance and subsequent use of the t-test showed that there were significant differences between the basal body temperature on certain days of the oestrous cycle in the bettong. Periodic regression (Bliss 1970) and the use of the Fourier series demonstrated that a number of rhythms occur and that one of these periods approximates to the duration of the oestrous cycle. Some form of periodic regression has been widely used in the analysis of cyclic phenomena in biology but computer programs that cope with large amounts

of data in a rapid fashion have simplified the procedures involved in such analysis. The Fourier series lends itself to the analysis of cyclic changes in body temperature whether over 24 h or longer periods. To my knowledge the Fourier series has only been applied to the diurnal body temperature rhythms of one marsupial, the opossum (Treagust *et al.* 1980) and has not been used at all in the analysis of BBT in relation to mammalian reproductive cycles. This type of computer analysis of the correlation between body temperature and reproduction warrants wider use and can be adapted to deal with hormonal or other correlates of the reproductive cycle.

Rhythmic changes in basal body temperature occur throughout the oestrous cycle of *B. gaimardi*. Most prominent are the variations that occur near oestrus and it seems probable that these are directly related to the behavioural and physiological changes associated with oestrus and ovulation. This is further substantiated by the fact that these cyclic changes were not apparent during lactation.

The small transient peak that occurs at oestrus is found in other mammals, e.g. the rat (Yochin and Spenser 1976) and the wombat (Peters and Rose 1979). This peak may be due to the hormonal fluctuations that occur at oestrus or it may be that 'true' basal body temperatures do not occur at oestrus when the animals are far more active.

The post-ovulatory rise in BBT that occurs during the human menstrual cycle is maintained for the life of the corpus luteum. In the bettong the transient peak in BBT at oestrus is followed by a more substantial but no less transient peak on day 3 after mating; the body temperature then gradually drops over the following 5 days. In the wombat BBT did not become elevated until 5 to 10 days after the transient peak at oestrus (Peters and Rose 1979). The low post-oestrous temperatures may be a true reflection of low progesterone levels during this

period, or perhaps a low progesterone to oestrogen ratio as Walker & Gemmell (1982) (in Gemmell comm.) has shown that oestrogen levels remain elevated for up to 7 days after oestrus in *M. rufogriseus* though this does not occur in the opossum (Harder and Fleming 1981). The statistically significant rise in BBT to a cycle maximum on day 3 after oestrus is interesting as it may be indicative of a similar peak in progesterone that is known to occur in a number of macropodid species, e.g. *S. brachyurus* (Cake *et al.* 1980) and *M. eugenii* (Hinds and Tyndale-Biscoe 1982). This progesterone peak also occurred in one bettong on day 4 after RPY (Appendix C). It appears unlikely that the BBT peak on day 3 reflects the occurrence of ovulation as ovulation most probably occurs within 24 h of oestrus as found in the quokka and tammar (Sharman 1955a; Tyndale-Biscoe and Roger 1972). In fact the curves presented in Figures 6.6 and 6.7 closely resemble the progesterone profiles obtained for the tammar wallaby (Tyndale-Biscoe *in litt.*; Hinds and Tyndale-Biscoe 1982).

The major events that characterise the changes in BBT during the oestrous cycle in the Tasmanian bettong can be summarised thus: the BBT reaches a mid-cycle elevated plateau that drops for 3 to 4 days until a small transitory peak on the day of oestrus; this is followed by a drop to a cycle minimum temperature immediately before the cycle maximum. Changes in basal body temperature in the sequence as detailed would allow oestrus to be pin-pointed without the need to handle the animal. In practice, however, oestrus could only be determined in retrospect, as the greatest changes in BBT occur on days 2 and 3 after oestrus.

In some eutherian mammals, e.g. the pig (Elmore *et al.* 1979), body temperature remains elevated throughout pregnancy. This is partly due to the relatively high levels of progesterone that are found during

pregnancy and (at least in the final trimester) to the actual presence of a large fetus. Pregnancy in marsupials is not comparable to that in eutherians, as there appears to be little hormonal difference between pregnant and non-pregnant animals and the small size of the fetus is unlikely to directly affect the maternal body temperature. Thus it is not surprising that pregnancy in the bettong had little apparent effect on BBT values.

Although it appears unlikely that the use of BBT measurements (by telemetry or other methods) of captive animals will supplant the standard method of vaginal smears, it may form the basis for monitoring reproductive events, e.g. oestrus, breeding season and lactation in wild populations of marsupials. Telemetry is widely used by mammal ecologists in tracking and determining animal movements; temperature sensitive telemeters could serve the multiple function of providing data on movement, temperature regulation and reproductive events.

CHAPTER 7

POUCH VACATION IN THE TASMANIAN BETTONG

CHAPTER 7 POUCH VACATION IN THE TASMANIAN BETTONG

7.1 INTRODUCTION

The possession of a pouch readily distinguishes most marsupials from their eutherian counterparts. After birth, the young marsupial spends a variable period of time wholly within the pouch before leaving this safe but confined space for the outside environment. Initially, the young is able to return to the inside of the pouch but this it does less and less frequently until, one day, it no longer returns. The first day that the young remains totally outside the pouch may be termed the 'day of final pouch vacation'.

Neither the initial emergence nor the final vacation from the pouch can be easily equated with parturition in those eutherian mammals which have precocial young. Marsupial birth followed by the attainment of the safe confines of the pouch also has no eutherian equivalent. In fact, the parental strategies of these two mammalian groups have considerable differences (Parker 1977; Low 1978; Russell 1982).

In most polyoestrous marsupials, reproductive cycles are inhibited by the presence of a sucking young in the pouch (Sharman 1970; Tyndale-Biscoe 1973). In the Tasmanian bettong, as in the majority of macropodid marsupials, there is a post-partum oestrus immediately after birth which usually results in the fertilised egg developing to a unilaminar blastocyst stage before entering embryonic diapause. In some kangaroos, this quiescent blastocyst resumes development near the end of pouch life (while a young still resides within the pouch). The end result is that birth occurs shortly after final pouch vacation.

In several macropodid species, young vacate the pouch during the non-breeding season and the delayed blastocyst remains dormant until the next breeding season, e.g. the tammar *Macropus eugenii* and Bennetts wallaby, *M. rufogriseus rufogriseus* (in Tasmania). In other wallabies, e.g. the red-necked wallaby *M. r. banksianus*, the blastocyst remains dormant until the pouch is empty, i.e. after final emergence (Merchant and Calaby 1981).

However, in some continuously breeding macropodids, ^{it is possible, theoretically, that} the pouch may be occupied simultaneously by successive young (the large furred young soon to vacate the pouch and the new young born as a result of the activation of the delayed blastocyst); alternatively, an egg fertilised at post-partum oestrus may continue development and not enter diapause, resulting in two pouch young separated in age by the length of one oestrous cycle. In reality the possibilities rarely occur, and Sharman and Berger (1969) have proposed that embryonic diapause has evolved in macropodids to prevent just such an occurrence.

Within the Macropodidae, efficient reproduction involves more than merely the growth in the pouch of the largest young possible. For example, it may be advantageous to produce and release smaller young as a result of a shorter pouch life and hence have an opportunity to breed again more quickly. Presumably, Natural Selection has acted upon the length of pouch life in all marsupials, and this implies some sort of genetic control over its duration. Support for this suggestion comes from studies by Merchant and Sharman (1966) who showed that length of pouch life ^(donor species) did not vary when pouch young of one species of kangaroo were fostered in the pouch of another. This observation not only suggests that genetic factors control the length of pouch life, but that environmental factors may have little influence.

In some cases Merchant and Sharman found that although pouch young were maintained in the pouch for their usual time period, they were much heavier and larger than normal. This may have been due to a more nourishing milk supply from the foster mother and/or a different sucking pattern. These observations suggest that young are programmed to leave the pouch when they have occupied it for a certain period of time rather than when they have achieved a particular weight.

There is another possibility which has not been examined to date: that the mother herself is influential in determining the length of pouch life. The strategy of carrying a large young in the pouch obviously has some maternal disadvantages - reduced mobility must affect her ability to evade predators and gather food, for example. Low (1978) claims that mothers are able to curtail the pouch occupancy when they are under stress. She instances mothers ejecting young whilst being pursued, arguing that this has the advantage of confusing the predator so that both mother and young have a better chance of escaping. There is no evidence to establish whether this ability to jettison young is voluntary and deliberate or involuntary and accidental.

In their study of the reproductive behaviour of the red kangaroo *M. rufus*, Sharman and Calaby (1964) hypothesised that pouch vacation was associated with physiological and morphological changes in the mother. The implications of their hypothesis have been ignored until the present study. One of the main purposes of this chapter is to examine the relationship between physiological and morphological changes in the mother and pouch vacation.

The bettong is an ideal subject for such a study because there is a close temporal relationship between pouch vacation, parturition and oestrus. It is proposed that physiological events in the mother during parturition and oestrus are significant determinants of the

duration of pouch life in the bettong. This hypothesis may be examined by altering the timing of birth and/oestrus in an experimental situation. In other studies of macropodids it has been usual to initiate oestrous cycles and parturition by removal of the young from the pouch (see Chapter 4). Obviously, employing this technique in the present study would be of no use. Earlier work by Clark (1968), Tyndale-Biscoe (1963a,b) and Renfree and Tyndale-Biscoe (1973) has shown that injection of progesterone into lactating kangaroos (with pouch young) can lead to the activation of the diapausing blastocyst. More recently Tyndale-Biscoe and Hinds (1981) have induced the activation of the delayed blastocyst and its eventual birth with a single intramuscular injection of the prolactin antagonist, bromocriptine (CB154), in animals carrying small young in their pouches.

In the present study both progesterone and bromocriptine were used in an attempt to precipitate birth and/or oestrus earlier than normal, whilst young were still in the pouch. If the proposed hypothesis is correct, inducing the early onset of parturition or oestrus by the abovementioned methods in mothers with medium to large size young should result in an early departure from the pouch. Similarly, delaying or inhibiting these two reproductive events should prolong pouch life. Evans, Tyndale-Biscoe and Sutherland (1980) and Renfree, Wallace and Young (1982) demonstrated that small amounts of oestrogen were capable of inhibiting follicular growth in the tammar wallaby with the result that ovulation and oestrus were delayed or failed to occur altogether. One experiment in the present series employed this technique in an attempt to inhibit the ensuing oestrus in the bettong.

For reasons that will become apparent later (Section 7.3.6), a further set of experiments was designed to achieve birth and/or oestrus at abnormal or inappropriate times during pouch life. This involved

the substitution of pouch young. The young were introduced into the pouch of a foster mother at a time when her uterus contained a blastocyst that had become activated or could be expected to become so at the commencement of the experiment. In these circumstances, according to the hypothesis, the fostered young should remain in the pouch only until birth of a new young and/or oestrus.

In this series of experiments it was necessary to use tame bettongs (in fact the subject mothers were born in captivity) with pouch young of known birth dates.

7.2 METHODS

7.2.1 Pouch Young

All female bettongs were checked weekly. Any furred young found in the nest next to its mother were placed under daily observation for its ability to return to the pouch. The disturbing presence of the observer was usually sufficient to provoke a return to the pouch. In other cases the young were removed from the nest and the mother roused. Mothers whose pouch young failed in their attempts to return to the pouch were examined for the presence of a new young or signs of mating or oestrus. The degree of distension of the lips of the pouch was noted at this time, as was the age and weight of all furred young.

7.2.2 Experimental

Progesterone

10 mg Progesterone [DepoProvera] was given intramuscularly every three days (total of three injections per animal, B180, B15, B280, B6).

Bromocriptine [Parlodel tablets or CB154 powder - two batches].

Three bettongs were given an oral dose of bromocriptine in tablet form

(Parlodel, 5 mg kg^{-1}). Separately obtained powdered bromocriptine (two batches) was prepared according to instructions provided by Sandoz Australia as 150 mg of CB154 in 6 ml of a warm 70% alcohol/saline mixture containing 150 mg tartaric acid. A single intramuscular injection at a dose rate of 5 mg kg^{-1} was given to nine bettongs with pouch young. In addition, nine female Tasmanian pademelons, *Thylogale billiardieri* (Macropodinae) received a single intramuscular injection of bromocriptine (batch two) at a dose rate of 5 mg kg^{-1} .

Oestrogen [Oestradiol benzoate]. Over a period of 3-7 days four bettongs with pouch young received intramuscular injections (0.1 ml) of $5 \text{ } \mu\text{g} \cdot \text{day}^{-1}$ and one animal a lower dose of $1 \text{ } \mu\text{g} \cdot \text{day}^{-1}$.

All of the experimental animals were observed regularly for pouch vacation, birth and oestrus.

7.2.3 Foster Young

Pouch young (day of birth known) were removed from six female bettongs and were replaced by young of different ages. In practice this involved the 'swapping' of young between two mothers, one mother receiving a foster young that was smaller than her own and the other receiving a foster young larger than her own. The six pouch young were weighed and measured each week and on the day of final pouch vacation. Growth rates before and after the swap were calculated.

7.3 RESULTS

7.3.1 Pouch Vacation: Untreated Animals

In the Tasmanian bettong, *B. gaimardi*, a precise sequence of events occurs on the night of final pouch vacation. This sequence

is initiated when the pouch of the mother tightens as if drawn by purse strings (there is a contracting of a sphincter muscle and some eversion of the inner lining; Plate 7.1b). This tightening prevents the re-entry of the young (they can be observed making repeated attempts to return to the pouch over a period of several days; Plate 7.1a). Birth and a post-partum oestrus usually follow pouch vacation (Group 1, Table 7.1, Plate 7.1c). On most occasions both events occur on the same night as final pouch vacation. In those cases where birth occurs on the night before oestrus the young always vacates the pouch on the night of parturition.

When birth fails to occur, but oestrus takes place, the young remains in the pouch until the night of oestrus (Group 2, Table 7.1). On three occasions young vacated the pouch without birth or oestrus occurring. The mothers entered anoestrus immediately and their pouches became dry and scaley (Group 3, Table 7.1).

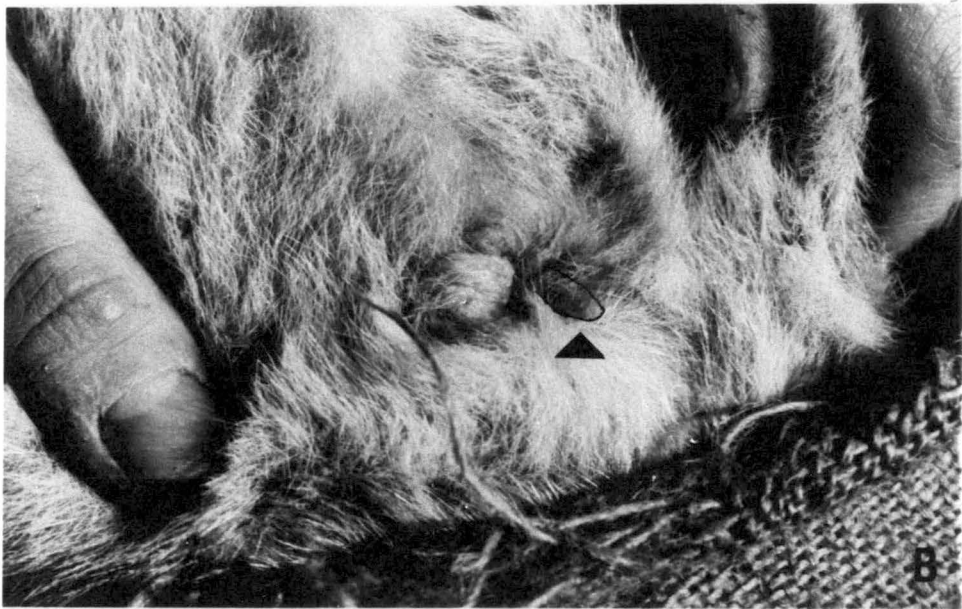
TABLE 7.1 Mean age and weight (\pm standard deviation) at pouch vacation in three groups of bettong

Associated Events	Group 1	Group 2	Group 3
	Birth & Oestrus N=10	Oestrus only N=5	Neither N=3
Age (days) \pm S.D. (Range)	106.8 \pm 1.7 (104-109)	105.4 \pm 3.6 (101-111)	112.7 \pm 3.5 (109-116)
Weight (g) \pm S.D. (Range)	333.5 \pm 27.7 (291-371)	369.0 \pm 67.1 (298-460)	430.7 \pm 6.4 (427-438)

These data are summarised in Table 7.1. The analyses of variance for both age and weight between the three groups in Table 7.1 are presented in Table 7.2 (a and b). These analyses show that there are



7.1(a) Day of final pouch vacation. Young unable to return to pouch. Mother's fur ruffled due to mating.



7.1(b) Same animal as above. Pouch tightly closed. Arrow points to protruding teat.



7.1(c) Inside the above pouch. Note large teat and neonate sucking on another teat.

significant differences between the groups being tested. Student t-tests show that there are significant differences between the mean weight ($t = 10.2$, $P < 0.01$) and age ($t = 2.8$, $P < 0.05$) of Group 3 (pouch vacation not accompanied by oestrus in mother or by birth of a new young) and that of Group 1. Significant differences were not found between the mean weights or ages of Groups 1 and 2, although Group 2 (oestrus but no birth at pouch vacation) appeared much more variable. The greater variability was not due to the lower sample size alone, as the range was also greater in Group 2.

TABLE 7.2 Analysis of variance of the data presented in Table 7.1
(a) Weight

Source of Variation	Sum of Squares	Degrees of Freedom	Variance Estimate
Between groups	22310.8	2	11155.4
Within groups	20497.2	14	1464.1
Total	42808.0	16	

F.statistic = 7.62, $P < 0.01$ (significant)

(b) Age

Between groups	106.7	2	53.4
Within groups	99.4	14	7.1
Total	206.1	16	

F.statistic, $P < 0.01$ (significant)

For both parameters (age and weight) there is a greater (and statistically significant) variation between the groups than within the groups.

7.3.2 Progesterone

None of the female bettongs gave birth within 28 days of the progesterone injection. All females continued with lactation but failed to give birth at pouch vacation.

7.3.3 Oestrogen**TABLE 7.3** Effects on bettong young of 7 daily injections of oestrogen (1^f or 5 µg) given to their mother during lactation

Animal	Age of young at time of initial injection	Age at vacation days	Weight (g)	Comments (re. mother)
1*	98	103	303	Mating and birth night of vacation
2	42	107	324	Mating and birth night of vacation
3	95	116	491	Mated night of vacation
4	96	113	423	Mated 4 days after vacation
5	105	114	311	Mated night of vacation
Control	-	106	345	Mating and/or birth night of vacation

The control values are the mean values obtained from Groups 1 and 2 (Table 7.1). Animal 1 received the low dose of oestrogen and showed no response. The young of three out of the four animals injected with oestrogen at a dose rate of 5 µg.day⁻¹ had a pouch life longer than normal and the pouch young were generally heavier (although animal 5 had an abnormally light pouch young). Parturition failed to occur in these three animals (3, 4 and 5), but two young vacated the pouch at the time their mother came onto 'heat' and one left the pouch 4 days before oestrus (4). Within 3-5 days of the first injection of oestrogen there was a noticeable tightening of the pouch sphincter muscles, although the degree of contraction was insufficient to prevent re-entry by the young. The two large young (mothers 3 and 4) had

difficulty gaining full access to the pouch towards the end of their pouch life. The mothers would often move around with the hind quarters of the young protruding outside the pouch. This behaviour was never noted with the control animals.

Experimental animal No. 2 showed no response to the higher dose of oestrogen but this animal received its injections early in lactation (in contrast to all other females which were injected near the end of pouch life when the previously quiescent blastocyst had been activated).

7.3.4 Bromocriptine

Three bettongs with pouch young received bromocriptine in an oral tablet form. They were examined daily. None of them gave birth during the following 28 days.

Five females (with pouch young) injected with bromocriptine (5 mg kg^{-1}) also failed to give birth or enter oestrus during the subsequent 28 day period. Animal B6 was reinjected with bromocriptine 60 days after her initial injection and after her young had vacated the pouch and been replaced by a new young. She again failed to react to the bromocriptine. All five animals gave birth and/or came onto heat at the usual time coincident with the end of pouch life.

This singular lack of success posed the question: had the bromocriptine been prepared incorrectly, or was the bettong insensitive to it? A fresh batch of bromocriptine was obtained. It was administered to another kangaroo species, the Tasmanian pademelon (*Thylogale billardierii*, Macropodinae) and to five bettongs, only one of which had been previously injected with bromocriptine. Two of the bettongs showed some response. One (B381) mated 20 days after injection (while a young was present in the pouch), but there was no evidence of birth.

A second bettong (B26Y) may have given birth 20 days after injection as there was a 'path' leading from the cloaca to the pouch and along part of this path, orange crusty material adhered. However, no new young were found in the pouch and the mother did not come into oestrus. Both bettongs held their pouch young for the normal time but failed to give birth to a young at the time of pouch vacation. Due to a lack of controls it is not certain that these results are due to the bromocriptine injection or to some other factor.

7.3.5 Bromocriptine and the Tasmanian Pademelon

The general reproductive characteristics of this species are summarised in Table 7.4 from Rose and McCartney (1982a), and the results of bromocriptine injection (5 mg kg^{-1} , batch two) are summarised in Table 7.5. A positive result was obtained with six of the nine pademelons. Animals T8 and T9 had relatively large young at the time of injection and in both cases these young prematurely vacated the pouch on the night that the new young were born. These young left the pouch 10-12 days earlier than normal and their body weight was 20-30% below expected values.

TABLE 7.4 Reproduction in *Thylogale*

	N	Mean \pm S.D. (Days)
Oestrous cycle length	12	30.4 ± 0.7
Gestation length	6	30.3 ± 5
RPY - birth	6	28.7 ± 1.2
RPY - oestrus	6	29.2 ± 1.4
Pouch life	6	202 ± 7
Weight at vacation (g)	6	$1029 \pm 112 \text{ (g)}$

TABLE 7.5 Injection of bromocriptine (5 mg kg^{-1}) into *Thylogale*

Animal No.	Pouch Young Weight (g)	Results
T1	?	Small young (approx. 3 weeks old at injection) NIL
T2	48	NIL
T3	150	Oestrus 26 days after injection
T4	255	Oestrus 30 days after injection, no young found
T5	285	Pouch young lost 14 days after injection. Birth and oestrus 28 days after injection
T6	300	NIL
T7	425	Oestrus 27 days after injection
T8	507	Birth and oestrus 26 days after injection. Pouch vacated same night, age 188 days, weight 810 g
T9	580	Birth and oestrus 31 days after injection. Pouch young vacated same night, age 190 days, weight 725 g

7.3.6 Foster Experiments

The failure of the experiments reported in Section 7.3.4 made it necessary to develop an alternative technique to test the hypothesis that the mother is primarily responsible for pouch vacation by the young. Table 7.6 summarises the results from these foster experiments. The mothers selected for use in Group A had large furred young due to vacate the pouch within one or two weeks. Those selected for use in Group B had smaller young approximately three weeks from final pouch vacation and (very likely) a quiescent, or only recently activated, blastocyst. The young from Group A were

transferred to the pouches of the mothers in Group B, and *vice versa*.

All young survived the transfer and were reared by their foster mothers. Mothers would retrieve their foster young from the nest within a day of the transfer and allow them to return to the pouch.

Foster young grew at rates that differed from their growth rates prior to the swap (Table 7.7 and Figure 7.1). Smaller young grew at an increased rate when fostered into the pouches of mothers that had previously had larger young. The reverse occurred when large young were placed into pouches occupied previously by smaller young. Foster young Ba grew particularly slowly and this accounts for its relatively low weight (285 g) at age 124 days when the pouch was finally vacated.

On the night of birth and oestrus all foster young were excluded from the pouch by the characteristic contraction of the pouch.

The three pouch young in Group A left the pouch prematurely at ages between 91 and 95 days (compared with the mean of 106 days established by the control group). Young in Group B stayed in the pouch for between 116 and 124 days (considerably longer than the controls). Although the data base is small an analysis of variance showed that there were significant differences in weight and age at pouch vacation.

The foster young did not appear to affect the timing of parturition or oestrus. The foster mothers gave birth and/or entered oestrus on average 105.8 ± 2.5 days after the birth of their original pouch young. This was not significantly different from the control group (106.8 ± 2.1 days, $t = 0.9$, $P < 0.1$). On the basis of these results it appears that the foster young did not affect the development of the embryo or the onset of oestrus.

TABLE 7.6 Results of the foster experiments. Age and weight of young at pouch vacation

		Young			Mother
		Age of Foster Young at Swap (days)	Age at vacation (days)	Weight at vacation (g)	Oestrus to birth/or oestrus (days)
Group A Small for large	a	77	91	213	110
	b	84	95	215	107
	c	85	93	226	104
Group B Large for small	a	98	124	285	103
	b	98	117	395	105
	c	96	116	453	106
Control (N=16)		-	106.3	345.0	106.8

TABLE 7.7 Growth rates (K^*) after fostering ($K = \log g/\text{day}$)

		K before exchange	K after swap
Group A large young into smaller pouch	a	5.0×10^{-2}	0.6×10^{-2}
	b	4.88×10^{-2}	3.0×10^{-2}
	c	4.78×10^{-2}	3.32×10^{-2}
Group B small young into larger pouch	a	4.16×10^{-2}	5.89×10^{-2}
	b	4.17×10^{-2}	6.85×10^{-2}
	c	4.86×10^{-2}	6.22×10^{-2}

*Growth rate (K) calculated as in Chapter 3

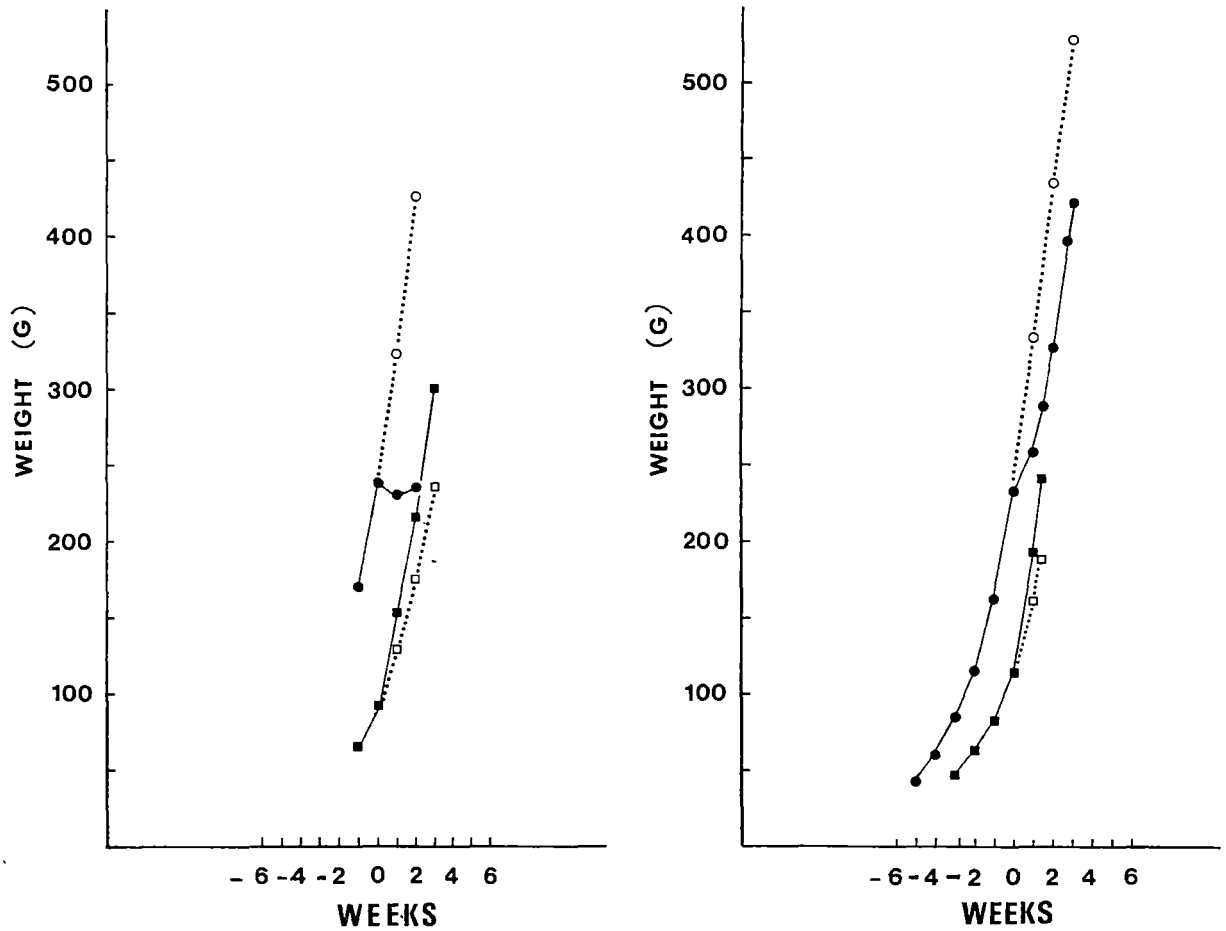


Figure 7.1 Growth before and after two pouch transfers (at week 0). Dotted lines and open symbols indicate the expected growth had pouch transfer not taken place.
 ● = larger young, ■ = smaller young at transfer.

7.4 DISCUSSION

7.4.1 Pouch Vacation

This series of experiments has shown ~~an~~ extensively that changes in maternal physiology affect the time when young leave the pouch.

The final vacation of the pouch by the young bettong is in almost all cases directly associated with a contraction of the pouch, followed by birth and/or oestrus. In those few cases when pouch vacation is not associated with these phenomena (e.g. after experimental manipulation or the mother becoming anoestrus) the young stays in the pouch for longer than would be expected.

It should not be surprising that, in those species in which the end of pouch life is associated with the onset of reproductive cycles, there is a mechanism to prevent successive young from occupying the pouch simultaneously. Should this not occur, it would be likely to result in the loss of the newborn and hence lead to reproductive inefficiency.

The precise sequence of events that occurs at pouch vacation in the bettong has previously been observed definitely in only two marsupials, the red kangaroo (*Macropus rufus*, Sharman and Calaby 1964) and the Tasmanian pademelon (*Thylogale billardierii*, Rose and McCartney 1982a) though it may occur in some agile wallabies (*M. agilis*, Merchant 1976) and captive quokkas (*S. brachyurus*, Sharman 1955b). Sharman and Calaby (1964) found that newborn young were present in the pouch of *M. rufus* within 24 h of pouch vacation in seven out of eight animals. They also noted a dramatic tightening of the pouch musculature prior to birth and oestrus. They found that the pouch was not vacated in order to make room for another offspring since females carried their young for the same length of time whether or not they gave birth. A similar result was obtained with the bettong. However, there was more variability about the mean pouch life of those bettongs which did not give birth. In the cases where birth occurred before oestrus,

pouch vacation always occurred on the night of parturition.

Consequently, it appears that in the bettong the physiological events associated with parturition are a primary stimulus to pouch vacation but that the advent of oestrus is also involved. The greater variability observed in the mean pouch life of young whose mothers failed to give birth at pouch vacation (Table 7.1) may be due in part to the 'Merchant effect' (Merchant 1976, 1979, see Chapter 4), whereby the timing of reproductive events is influenced by the presence or absence of a fetus. There also was greater variability in the mean length of infertile oestrous cycles (Chapter 4).

The data available from the Tasmanian pademelon are not as substantial as those for the red kangaroo or the bettong, but in the pademelon, as with the other two species, pouch vacation is always associated with pouch contraction, birth and oestrus (Rose and McCartney 1982a,b). This association of reproductive events remained after the bromocriptine injections and subsequent premature pouch vacation.

The hypothesis that birth and/or oestrus influence pouch vacation implies that experimentally induced birth or oestrus will cause pouch vacation even at times inappropriate to the young. The pademelon data on premature pouch vacation to some extent provide this validation. The premature vacation only occurred with older furred young during their interim pouch life; hence it appears that the mechanism of pouch contraction functions to prevent re-entry to the pouch rather than to exert a 'squeezing' effect on the young in the pouch. The red kangaroo (which shows a similar sequence of events to the bettong at pouch vacation) can enter oestrus during pouch occupancy in the wild (Newsome 1964). Newsome found that those red kangaroos that were shot 'immediately after oestrus' had only quite young pouch occupants (mean age = 35.6 days, range 22-80) whereas those approaching oestrus had some

young that were relatively large (\bar{X} = 72 days, range 18-182). Red kangaroos that underwent oestrus while carrying large pouch young would most probably demonstrate pouch contraction and loss (or prevention of re-entry) of the young. This may account for the fact that only small young were present in the pouches of kangaroos that were obtained shortly after oestrus. Newsome (1964) describes an interesting sequence of events in which a red kangaroo young (170 days old) was lost from the pouch and replaced by a new young between 1-32 days later. As this kangaroo had not mated after the birth of the first young it had no quiescent blastocyst. It seems quite possible that this animal came onto heat on the night that the large young was 'lost', and that oestrus may have resulted in pouch contraction and prevention of re-entry by that young.

7.4.2 Progesterone and Oestrogen

The fact that none of the three bettongs gave birth after the progesterone injections suggests that although the treatment may have resulted in activation of the quiescent blastocysts it was insufficient to allow ^{parturition} . Berger and Sharman (1968) came to similar conclusions in a series of experiments with the tammar wallaby. Alternatively it may be that none of the mothers had blastocysts, but this seems less likely as all three had a post-partum mating.

After a transient contraction observed in some pouches the young of females injected with oestrogen remained in their pouches for longer than normal. Associated with this phenomenon was a delay in the onset of maternal oestrus and a failure of parturition in a number of the females.

Low levels of oestrogen have been shown to inhibit the occurrence of oestrus in the tammar wallaby, *M. eugenii* (Evans *et al.* 1980;

Renfree, Wallace and Young 1982) and the hormone appears to have a similar effect in the bettong. Csapo (1972) has suggested that oestrogen may act upon the levels of calcium ions with resultant changes in either the thresholds or the excitability of uterine and striated muscle. Perhaps natural (or experimentally induced) levels of oestrogen at the time of birth and subsequent oestrus are associated with changes in the pouch musculature that result in sphincter contraction. Sex hormones (in particular testosterone) are known to affect both fibre growth and size (Cihak *et al.* 1970; Dubowitz and Brook 1973) and the firing rate of motor neurons supplying striated muscle (Erulkar *et al.* 1981). Also, the experimental injections may have caused uterine contractions which resulted in elimination of the uterine blastocysts.

There are few published reports on the plasma levels of oestrogen in macropodids near birth or oestrus ^{Flint and Renfree 1982;} (Walker and Gemmell 1983). It seems highly likely that oestrogen levels will be elevated when associated with oestrus (Shaw pers. comm.). One consequence of this elevation may be pouch contraction at the end of pouch life (at least in kangaroos in which pouch vacation is associated with oestrus).

7.4.3 Bromocriptine (CB154)

The anterior pituitary hormone, prolactin, has been shown to inhibit the corpus luteum during embryonic diapause in the tammar wallaby (Tyndale-Biscoe and Hawkins 1977). Prolactin is most probably released in response to sucking by the pouch young, as denervation of the mammary gland during lactation results in activation of the corpus luteum and the quiescent blastocyst (Renfree 1979). However recently Tyndale-Biscoe and Hinds (1984) have shown that at certain times in the year bromocriptine did not depress prolactin levels but did induce development of the corpus luteum and diapausing embryo was

reactivated.

Macropodid marsupials exhibit two forms of embryonic diapause: facultative diapause, associated with sucking, and obligate diapause, associated with seasonal factors (Tyndale-Biscoe *et al.* 1974).

Both types of diapause can be seen in the tammar wallaby *M. eugenii*, and it has been shown that administration of bromocriptine during facultative diapause can result in activation of both the quiescent corpus luteum and the blastocyst (Tyndale-Biscoe and Hinds 1981, 1984). However, Tyndale-Biscoe and Hinds failed to obtain activation of the corpus luteum or embryo during obligate diapause.

During obligate quiescence in the tammar the pouch young continues to suck for some time, but when it leaves the pouch it is not replaced immediately by a new young. The quokka (*S. brachyurus*) exhibits facultative diapause during lactation but, unlike the tammar, does not have a seasonal embryonic diapause. Outside of the breeding seasons quokkas do not retain a blastocyst but enter seasonal anoestrus (Sharman 1955b). Wallace and Renfree (1979) have shown that bromocriptine will cause activation of the corpus luteum and blastocyst during the breeding season in the quokka; however, during seasonal anoestrus, bromocriptine was unable to initiate reproductive cycles.

The present study is the first to demonstrate an effect of bromocriptine near to the time of natural pouch emergence (in *T. billardieri*). It is also the first to be unable to elicit a clear positive response to bromocriptine by a macropodid marsupial (*B. gaimardi*), albeit from a different sub-family (Potoroinae).

The positive response exhibited by the pademelon suggests that the bromocriptine was prepared correctly. The lack of response by the bettong (even though CB154 was administered during most months of the year and periods of pouch life) is difficult to explain. Perhaps the hormonal levels of prolactin during facultative diapause in the bettong

are similar to those found during obligate diapause in the tammar. However, Tyndale-Biscoe and Hinds (1981, 1984) have suggested that the failure of tammars to respond to bromocriptine (during December, when prolactin levels were low) indicates that another factor unaffected by bromocriptine may be involved. The pineal gland is involved in the control of embryonic diapause (during seasonal quiescence, Renfree *et al.* 1981) and may be involved with the failure of bromocriptine in the tammar during December and the bettong throughout the year.

7.4.4 Foster Experiments

The results from experiments that involved the fostering of bettong young are interesting for a number of reasons. Merchant and Sharman (1966) showed that fostering could be accomplished between larger kangaroos. These experiments have shown that it can also be accomplished with much smaller members of the family, and that the appropriate maternal responses develop rapidly.

The growth rate of the foster young changed after the exchange. This may have been because the composition of the milk differed from that of their previous diet (i.e. it may have been richer, or poorer), or because the amount of time spent sucking changed. Possibly both factors were involved.

Messer and Green (1979) and Green *et al.* (1981) showed that in the tammar wallaby the composition of milk changed substantially throughout lactation and that peaks in carbohydrate and protein levels coincide with the time when the young commenced to leave the pouch and the final pouch vacation. Changes in milk composition may be associated with the young beginning to move outside the mother's pouch

(either as a stimulus or a consequence). But these changes are insufficiently discrete to make them a likely factor in the precision involved in final pouch vacation in the bettong.

The fact that every one of the six foster young left the pouch on the night of parturition and/or oestrus is strong support for the hypothesis that the final vacation of the pouch is temporally related and directly controlled by both birth and oestrus. All transfer of young took place towards the end of pouch life. In Group A (Table 7.6) the diapausing blastocyst had almost certainly been activated and in Group B it probably had been so at the time of the swap due to the decreased time spent sucking by the large young. It would be interesting to test the hypothesis further by exchanging much smaller and younger young in whose mothers the blastocyst would presumably remain in diapause for some time after the transfer. As the initial development of the blastocyst is stimulated by decreased sucking towards the end of pouch life (Sharman 1965) it seems likely that the smaller pouch young would remain in the pouch for the normal time.

7.4.5 Pouch Contraction

The contraction of the pouch on the night the young finally vacates the pouch appears to be an event of great importance. Obviously it prevents the older young returning to the pouch. The stimulus for the contraction is unknown. If the mother contracts the pouch voluntarily it seems odd that she should do so regardless of whether or not a new young is born. It is likely that the bettong is capable of some voluntary control of pouch musculature since stimulation of the anterior thoracic nerves in the opossum results in the apposition of the lips of the pouch (Langworthy 1932), and similar results have been obtained for the bettong (Rose, unpublished data).

Sharman and Calaby (1964) have suggested that there is hormonal control of pouch contraction in the red kangaroo at final pouch vacation and they have postulated a role for oestrogen as pouch contraction occurred at oestrus. This study has come to similar conclusions in regard to oestrogen (Section 7.4.2). However, the pouch musculature would be unusual if it has both motor and hormonal pathways involved in the control of contractions. Although steroids can result in hypertrophy of certain muscles, hence the larger muscle mass of many human males, there are no reports of the presence of smooth muscle in the pouch. Liggins (1982) discusses the importance of the effect of prostaglandins on the collagen fibres of the cervical connective tissue during the relaxation of the cervix prior to parturition. Perhaps an opposite action occurs in the bettong pouch, i.e. a retraction of connective tissues near parturition or oestrus. Histological studies of the pouch would be a worthwhile study in this regard.

Bolliger and Canny (1941) attempted a number of experiments to investigate the effect of sex hormones on the pouch of the brushtail possum *Trichosurus vulpecula*. They were able to show that injections of various forms of oestrogens and gonadotrophins could result in pouch contraction. Although the doses of hormone used by Bolliger were well above the levels regarded as physiological, e.g. he used oestrogen at milligram levels, the effects of sex hormone on pouch contraction in the bettong, and perhaps other marsupials, cannot be discounted and should be investigated further.

In the published literature pouch vacation in marsupials is rarely associated with contraction of the pouch, birth or oestrus. In some species oestrus can occur during pouch occupancy, e.g. the grey kangaroo *M. giganteus* (Clark and Poole 1967) and the bandicoot (Stodart 1977). In the grey kangaroo it is interesting to note that

pouch vacation is associated with relaxation of the pouch rather than with contraction. This has the effect of causing the young to tumble out of the pouch.

Parker (1977) and Low (1980) have stated that some marsupial mothers are able to 'eject' their pouch young during periods of environmental stress or predation. Russell (1982), in a critique of these two papers, states that desertion of young in such situations may be beneficial for only relatively few extremely specialised species (e.g. the red kangaroo). However, Ealey (1963) reports that the euro *M. robustus* ejects its young in times of stress. Furthermore, during the present study the bettong often ejected its young in the course of pursuit or after being bagged.

The causes of pouch vacation are numerous and may differ in each marsupial species. For the purposes of the following discussion a number of these 'causes' have been drawn together under the five major headings of behaviour, morphology, physiology, and genetics and environment (Figure 7.2). There is evidence for some of these 'causes'; others are speculative and require further investigation.

7.4.6 Causes of Pouch Vacation

MORPHOLOGY

There must be a limit to the size to which the pouch can expand to accommodate single or multiple young. The larger the young the more locomotion of the mother is inhibited and the greater the risk of predation. However, Reynolds (1952) has observed in the opossum that young of small litters leave the pouch at the same age as the young of large litters, and not later as might be expected if lack of space were the primary stimulus (Tyndale-Biscoe 1973). The size of young is less likely to be a pouch occupancy problem in the macropodids than it is in marsupial families which have larger litters (Russell 1982).

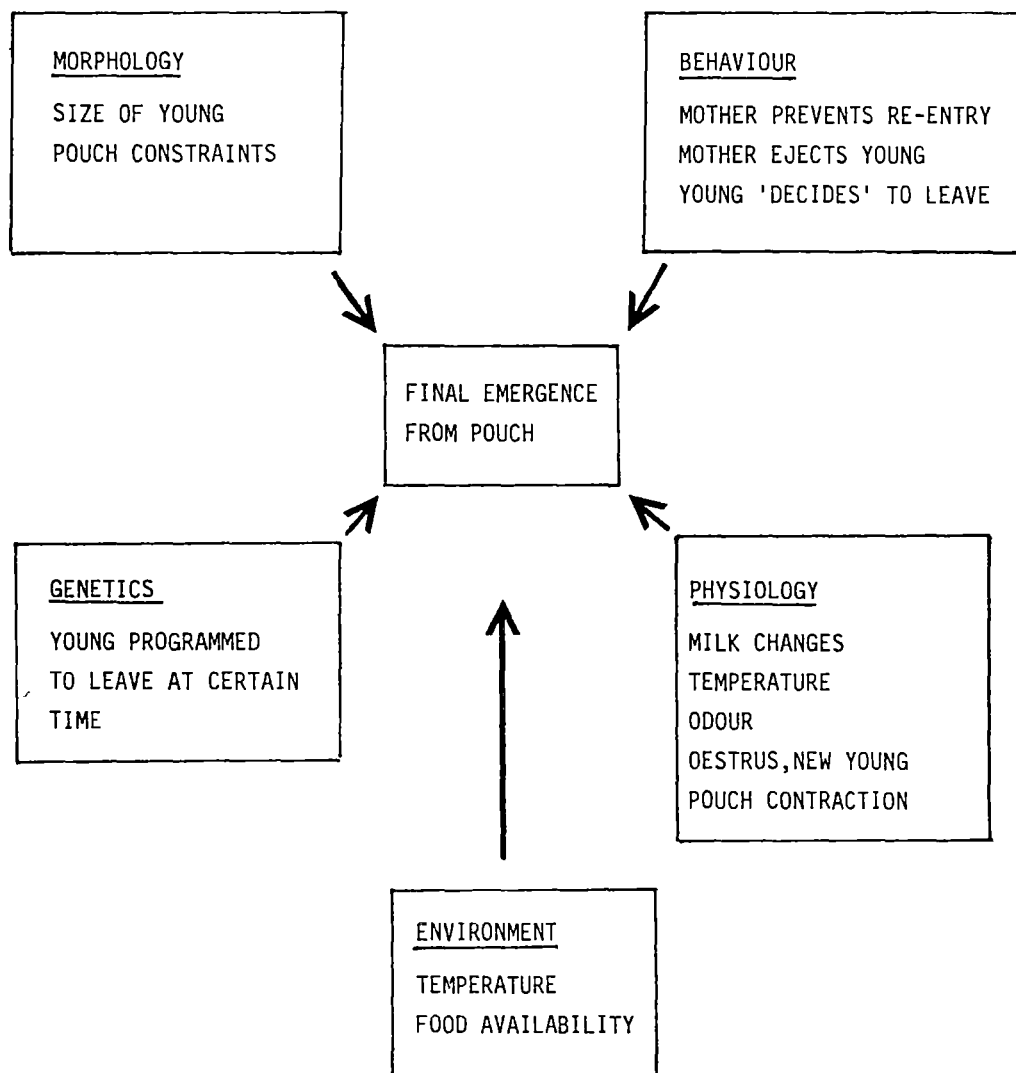


Figure 7.2 Some 'causes' of pouch emergence by marsupial young.

However, the longer pouch life, coupled with the increased size of the mammary gland, does result in a shortage of space in the pouch near the time of final vacation. In the bettong, as with some other kangaroo species, the feet or tail may constantly protrude from the pouch near the end of pouch life.

BEHAVIOUR

In some species the young may be prevented from returning to the pouch by the mother actively discouraging them or simply failing to keep still, e.g. the reg kangaroo (Sharman and Calaby 1964).

In the present study, the bettong mother remained still whilst the young attempted to open the tightly closed pouch (Plate 7.1a) but moved away before the young had desisted from its endeavours.

The contraction of the pouch may be used to eject or prevent re-entry in some species, although it is uncertain whether voluntary action by the mother is involved. In some species, young show little interest in the pouch after final vacation, e.g. *M. agilis* (Merchant 1976).

PHYSIOLOGY

This chapter has already considered some physiological factors, e.g. pouch contractions, associated with the hormonal changes of parturition or oestrus. There are changes in milk constituents near pouch vacation (Green *et al.* 1981) and these may well result in the young leaving the pouch to obtain other forms of nutrition.

The approach of oestrus in some marsupials is associated with changes in the lining of the pouch and its odour (Sharman and Calaby 1964) this may be distasteful to the young. The effect of the body

temperature (of the young and the mother) and the ambient temperature may contribute to the young leaving the pouch, as suggested by Tyndale-Biscoe (1973). Both Reynolds (1952) and Shields (1966) have shown that the young of the opossum and quokka, respectively, have a preferred body temperature about one degree below that of the temperature in the pouch. Presumably, as the ambient temperature warms during spring in temperate climates and the pouch young's regulatory functions mature, the outside environment becomes more attractive. This would also be related to the development of hair, insulation and locomotory powers.

ENVIRONMENT

For a well-furred pouch young, living in the pouch must be like wearing two fur coats, but there is little evidence (apart from that mentioned above) to suggest that the young leave the pouch because of excessive heat.

Other environmental factors, such as the ready availability of food outside the pouch, may be important. Near the time of pouch vacation, the digestive system of the young red kangaroo undergoes changes that allow it to digest cellulose (Griffiths and Barton 1966). These are associated with dental development. The bettong also develops its first molar tooth shortly after pouch vacation (Chapter 3).

GENETICS

Work by Merchant and Sharman (1966) has shown that the pouch life of some kangaroo young has a fixed duration, even when young are fostered out to mothers of other species whose young normally have a quite different pouch life duration. It seems likely that some

young are programmed to leave the pouch at a certain age or size. In the bettong though, this cannot be a primary cause of pouch vacation.

In summary: the female bettong mother demonstrates a tightening of the pouch musculature on the night of final pouch vacation. This may be a result of the elevated levels of oestrogen (or prostaglandins) associated with parturition or oestrus. Behavioural changes are unlikely to be important as, after final vacation, the young still attempts to return to the pouch while the mother remains still.

The young's developing ability to regulate its temperature may play some role in pouch vacation; so too, there may be changes in the body temperature of the mother near oestrus (Chapter 6). The variations in pouch life achieved by the experimental fostering of young of different ages seems to preclude the possibility that genetic factors exert a precise control over pouch life in the bettong.

The proximal stimuli for pouch vacation are maternal (birth and/or oestrus), but it is the pouch young that ultimately determines when these events occur. It is the reduction in their sucking near the end of pouch life that allows reproductive cycles to be re-established.

Further work is required on this topic, and it should involve more species. A good starting point would be detailed morphological study of the pouch throughout and beyond pouch life. The stimuli that cause changes in the pouch as the young grows, and finally leaves the pouch, may provide further answers to the question, "Why does the young leave the pouch?".

CHAPTER 8

CONCLUSIONS

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8.1 CONSERVATION

There has been no recorded sighting of the Tasmanian bettong, *Bettongia gaimardi*, on the Australian mainland during the past 60 years (Poole 1979). The species still exists in reasonable security on the island of Tasmania, though its conservation status would have to be described as 'vulnerable'.

Fifty per cent of its habitat is in areas under private ownership and susceptible to forestry and agricultural development; 45 per cent is in areas owned and controlled by the Crown (though also susceptible to forestry and other development); and only five per cent lies within lands designated National Parks (Rose 1983).

The bettong is a wholly protected species. Nevertheless it is prey to feral cats, and the introduction of other eutherian predators (such as the fox) could endanger it.

The information produced by this detailed study of the species' reproductive biology should be valuable to those authorities charged with responsibility for the conservation of native fauna. However, further work is required to produce detailed information about the ecology of the bettong's habitat and the effects of environmental disturbance.

8.2 AGE ESTIMATION AND GROWTH

Monitoring the age structure and dynamics of a vulnerable animal population is only possible when reliable methods of estimating age have

been developed. The work outlined in Chapter 3 of this thesis provides an accurate method of estimating the age of bettongs from birth to 3-plus years. No detailed studies have been undertaken on the degree of enamel wear as an indicator of age. However, animals with extreme enamel wear are likely to be aged four to five years and those with a fifth molar are likely to be aged 5-plus years. Bettongs in captivity (and, presumably, those in the wild) seldom live beyond five or six years of age.

Estimations of the age of pouch young in the field provide information about the timing and duration of the breeding season. When all the data gathered in this study were pooled it appeared that the bettong had no distinct breeding season (though there was a tendency for more young to be born in the cooler months and to vacate the pouch in spring or early summer).

Continuous breeding is unusual in marsupials (Tyndale-Biscoe 1973), particularly in the temperate regions of south-eastern Australia. This aspect of the reproductive biology of the bettong could profitably be studied in more detail.

The reliability of the age estimation method mentioned above has not been tested with animals living in the wild. Larger populations probably mean less accuracy, since the confidence limits about the mean values of the various measurements are greater. However, measurements with an accuracy greater than plus or minus one week in pouch young, and plus or minus a few months in adults are rarely required. Therefore the method ought to be capable of useful application in studies of animals in the wild as well as in captivity.

The finding that simple interpolation in the growth curves gave better estimates of age than substitution of values into mathematically fitted regression equations is not new. Shield and Woolley reached

similar conclusions in 1961. Work by Poole *et al.* (1982a,b) and others has demonstrated that a high degree of accuracy can be achieved using fitted equations, but the computations involved are not simple enough to have encouraged widespread use of the technique.

The finding that the growth rate of the bettong during pouch life (k) is much higher than that exhibited in other macropodids is interesting. It suggests that further studies should be carried out on lactation in this species (and other Potoroinae) to establish the constituency of the milk. The faster growth rate allows the production and weaning of more than one young per year, an uncommon event in larger macropodids.

8.3 REPRODUCTION

The reproductive biology of the Tasmanian bettong conforms to the pattern found in most macropodid marsupials. Gestation and the oestrous cycle are of a similar duration, and a post-partum oestrus may result in the formation of a blastocyst that remains quiescent (embryonic diapause) throughout most of the pouch life.

The actual parameters of reproduction are strikingly similar to those found in two other members of the genus, *B. lesueur* and *B. penicillata*. This suggests that selection has acted in an even-handed manner and that all three species have evolved in somewhat similar environments. It could also indicate that the members of this genus have speciated relatively recently. Studies based on protein analysis could further test this idea.

The short oestrous cycle and gestation of the bettong (compared with those of other macropodids) belies the suggestion of Flynn (1930) that *B. cuniculus* (= *gaimardi*) had an oestrous cycle of six weeks. The duration of pouch life (fifteen weeks) was also found to be similar to

other members of the genus *Bettongia* and conflicted with Flynn's even more surprising claim of a six-week pouch life.

It was interesting to note that the 'Merchant effect' (Merchant 1976, 1979) occurs in the bettong. This is the first evidence of its occurrence in the Potoroinae. The biological significance of this phenomenon (which resulted in a difference of 1.5 days in the length of the oestrous cycle) is not clear. It may promote the synchrony of birth and post-partum oestrus (in the absence of such synchrony the sucking of the new young could inhibit further reproductive cycles and the formation of a blastocyst).

In relation to the 'Merchant effect', it is particularly interesting to note that an embryo/maternal interaction is capable of influencing the timing of reproductive events in the mother. This interaction does not merely affect the duration of the oestrous cycle, it is also involved in control of the duration of pouch life.

8.4 REPRODUCTIVE TRACT HISTOLOGY AND EMBRYONIC GROWTH

The relatively early initiation of the luteal phase after RPY in the bettong is reminiscent of the same phenomenon in the potoroo (it would allow early activation and development of the blastocyst in both species). However, the 18-day delayed gestation period of the bettong is considerably shorter than the 29-day period of the potoroo (Shaw and Rose 1979). This difference can now be attributed to the more rapid growth of the bettong embryo, not to variations in the initiation of embryo development proposed by Tyndale-Biscoe (1973) and Shaw and Rose (1979).

The local effects upon the histology of the uterine glands associated with the presence of the corpus luteum early in the cycle,

and the feto-placental unit later in gestation are similar to those found in the potoroo by Shaw and Rose (1979), and the tammar wallaby by Renfree and Tyndale-Biscoe (1973). Further study is needed (including the measurement of the number of receptor sites in each of the paired uteri) to establish whether or not there is a direct causal relationship between these localised phenomena and histological appearance in the bettong and the other species.

8.5 BODY TEMPERATURE

It is not surprising to find that the Tasmanian bettong displays a circadian rhythm in body temperature. It has been demonstrated in other marsupials, though not with such substantial data. The degree of daily fluctuations in body temperature varied according to the phases of the reproductive cycle. This was a consequence of a relatively stable T_{\max} but a more labile BBT. Attention has been drawn to the practice of assigning a single temperature as the body temperature of a marsupial in spite of the fact that body temperature fluctuates over a 24-hour period. If single body temperatures are to be used for comparison then they should be either basal temperatures, or temperatures taken during the same time period. If female animals are used in temperature studies, their reproductive status must be taken into account.

Mammalian reproductive cycles are usually monitored by the standard techniques of vaginal smears and histological changes in the reproductive tract detailed in Chapters 4 and 5. The simple alternative technique of measuring body temperature has rarely been applied to non-domesticated animals. This thesis has demonstrated that statistically significant changes in basal body temperature occur around the time of

oestrus and the transient peak in progesterone that follows oestrus in some other macropodids (including the bettong, Appendix C). The changes in BBT may be a true reflection of hormonal variations, and further work on this topic is warranted.

Apart from its usefulness as a method of monitoring reproduction in fragile or nervous animals in captivity, biotelemetric measurement of temperature could also be usefully applied to wild populations (e.g., to provide an indication of the onset of the breeding season). Studies of the ecological physiology of marsupial species in the wild are sparse in number.

The computer analysis of temperature fluctuations undertaken in this thesis could also be adapted to take account of long-term environmental and seasonal changes occurring in the wild.

Changes in body temperature related to the presence of pouch young may well be involved in the mechanism of pouch vacation, as suggested by earlier workers (Reynolds 1952; Shield 1966) and further work is contemplated.

8.6 POUCH VACATION

The causes of pouch vacation in the bettong were examined in detail in Chapter 7. The conclusion that physiological events in the mother are directly responsible for final pouch vacation is relatively novel. The results of most previous studies (though few in number) have suggested that the young itself determined the duration of pouch life.

During the last few days of pouch life the young bettong spends most of its time outside the pouch. In captivity at least, it usually returns to the pouch only when startled by sudden movements or loud noises. The contraction of the pouch sphincter muscle on the day of

parturition and/or oestrus prevents re-entry. The young must then fend for itself, though it is still able to suck from its mother's teat which protrudes through the tight entrance to the pouch.

That some macropodids should have evolved such a precise sequence of events ensuring separation of successive pouch occupants is not really surprising; it is essential for the survival of the new young. The causes of pouch vacation in those marsupials which do not display this synchrony of reproductive events may be less obvious, but nonetheless worthy of further study.

The exact proximate cause of final pouch vacation in the bettong has not been determined. As speculated upon in Chapter 7, higher levels of oestrogen (presumably) associated with parturition and oestrus may be involved in changing the thresholds of the motor nerves supplying the pouch musculature or the configuration of pouch connective tissue. The control of the varying size and muscle tone of the marsupial pouch merits further study, particularly from a neurophysiological and an endocrinological point of view.

In some cases, the pouch young vacated the pouch at oestrus without there being an association with birth. Why this should occur is still unclear; perhaps it represents a failsafe mechanism. There is little point in pouch life being extended once the young has obtained a certain size and weight. The large young must find the pouch an increasingly uncomfortable environment (both cramped and hot). Nevertheless, in those instances where the mother failed to give birth or enter oestrus (either as a result of experimental procedures or natural anoestrus), the pouch life was extended! It appears that the duration of pouch life is, in effect, a compromise between the wellbeing of the existing pouch young and the mother's capacity to maximise her reproductive output during her life span.

REFERENCES

- Abrams, R., D. Caton, J. Clapp and D. Barron (1971). Temperature differences in the reproductive tract of the non-pregnant ewe. *Am. J. Obstet Gynec.* 110: 370-375.
- Archer, M. (1976). The dasyurid dentition and its relationship to that of Didelphids, Borhyaenids (Marsupicarnivora) and Peramelids (Peramelina: Marsupialia). *Aust. J. Zool. Supp. Ser.* 39: 1-34.
- Archer, M. (1981). A review of the origins and radiations of Australian mammals. In *Ecological Biogeography of Australia*. Ed A. Keast, pp 1435-1489. Junk, The Hague.
- Benoit, H.J., R. Borth, A. Ellicot, and C. Woolever (1976). Perioovulatory changes in ovarian temperature in ewes. *Am. J. Obstet. Gynecol.* 124: 356-360.
- Berger, P.J. (1966). Eleven-month "embryonic diapause" in a marsupial. *Nature* 211: 435-436.
- Berger, P.J. and G.B. Sharman. (1969). Progesterone-induced development of dormant blastocysts in the tammar wallaby *Macropus eugenii*. *J. Reprod. Fert.* 20 201-210.
- Bertalanffy L. von. (1960) Principles and theory of growth. In *Fundamental Aspects of Normal and Malignant Growth*. Ed W.W. Nowinski, pp 137-259. Elsevier Pub. Co., Amsterdam
- Bick, Y.A.E. and J.K. Brown. (1969). Variations in radio-sensitivity during the cell cycle in a marsupial cell line. *Mutation Res.* 8: 613-622.
- Bligh, J. and A.M. Harthoorn. (1965). Continuous radiotelemetric records of the deep body temperature of some unrestrained African mammals under near-natural conditions. *J. Physiol.* 176: 145-162.
- Bliss, C.I. (1970). *Statistics in Biology (Vol. Two)*. McGraw Hill, New York.
- Bolliger, A. and A.J. Canny. (1941). The effect of the synthetic and oestrogens, stilboestrol and hexoestrol on the pouch and scrotum of *Trichosurus vulpecula*. *J. and Proc. R. Soc. N.S.W.* 75: 21-26.
- Bradshaw, S., I.R. McDonald, R. Hahnel and H. Heller. (1975). Synthesis of progesterone by the placenta of a marsupial. *J. Endocrin.* 65: 451-452.
- Brody, S. (1945). 'Bioenergetics and Growth'. Hefner Co., New York.
- Brown, G.D. and T.J. Dawson. Seasonal variations in the body temperatures of unrestrained kangaroos. (Macropodidae: Marsupialia). *Comp. Biochem. Physiol.* 56A: 59-67.
- Buxton, C.L. and W.B. Atkinson. (1948). Hormonal factors involved in the regulation of basal body temperature during the menstrual cycle. *J. Clin. Endocr.* 8: 544-549.
- Buxton, C.L. and E.T. Engle. (1950). Time of ovulation: a correlation between basal temperatures, the appearance of the endometrium and the appearance of the ovary. *Amer. J. Obstet. Gynec.* 60: 539.

- Cake, M.H., F. Owens and S. Bradshaw. (1980). Difference in concentration of progesterone in plasma between pregnant and non pregnant quokkas (Setonix brachyurus). J. Endocr. 84: 153-158.
- Calaby, J.H. and W.E. Poole. (1970). Keeping kangaroos in captivity. Int. Zoo. Yb. 11: 5-12.
- Caughley, G. and R.I. Kean. (1964). Sex ratios in marsupial pouch young. Nature, Lond. 204: 491.
- Christensen, P. (1980). The biology of Bettongia penicillata Gray, 1837 and Macropus eugenii Desmarest, 1834 in relation to fire. Forest Department of Western Australia Bulletin 91.
- Christie, D.W. and E.T. Bell. (1971). Changes in rectal temperature during the normal oestrous cycle in the beagle bitch. Br. Vet. J. 127: 93-8.
- Cihak, R., E. Gutman and V. Hanzliikova. (1970). Involution and hormone-induced persistence of the M.sphincter (levator) ani in female rats. J.Anat. 106:93-110
- Clark, M.J. (1966). The blastocyst of the red kangaroo (Megaleia rufa (Desm) during diapause. Aust. J. Zool. 14: 19-25.
- Clark, M.J. (1967). Pregnancy in the lactating pigmy possum Cercartetus concinnus. Aust. J. Zool. 15: 673-83.
- Clark, M.J. (1968). Termination of embryonic diapause in Megaleia rufa by injection of progesterone and oestrogens. J. Reprod. Fert. 15: 347-355.
- Clark, M.J. and W.E. Poole (1967). The reproductive system and embryonic diapause in the female grey kangaroo, Macropus giganteus. Aust. J. Zool. 15: 441-59.
- Collins, L.R. (1973). Monotremes & Marsupials. Smithsonian Institution Press.
- Cook, P. (1973). Some observations on bettongs. Discovery 8: 93-96.
- Cook, P. (1975). Aspects of the Biology of Bettongia penicillata, a Rat Kangaroo with Plagiaulacoid Dentition. Ph.D Thesis, Yale Univ.
- Csapo, A.I. (1973). The regulatory interplay of progesterone and PGF₂ in the control of the pregnant uterus. In Uterine Contraction. Ed. J.B. Josimovich, pp 223-256. Wiley, New York.
- Cumming, I.A. and J.K. Findlay (1977). Evolution of ovarian function in sheep and cattle. In Reproduction and Evolution. Eds J.H. Calaby and C.H. Tyndale-Biscoe pp 225-234 Australian Academy of Science, Canberra.
- Darlington, P.J. (1965). Zoogeography: Biogeography of the Southern End of the World. Harvard Uni Press. Camb., Mass.
- Davis, M.E. and N.W. Fugo. (1948). A use of physiological basal temperature changes in women. J. Clin. Endocr. 8: 550.

- Dawson, T.J. and A.J. Hulbert. (1970). Standard metabolism, body temperature and surface area of Australian marsupials. *Am. J. Physiol.* 218: 1233-1238.
- De Bavay, J.M. (1950). Notes on the female urogenital system of *Tarsipes spenserae* (Marsupialia). *Pap. Proc. Roy. Soc. Tasmania.* 1950, 143-150.
- Desmarest. (1822). *Mammalogie ou description des especes de mammiferes.* Tabl. Encyc. Meth., Mamm. Suppl. ii p 542.
- Dickman, C.R. (1982). Some ecological aspects of seasonal breeding in *Antechinus* (Dasyuridae, Marsupialia). In *Carnivorous marsupials.* Vol. 1. Ed M. Archer, pp 139-150. Roy. Zoo. Soc. N.S.W.
- Dubowitz, V., and J. Brooke. (1973). *Muscle Biopsy: A Modern Approach.* Saunders, Philadelphia, PA.
- Dudzinski, M.L., A.E. Newsome, J.C. Merchant and B.L. Bolton. (1977). Comparing the two usual methods for ageing macropodidae on tooth-classes in the agile wallaby. *Aust. Wildl. Res.* 4: 219-222
- Ealey, E.H.M. (1963). The ecological significance of delayed implantation in a population of the hill kangaroo, (*Macropus robustus*). In *Delayed Implantation.* Ed A.C. Enders. Rice Univ. Semicentennial Press.
- Ealey, E.H.M. (1967). Ecology of the euro, *Macropus robustus*. (Gould) in north-western Australia. IV. Age & Growth. *CSIRO Wildl. Res.* 12: 67-80.
- Elmore, R.G., C.E. Martin, J.L. Riley and T. Littledike (1979). Body temperatures of farrowing swine. *J. Am. Vet. Med. Assoc.* 174: 620-2.
- Erulkar, S.D., D.B. Kelley, M.E. Jurman, F.P. Zelman, G.T. Schneider and N.R. Kreiger. (1981). Modulation of the neural control of the clasp reflex in male *Xenopus laevis* by androgens: A multidisciplinary study. *Proc. Natl. Acad. Sci. USA.* 78: 5876-5880.
- Evans, S., C.H. Tyndale-Biscoe and R. Sutherland. (1980). Control of gonadotrophin secretion in the female tammar wallaby (*Macropus eugenii*). *J. Endocr.* 86: 13-23.
- Fisher, J. (1969). *The Red data book. Wildlife in Danger.* I.U.C.N.
- Fleming, M.W., J.P. Harder and R. Wukie (1981). Reproductive energetics of the virginia opossum compared with some eutherians. *Comp. Biochem. Physiol.* 70B: 645-648.
- Flynn, T.T. (1922). Notes on certain reproductive phenomena in some Tasmanian marsupials. *Ann. Mag. N. Hist. Ser 9. X*, pp 225-231.
- Flynn, T.T. (1930). The uterine cycle of pregnancy and pseudo-pregnancy as it is in the diprotodont marsupial *Bettongia cuniculus*. *Proc. Linn. Soc. N.S.W.* 55: 506-531.
- Freeman, M.E., J.K. Crissman, Jr., G.N. Louw, R.L. Butcher and E.K. Inskeep (1970). Thermogenic action of progesterone in the rat. *Endocr.* 86: 717-720.

- Gaimard. (1824). Bull. Sci. Nat. i. p 271. (Quoted from Wakefield, 1967).
- Graham, C.E., H. Warner, J. Misener, D.C. Collins and J.R.K. Preedy. (1977). The association between basal body temperature, sexual swelling and urinary gonadal hormone levels in the menstrual cycle of the chimpanzee. J. Reprod. Fert. 50: 23-28.
- Gray, J.E. (1837). Mag. Nat. Hist. (Charlesworth) Vol. 1. p.584.
- Green, B., K. Newgrain and J. Merchant. (1980). Changes in milk composition during lactation in the tammar wallaby (Macropus eugenii). Aust. J. Biol. Sci. 33: 35-42.
- Greulich, W.W., E.E. Morris and M.I. Black. (1943). Problems of Human Fertility. Proceedings of the Conference Sponsored by the National Committee on Maternal Health. pp37-63. Menasha. Wis. George Banta Publishing Co.
- Griffiths, M.E. and A.A. Barton. (1966). The ontogeny of the stomach in the pouch young of the red kangaroo. CSIRO Wildl. Res. 11: 164-185.
- Guiler, E.R. (1960). The pouch young of the potoroo. J. Mammal. 41: 441-51.
- Guiler, E.R. and R.W.L. Heddle. (1974). Body temperatures in the Tasmanian devil, Sarcophilus harrisii (Marsupialia:Dasyuridae). Comp. Biochem. Physiol. 47A: 981-989.
- Hammel, H.T. (1968). Regulation of internal body temperature. Ann. Rev. Physiol. 30: 641-693.
- Harbert, G.M. Jr., G.W. Cornell and W.N. Thornton. (1970). Diurnal variation of spontaneous uterine activity in nonpregnant primates (Macaca mulatta). Sci. 170: 82-85.
- Harder, J.D. and M.W. Fleming. (1981). Estradiol and progesterone profiles indicate a lack of endocrine recognition of pregnancy in the opossum. Sci. 212: 1400-1402.
- Heap, R.B., M.B. Renfree and R.B. Burton. (1980). Steroid metabolism in the yolk sac placenta and endometrium of the tammar wallaby, Macropus eugenii. J. Endocr. 87: 339-349.
- Hill, J.P. and C.H. O'Donoghue. (1913). The reproductive cycle in the marsupial Dasyurus viverrinus. Quart. J. Microsc. Sci. 59: 133-174.
- Hinds, L.A. and C.H. Tyndale-Biscoe. (1982). Plasma progesterone levels in the pregnant and non-pregnant tammar, Macropus eugenii. J. Endocr. 93: 99-107.
- Hogarth, P.J. (1978). Biology of Reproduction. Blackie, Glasgow and London.
- Hope, J.H. (1973). Mammals of the Bass Strait Islands. Proc. Roy. Soc. Vict. 85: 163-196.
- Hudson, J.W. and T.J. Dawson. (1975). Role of sweating from the tail in the thermal balance of the rat-kangaroo, Potorous tridactylus. Aust. J. Zool. 23: 453-461.

- Hughes, H.E. and T.C. Dodds. (1968). Handbook of Diagnostic Cytology. E. and S. Livingston Ltd., Edinburgh.
- Hughes, R.L. (1962). Reproduction in the macropod marsupial Potorous tridactylus. Aust. J. Zool. 10: 193-224.
- Hughes, R.L. (1974). Morphological studies on implantation in marsupials. J. Reprod. Fert. 39: 173-186.
- Huxley, J.S. (1932). Problems of Relative Growth. Methuen, London.
- Israel, S.L. and O. Schneller. (1950). The thermogenic property of progesterone. Fert. Steril. 1: 53-64.
- Jackson, W. (1965). Vegetation. In Atlas of Tasmania. Mercury Walsh, Hobart.
- Johnson, C.N. and P.J. Jarman. (1983). Geographical variation in offspring sex-ratio in kangaroos. Search. 14: 152-154.
- Johnson, K.A. and R. Rose. (1983). Tasmanian bettong, Bettongia gaimardi. In Complete Book of Australian Mammals. Ed R. Strahan. Angus & Robertson, Syd.
- Johnson, P.M. (1978). Reproduction in the rufous rat-kangaroo (Aepyprymnus rufescens) (Gray) in captivity with age estimation of pouch young. Qld. J. Agric. Anim. Sci. 35: 69-72.
- Johnson, P.M. (1979). Reproduction in the plain rock wallaby Petrogale penicillata inornata in captivity with age estimation of the pouch young. Aust. Wildl. Res. 6: 1-4.
- Johnson, P.M. and R. Strahan. (1982). A further description of the musky rat-kangaroo, Hypsiprymnodon moschatus. Ramsay 1876, (Marsupialia, Potoroidae), with notes on its biology. Aust. Zool. 21: 27-46.
- Kaufmann, K.W. (1981). Fitting and using growth curves. Oecologia 49: 293-299.
- Keast, A. (1968). Australian mammals: zoogeography and evolution. Quart. Rev. Biol. 43: 373-408.
- Kerr, T. (1934). Notes on the development of the germ-layers in diprotodont marsupials. Quart. J. Microsc. Sci. 77: 305-315..
- Kerr, T. (1936). On the primitive streak and associated structures in the marsupial Bettongia cuniculus. Quart. J. Microsc. Sci. 78: 687-698
- Kihlstrom, J.E. and C. Lundberg. (1971). Cyclic variation of body temperature in female rabbits before and after ovariectomy. Acta. Physiol. Scand. 82: 272-276.
- King, D.R., A.J. Oliver and R.J. Mead. (1981). Bettongia and Fluoroacetate: a role for 1080 in fauna management. Aust. Wildl. Res. 8: 529-536.
- King, J.O. (1977). The effect of oestrus on milk production in cows. Vet. Rec. 101: 107-108.

- Kirkpatrick, T.H. (1965). Studies of Macropodidae in Queensland. 2. Age determination in the grey kangaroo, the red kangaroo, the eastern wallaroo and the red-necked wallaby, with notes on dental abnormalities. Qld. J. Agric. & Anim. Sci. 22: 301-17.
- Kirsch, J.A.W. and W.E. Poole. (1972). Taxonomy and distribution of the grey kangaroo Macropus giganteus (Shaw) and Macropus fuliginosus (Desmarest) and their subspecies. Aust.J.Zool. 20: 315-339
- Langworthy, O.R. (1932). The panniculus carnosus and pouch musculature of the opossum, a marsupial. J.Mammal. 13: 241-251.
- Leavitt, W.W., D.O. Taft, C.A. Strott and B.W. O'Malley. (1974). A specific progesterone receptor in the hamster uterus. Endocr. 94: 1041-1053.
- Lee, C.S. and J.D. O'Shea. (1977). Observations on the vasculature of the reproductive tract in some Australian marsupials. J. Morphol. 154: 95-114.
- Lemon, M. (1972). Peripheral plasma progesterone during pregnancy and the oestrous cycle in the tammar wallaby, Macropus eugenii. J. Endocr. 55: 63-71.
- Le Soeuf (1929). Notes on some mammals from Bass Strait islands, including a new subspecies of Pseudocheirus. Aust. Zool. 5: 329-332.
- Liggins, G.C. (1972). The fetus and birth. In Reproduction in Mammals. Vol. 2. Eds Austin, C.R. and R.V. Short. Camb. Univ. Press.
- Liggins, G.C. (1982). The fetus and birth. In Embryonic and fetal development: 2nd edition. Reproduction in mammals. Vol. 2. Eds Austin, C.R. and R.V. Short. Camb. Univ. Press.
- Lilliegraven, J. (1969). Review of the marsupial-placental dichotomy in mammalian evolution. Univ. Kansas Paleon. Contrib. Art 50. (Vertebrata 12) 1-122.
- Lillegraven, J.A. (1975). Biological considerations of the marsupial - placental dichotomy. Evolution 29: 707-722.
- Lilliegraven, J. (1979). Reproduction in mesozoic mammals. In Mesozoic Mammals: The First Two Thirds of Mammalian History. Eds Lilliegraven, J.A., Kielan-Jaworoska, Z., and Clemens, W.A., pp 259-279. Univ. California Press, Berkeley.
- Low, B.S. (1978). Environmental uncertainty and the parental strategies of marsupials and placentals. Amer. Nat. 112: 197-213.
- Lukschanderl, L and K. Lukschanderl. (1969). Einige beobachtungen zur jugendentwicklung von Bettongia cuniculus. Der Zoologische Garten. 37: 117-126.
- Lyne, A.G. (1959). The systematic and adaptive significance of the vibrissae in the marsupialia. Proc. Zool. Soc. Lond. 133: 79-133.
- Lyne, A.G. and D.E. Hollis. (1977). The early development of marsupials, with special reference to bandicoots. In Reproduction and Evolution. Eds

- Calaby, J.H. and C.H. Tyndale-Biscoe, pp 293-302 Australian Academy of Science. Canberra.
- Lyne, A.G. and A.M. Verhagen. (1957). Growth of the marsupial Trichosurus vulpecula and a comparison with some higher mammals. Growth 21: 167-195.
- Marrone, B.L., Gentry, R.T. and G.N. Wade. (1976). Gonadal hormones and body temperature in rats: effects of estrous cycles, castration and steroid replacement. Physiol. Behav. 17: 419-425.
- Martin, R.D. (1969). Evolution of reproduction in primates. J. Reprod. Fert. Suppl. 19: 203-220.
- May, E. (1982). Thermoregulation of the sugar glider Petaurus breviceps. Unpublished honours thesis. University of N.S.W.
- Maynes, G.M. (1972). Age estimation in the parma wallaby Macropus parma Waterhouse. Aust. J. Zool. 20: 107-18.
- Maynes, G.M. (1973). Reproduction in the parma wallaby. Macropus parma. Aust. J. Zool. 21: 331-51.
- Maynes, G.M. (1976). Growth of the parma wallaby Macropus parma. Aust. J. Zool. 24: 217-235.
- Merchant, J.C. (1976). Breeding biology of the agile wallaby Macropus agilis (Gould) in captivity. Aust. Wildl. Res. 3: 93-103.
- Merchant, J.C. (1979). The effect of pregnancy on the interval between one oestrus and the next in the tammar wallaby Macropus eugenii. J. Reprod. Fert. 56: 459-464.
- Merchant, J.C. and G.B. Sharman. (1966). Observations on the attachment of marsupial pouch-young to the teats and on the rearing of pouch young by foster-mothers of the same or different species. Aust. J. Zool. 14: 593-609.
- Merchant, J. and J. Calaby. (1981). Reproductive biology of the red-necked wallaby (Macropus rufogriseus banksianus) and bennetts wallaby (M.r. rufogriseus) in captivity. J. Zool. 194: 203-217.
- Messer, M. and B. Green. (1979). Milk carbohydrates of marsupials. II. Quantitative and qualitative changes in milk carbohydrates during lactation in the tammar wallaby (Macropus eugenii). Aust. J. Biol. Sci. 32: 519-531.
- Moghissi, K.S., Sydney, F.N. and Evans, T.N. (1972). A composite picture of the menstrual cycle. Amer. J. Obstet. Gynec. 114: 405.
- Moors, P.J. (1974). The foeto-maternal relationship and its significance in marsupial reproduction: a unifying hypothesis. Aust. Mamm. 1: 263-266.
- Moors, P.J. (1975). The urogenital system and notes on the reproductive biology of the female rufous rat-kangaroo Aepyprymnus rufescens (Gray) (Macropodidae). Aust. J. Zool. 23: 355-61.

- Morris, N.M., L.E. Underwood and W. Easterling. (1976). Temporal relationship between basal body temperature nadir and luteinizing hormone surge in normal women. *Fert. Steril.* 27: 780-783.
- Murphy, C.R. and J.R. Smith. (1970). Age determination of pouch young and juvenile kangaroo island wallabies. *Trans. R. Soc. S. Aust.* 94: 15-20.
- Nagy, K.A. (1980). Energy metabolism of free-living marsupials. *Bull. Aust. Mamm. Soc.* 6: 54.
- Newsome, A.E. (1964). Oestrus in the lactating red kangaroo, *Megaleia rufa*. *Aust. J. Zool.* 12: 315-21.
- Newsome, A.E., J.C. Merchant, B.L. Bolton and M.L. Dudzinski. (1977). Sexual dimorphism in molar progression and eruption in the agile wallaby. *Aust. Wildl. Res.* 4: 1-5.
- Nicol, S.C. (1978). Nonshivering thermogenesis in the potoroo (*Potorous tridactylus*). *Comp. Biochem. Physiol.* 59C: 33-37.
- Ogilby, W. (1838). On the species of the genus *Hypsiprymnus*. *Proc. Zool. Soc. (Lond)*. 1838: 63.
- Owen, R. (1834). On the generation of the marsupial animals with a description of the impregnated uterus of the kangaroo. *Phil. Trans. R. Soc.* 1834: 333-364.
- Parker, P. (1977). An ecological comparison of marsupial and placental patterns. In *The Biology of Marsupials*. Eds B. Stonehouse and D. Gilmore. MacMillan, London.
- Pearse, R.J. (1981). Notes on breeding, growth and longevity of the forester or eastern grey kangaroo *Macropus giganteus*, Shaw, in Tasmania. *Aust. Wildl. Res.* 8: 229-236.
- Pearson, J. (1944). The vaginal complex of the rat-kangaroos. *Aust. J. Sci.* 7: 80-83.
- Pearson, J. (1945). The female urogenital system of the Marsupialia with special reference to the vaginal complex. *Pap. Proc. R. Soc. Tasm.* 1944, 71-98.
- Pearson, J. (1946). The affinities of the rat-kangaroos (Marsupialia) as revealed by a comparative study of the female urogenital system. *Pap. Proc. R. Soc. Tasm.* 1945, 13-25.
- Pearson, J. (1950). The relationships of the Potoroidae to the Macropodidae (Marsupialia). *Pap. Proc. R. Soc. Tasm.* 1949, 211-227.
- Peters, D.G. and R.W. Rose. (1979). The oestrous cycle and basal body temperature in the common wombat (*Vombatus ursinus*). *J. Reprod. Fert.* 57: 453-460.
- Poole, S. and J.E. Stephenson (1977). Core temperature: Some shortcomings of rectal temperature measurements. *Physiol. and Behavior*. 18: 203-205.

- Poole, W.E. (1975). Reproduction in two species of grey kangaroos Macropus giganteus Shaw and M. fuliginosus (Desmarest). II. Gestation, parturition and pouch life. Aust. J. Zool. 23: 333-53.
- Poole, W.E. (1976). Breeding biology and current status of the grey kangaroo Macropus fuliginosus fuliginosus of Kangaroo Island, South Australia. Aust. J. Zool. 24: 169-187.
- Poole, W.E. (1979). The status of the Australian Macropodidae. In: The status of endangered Australasian Wildlife. Ed M.J. Tyler. Roy. Zoo. Soc. Sth. Aust.
- Poole, W.E., S.M. Carpenter and J.T. Wood (1982a). Growth of grey kangaroos and the reliability of age determinations from body measurements. I. The eastern grey kangaroo Macropus giganteus. Aust. Wildl. Res. 9: 9-20.
- Poole, W.E., S.M. Carpenter and J.T. Wood. (1982b). Growth of grey kangaroos and the reliability of age determination from body measurements II. The western grey kangaroos, Macropus fuliginosus fuliginosus, M.f. melanops and M.f. ocydromus. Aust. Wildl. Res. 9: 203-212.
- Poole, W.E. and P.C. Catling. (1974). Reproduction in the two species of grey kangaroos, Macropus giganteus Shaw and M. fuliginosus (Desmarest). I. Sexual maturity and oestrus. Aust. J. Zool. 22: 277-302.
- Poole, W.E., and P.E. Pilton. (1964). Reproduction in the grey kangaroo Macropus canguru in captivity. CSIRO. Wild. Res. 9: 218-34.
- Quoy and Gaimard. (1824). Zoologie, in Freycinet: Voyage autour du monde, Uranie et Physicienne, 1817-20.
- Ralls, K. (1978). When bigger is best. New Scientist. 9th Feb. 360-362.
- Renfree, M.B. (1972). Influence of the embryo on the marsupial uterus. Nature (Lond). 240: 475-477.
- Renfree, M.B. (1979). Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial Macropus eugenii. Nature (Lond). 278: 549-551.
- Renfree, M.B. (1980). Embryonic diapause in the honey possum Tarsipes spencerae. Search 11: 81.
- Renfree, M.B. (1981). Embryonic diapause in marsupials. In Embryonic Diapause. Eds Flint, A.P.F., M.B. Renfree and B.J. Weir. J. Reprod. Fert. Supp. 29: 67-78
- Renfree, M.B., S.W. Green, and I.R. Young. (1979). Growth of the corpus luteum and its progesterone content during pregnancy in the tamar wallaby, Macropus eugenii. J. Reprod. Fert. 57: 131-136.
- Renfree, M.B., Lincoln, D.W., Almeida, O.F.X. and R.V. Short. (1981). Abolition of seasonal embryonic diapause in a wallaby by pineal denervation. Nature (Lond). 293: 138-139.

- Renfree, M.B. and C.H. Tyndale-Biscoe. (1973). Intra-uterine development after diapause in the marsupial Macropus eugenii. Devl. Biol. 32: 28-40.
- Renfree, M.B., Wallace, G.I. and I.R. Young. (1982). Effects of progesterone, oestradiol-17 and androstenedione on follicular growth after removal of the corpus luteum during lactational and seasonal quiescence in the tammar wallaby. J. Endocr. 92: 397-403.
- Reynolds, H.C. (1952). Studies on reproduction in the opossum (Didelphis virginiana virginiana). Uni. Calif. Publs. Zool. 52: 223-283.
- Ride, W.D.L. (1970). A guide to the native animals of Australia. Oxford Uni. Press. Melb.
- Rose, R.W. (1978). Reproduction and evolution in female macropodidae. Aust. Mamm. 2: 65-72.
- Rose, R.W. (1982). Tasmanian bettong Bettongia gaimardi: maintenance and breeding in captivity. In The Management of Australian Mammals in Captivity. Ed D.D. Evans.
- Rose, R.W. (1983). The Tasmanian bettong - Bettongia gaimardi. File held by Australian National Parks and Wildlife Service. Canberra.
- Rose, R.W. and D.J. McCartney. (1982a). Reproduction of the red-bellied pademelon Thylogale billiardieri. Aust. Wildl. Res. 9: 27-32.
- Rose, R.W. and D.J. McCartney. (1982b). Growth of the red-bellied pademelon, Thylogale billiardieri and age estimation of pouch young. Aust. Wildl. Res. 9: 33-38.
- Rose, R.W. and Peters, D. (1977). Reproduction in Bettongia gaimardi. Bull. Aust. Mamm. Soc. 4:34.
- Rubenstein, B.B. (1937). Relation of cyclic changes in human vaginal smears to body temperature and B.M.R. Am. J. Physiol. 119: 635-641.
- Rubenstein, B.B. (1940). Vaginal smear - basal body temperature technique and its application. Endocr. 27: 843.
- Russel, E. (1982). Parental investment and desertion of young in marsupials. Am. Nat. 119: 744-748.
- Sadleir, R.M.F.S. (1963). Age estimation by measurement of joeys of the euro Macropus robustus Gould in Western Australia. Aust. J. Zool. 11: 241-249.
- Sadler, R.M.F.S. (1965). Reproduction in two species of kangaroos (Macropus robustus and Megaleia rufa) in the arid Pilbara region of Western Australia. Proc. Zool. Soc. Lond. 145: 239-61.
- Sadler, R.M.F.S. (1969). The Ecology of Reproduction in Wild and Domestic Mammals. Methuen & Co. Ltd.
- Sampson, J.C. (1971). The Biology of Bettongia penicillata Gray, 1837. Doctor of Philosophy Thesis. Univ. of W.A.

- Schmalhausen, J. (1930). Über wachstumsformen und wachstumstheorien. Biol. Zentralbl. 50: 292-307.
- Seebeck, J.H. and P.G. Johnston. (1980). Potorous longipes (Marsupialia: Macropodidae), a new species from eastern Victoria. Aust. J. Zool. 28: 119-34.
- Selwood, L. (1981). Delayed embryonic development in the dasyurid marsupial, Antechinus stuartii. J. Reprod. Fert. Suppl. 29: 79-82.
- Sharman, G.B. (1955a). Studies on Marsupial reproduction. II. The oestrous cycle of Setonix brachyurus. Aust. J. Zool. 3: 44-55.
- Sharman, G.B. (1955b). Studies on Marsupial reproduction. III. Normal and delayed pregnancy in Setonix brachyurus. Aust. J. Zool. 3: 56-70.
- Sharman, G.B. (1964). The female reproductive tract of the red kangaroo, Megaleia rufa. CSIRO Wildl. Res. 9: 50-57.
- Sharman, G.B. (1965). Marsupials and the evolution of viviparity. In Viewpoints in Biology. Eds J.D. Carthy and C.L. Duddington. pp 1-28 Butterworths, London.
- Sharman, G.B. (1970). Reproductive physiology of marsupials. Sci. 67: 1221-1228.
- Sharman, G.B. (1976). Evolution of viviparity in mammals. In Reproduction in Mammals. Vol. 6. The evolution of reproduction. Eds C.R. Austin and R.V. Short. Camb. Uni. Press.
- Sharman, G.B. (1981). Discussion In Embryonic diapause in marsupials. (Renfree 1981). J. Reprod. Fert., Suppl. 29: 67-78.
- Sharman, G.B. and Berger, P.J. (1969). Embryonic diapause in marsupials. In Adv. Reprod. Physiol. 4: 211-40.
- Sharman, G.B. and J.H. Calaby. (1964). Reproductive behaviour in the red kangaroo Megaleia rufa in captivity. CSIRO Wild. Res. 9: 58-85.
- Sharman, G.B., C.E. Murtagh, P.M. Johnson and C.M. Weaver. (1980). The chromosomes of a rat-kangaroo attributable to Bettongia tropica (Marsupialia: Macropodidae). Aust. J. Zool. 28: 59-64.
- Sharman, G.B., J. Calaby and W. Poole. (1966). Patterns of reproduction in female diprotodont marsupials. Symp. Zool. Soc. Lond. 15: p.129-37.
- Sharman, G.B., H.J. Frith and J.H. Calaby. (1964). Growth of the pouch young, tooth eruption and age determination in the red kangaroo Megaleia rufa. CSIRO Wildl. Res. 9: 20-49.
- Shaw, G. and R.W. Rose. (1979). Delayed gestation in the potoroo Potorous tridactylus (Kerr). Aust. J. Zool. 27: 901-912.
- Shield, J.W. (1966). Oxygen consumption during pouch development of the macropod marsupial Setonix brachyurus. J. Physiol. Lond. 187: 257-270.
- Shield, J.W. and P. Woolley. (1961). Age estimation by measurement of pouch young of the quokka (Setonix brachyurus). Aust. J. Zool. 9: 14-23.

- Shorey, C.D. and R.L. Hughes. (1972). Uterine glandular regeneration during the follicular phase in the marsupial Trichosurus vulpecula. Aust. J. Zool. 20: 235-47.
- Shorey, C.D. and R.L. Hughes. (1973). Cyclical changes in the uterine endometrium and peripheral plasma concentrations of progesterone in the marsupial Trichosurus vulpecula. Aust. J. Zool. 21: 1-20.
- Shorr, E. (1941). A new technique for staining vaginal smears. 3. A single differential stain. Sci. 94: 545.
- Siegler, S.L. and A.M. Siegler. (1951). Evaluation of basal body temperature and analysis of 1012 basal body temperature recordings. Fert. Steril. 2: 287-301.
- Smith, M.J. (1981). Morphological observations on the diapausing blastocyst of some macropodid marsupials. J. Reprod. Fert. 61: 483-486.
- Specht, R.L., E.M. Roe and V.H. Broughton. (1974). Conservation of major plant communities in Australia and Papua New Guinea. Aust. J. Bot. suppl. series No.7.
- Squire, W. (1868). Puerperal temperatures. Trans. Obstet. Soc. (Lond) 9: 129.
- Steiner, R.A., Schiller, H.S., Illner, P., Blandau, R., and C.C. Gale. (1977). Sex hormones correlated with skin swelling and rectal temperature during the menstrual cycle of the pigtail macaque (Macaca nemestrina). Lab. Anim. Sci. 27: 217-21.
- Stodart, E. (1966). Observations on the behaviour of the marsupial Bettongia lesueuri (Quoy & Gaimard) in an enclosure. CSIRO. Wildl. Res. 11: 91-99.
- Stodart, E. (1977). Breeding and behaviour of Australian bandicoots. In The Biology of Marsupials. Eds Stonehouse, B. and D. Gilmore pp 179-191 Macmillan Press, Lond.
- Tausk, M. and J. De Visser. (1971). The effects of progesterone on body temperature. In Int. Encyc. Pharmacology and Therapeutics, Vol. I, Section 48. Pharmacology of the Endocrine System and Related Drugs: Progesterone, Progestational Drugs and Antifertility Agents pp 353-359. Ed M. Tausk. Pergamon Press, New York.
- Thrasher, J.D. (1970). The cell cycle and turnover times of the small intestinal epithelia of the pouchless opossum Marmosa mitis. Experientia. 26: 871-872.
- Tompkins, P. (1945). Basal body temperature graphs as index to ovulation. J. Obstet. Gynaec. Brit. Emp. 52: 241.
- Treagust, D.F., Folk, Jr. G.E., Randall, W. and M.A. Folk. (1979). The circadian rhythm of body temperature of unrestrained opossums Didelphis virginiana. J. Therm. Biol. 3: 251-255.
- Treagust, D., W. Randall, G. Edgar and M.A. Folk. (1980). A fourier regression analysis of body temperature of the American Opossum Didelphis virginiana. J. interdiscipl. Cycle Res. 11: 135-144.

- Towers, P. (1980). Utero-ovarian blood vessels and local hormone levels in the Tammar (M. eugenii). Unpublished honours thesis. Murdoch Univ. W. Australia.
- Tyndale-Biscoe, C.H. (1963a). The role of the corpus luteum in the delayed implantation of marsupials. In Delayed Implantation. Ed A. Enders, pp 15-36, University of Chicago Press. Chicago.
- Tyndale-Biscoe, C.H. (1963b). Effects of ovariectomy in the marsupial Setonix brachyurus. J. Reprod. Fert. 6: 25-40.
- Tyndale-Biscoe, C.H. (1965). The female urogenital system and reproduction of the marsupial Lagostrophus fasciatus. Aust. J. Zool. 13: 255-67.
- Tyndale-Biscoe, C.H. (1966). The marsupial birth canal. Symp. Zool. Soc. Lond. 15: 233-250.
- Tyndale-Biscoe, C.H. (1968). Reproduction and post-natal development in the marsupial Bettongia lesueur (Quoy & Gaimard). Aust. J. Zool. 16: 577-602.
- Tyndale-Biscoe, C.H. (1973). Life of Marsupials. London. Edward Arnold.
- Tyndale-Biscoe, C.H. (1979). Hormonal control of embryonic diapause and reactivation in the Tammar wallaby. In Maternal Recognition of Pregnancy. Ciba Foundation Series. 64: 173-190.
- Tyndale-Biscoe, C.H., J.P. Hearn and M.B. Renfree. (1974). Control of reproduction in macropodid marsupials. J. Endocr. 63: 589-614.
- Tyndale-Biscoe, C.H. and J. Hawkins. (1977). The corpora lutea of marsupials: aspects of function and control. In Reproduction and Evolution. Eds J. Calaby & C.H. Tyndale-Biscoe, pp 245-251. Aust. Academy of Science. Canberra
- Tyndale-Biscoe, C.H. and J.C. Rodger. (1978). Differential transport of spermatozoa into the two sides of the genital tract of a monovular marsupial, the tammar wallaby Macropus eugenii. J. Reprod. Fert. 52: 37-44.
- Tyndale-Biscoe, C.H. and L. Hinds. (1981). Hormonal control of the corpus luteum and embryonic diapause in macropodid marsupials. J. Reprod. Fert., Suppl. 29: 111-117.
- Tyndale-Biscoe, C.H., L. Hinds, C. Horn and G. Jenkin. (1983). Hormonal changes at oestrus, parturition and post-partum oestrus in the tammar wallaby (Macropus eugenii). J. Endocr. 96: p155-161.
- Tyndale-Biscoe, C.H. and L.A. Hinds. (1984). Seasonal patterns of circulating progesterone and prolactin and response to bromocriptine in the female Tammar Macropus eugenii. Gen. Comp. Endocr. 53: 58-68.
- Van de Velde, T.H. (1930). Ideal Marriage, its Physiology and Technique. Covici, New York.
- Wakefield, N.A. (1967). Some taxonomic revision in the Australian marsupial genus Bettongia (Macropodidae) with descriptions of a new species. Vict. Nat. 84: 8-22.

- Walker, M.T. and R.L. Hughes. (1981). Ultrastructural changes after diapause in the uterine glands, corpus luteum and blastocyst of the red-necked wallaby, Macropus rufogriseus banksianus. J. Reprod. Fert., Suppl. 29: 151-158.
- Walker, M.T. and R.T. Gemmell. (1983). Plasma concentrations of progesterone, oestradiol - 17B and 13, 14-dihydro-15-oxo-prostaglandin F2 α in the pregnant wallaby (Macropus rufogriseus). J. Endocr. 97: 369-377.
- Walker, M.T. and R.W. Rose. (1981). Prenatal development after diapause in the marsupial Macropus rufogriseus. Aust. J. Zool. 29: p.167-87.
- Wallace, G. and M.B. Renfree. (1979). Reactivation of the quiescent blastocyst and corpus luteum of the Quokka during breeding and non-breeding seasons following removal of pouch young and bromocriptine injection. Proc. Aust. Soc. Reprod. Biol. 11: 3.
- Waring, H., G.B. Sharman, D. Lovat and M. Kahan. (1955). Studies on marsupial reproduction. I. General features and techniques. Aust. J. Zool. 3: 34-43.
- Waterhouse, G. (1846). Natural history of the Mammalia Vol. 1. Marsupialia, or pouched animals. London.
- White, R.J., C.R. Blaine and G.A. Blakley. (1973). Detecting ovulation in Macaca nemestrina by correlation of vaginal cytology, body temperature and perineal tumescence with laparoscopy. Am. J. Phys. Anthropol. 38: 189-94.
- Williamson, N.B., Morris, R.S., Blood, D.C. and C.M. Canon. (1972). A study of oestrous behaviour and oestrus detection methods in a large commercial dairy herd. I. The relative efficiency of methods of oestrus detection. Vet. Rec. 91: 50-8.
- Wood, J.T., S.M. Carpenter and W.E. Poole. (1981). Confidence intervals for ages of marsupials determined from body measurements. Aust. Wildl. Res. 8: 269-74.
- Woodburne, M.D. and W.J. Zinsmeister. (1982). Fossil land mammal from Antarctica. Sci. 218: 284-286.
- Wrenn, T.R., J. Bitman and J.F. Sykes. (1959). The thermogenic influence of progesterone in ovariectomised cows. Endocr. 65: 317-321.
- Yochim, J.M. and F. Spenser. (1976). Core temperature in the female rat: effect of ovariectomy and induction of pseudopregnancy. Am. J. Physiol. 231: 361-365.
- Young, I.R. and M.B. Renfree. (1979). The effects of corpus luteum removal during gestation on parturition in the tammar wallaby (Macropus eugenii). J. Reprod. Fert. 56: p.249-254.
- Zuckerman, S. (1953). The breeding seasons of mammals in captivity. Proc. Zool. Soc. Lond. 122: 827-954.
- Zuspan, F.P. and P. Rao. (1974). Thermogenic alterations in the women. I. Interaction of amines, ovulation and basal body temperature. Amer. J. Obstet. Gynec. 118: 671-679.

- Zuspan, F.P. and K.J. Zuspan. (1973). Ovulatory plasma amine (epinephrine and norepinephrine) surge in the woman. *Amer. J. Obstet. Gynec.* 117:654-659.
- Zuspan, K.J. and F.P. Zuspan. (1979). Basal body temperature. In *Human Ovulation: mechanisms, prediction, detection and induction*. Ed E.S.E. Hafez, Elsevier, North Holland.

Findlay, L., K. L. Ward and M. B. Renfree. (1983). Mammary gland lactose plasma progesterone and lactogenesis in the marsupial Macropus eugenii. J. Endocr. 97: 425-436

Flint, A. P. F. and M. B. Renfree. (1982). Oestradiol-17B in the blood during reactivation of the diapausing blastocyst in a wild population of tammar wallabies. J. Endocr. 95: 293-300.

Russel, E. M. (1982). Patterns of parental care and parental investment in marsupials. Biol. Rev. 57: 423-486.

APPENDICES

APPENDIX A

Growth of Bettongs in the Wild

This section details the results of a six month trapping survey near Kempton. Pouch young were measured from four females and additional data were obtained from one juvenile out of the pouch. About half the measured pouch young were not found at the next trapping of the mother and it may be that the act of measurement decreases the survival chances of the young.

Bettong F8 possessed a newborn young on 31/12/79; 35 days later, the measured head length of this young was similar to laboratory bred animals of that age (see Chapter 3). On 21/3/80 at an approximate age of 80 days the head length was again similar to captive animals of that age but the weight of this pouch young (60 g) was much lower than the expected, approximately 100 g.

Bettong F10 had a large pouch young near the end of pouch life (estimated age of 101.5 days). Though this is a period of rapid growth, 13 days later the weight of this young had increased by only 65 g. Captive young of similar age increase by about 150-200 g.

Bettong F15 had a large young present on 12/12/79, at an estimated age of 98 days. This young could be expected to have left the pouch in one week and to be replaced immediately by a new young (Chapter 3). Three weeks after initial capture, the female was recaptured with a young of an estimated age of 14 days. This animal was again trapped four weeks later and a pouch young of estimated age six weeks (42 days) was present, but weights were not obtained from this young.

Bettong F7 was trapped as a young male of an estimated age of 22-24 weeks. Six months later it was caught when it would have been

approximately one year old. It was a light animal (1.4 kg) and had increased in weight by only 200 g during that period.

APPENDIX B

T-test matrix.

This table compares the BBT of each day of the oestrous cycle with every other day.
Values of 1.96* or greater are significant ($P < 0.05$).

	Oestrus																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	0.58	0.29	0.36	0.37	0.34	-0.27	0.42	*2.16	*2.03	1.13	1.31	*2.60	-0.38	-0.37	0.24	1.08	0.75	1.23	1.10	-0.58
2		-	-0.34	-0.24	-0.21	-0.24	-0.88	-0.17	1.65	1.52	0.56	0.76	*2.93	-0.94	-0.97	-0.34	0.54	0.19	0.69	0.57	-1.17
3			-	0.09	0.11	0.08	-0.59	0.16	*2.07	1.93	0.94	1.13	*2.54	-0.67	-0.69	-0.03	0.88	0.52	1.05	0.91	-0.90
4				-	0.03	-0.01	-0.66	0.07	1.94	1.81	0.83	1.02	*2.41	-0.74	-0.76	-0.11	0.78	0.43	0.94	0.82	-0.97
5					-	-0.03	-0.66	0.04	1.82	1.70	0.76	0.95	*2.29	-0.74	-0.75	-0.13	0.73	0.39	0.88	0.76	-0.95
6						-	-0.63	0.08	1.86	1.73	0.80	0.99	*2.32	-0.71	-0.72	-0.10	0.76	0.42	0.91	0.79	-0.92
7							-	0.72	*2.51	*2.39	1.46	1.63	*2.95	-0.14	-0.11	0.53	1.39	1.04	1.54	1.40	-0.33
8								-	1.82	1.70	0.74	0.93	*2.30	-0.79	-0.81	-0.18	0.70	0.35	0.86	0.73	-1.01
9									-	-0.13	-1.15	-0.87	0.51	*-2.40	*-2.55	*-1.96	-1.06	-1.42	-0.90	-0.98	*-2.70
10										-	-1.02	-0.74	0.69	*-2.29	*-2.43	-1.83	-0.94	-1.30	-0.78	-0.86	*-2.58
11											-	0.24	1.67	-1.46	-1.53	-0.90	0.01	-0.35	0.17	0.07	-1.72
12												-	1.39	-1.61	-1.69	-1.09	-0.21	-0.56	-0.05	-0.15	-1.87
13													-	*-2.81	*-2.98	*-2.42	-1.56	-1.90	-1.41	-1.47	*-3.11
14														-	0.03	0.62	1.39	1.08	1.53	1.41	-0.17
15															-	0.62	1.46	1.13	1.61	1.47	-0.22
16																-	0.86	0.52	1.01	0.89	-0.83
17																	-	-0.34	0.15	0.05	-1.64
18																		-	0.50	0.38	-1.31
19																			-	-0.10	-1.78
20																				-	-1.65
21																					-

Oestrus

APPENDIX CPlasma Progesterone Levels in the Bettong

Bettongs 1 and 2

Births 3rd November 1982

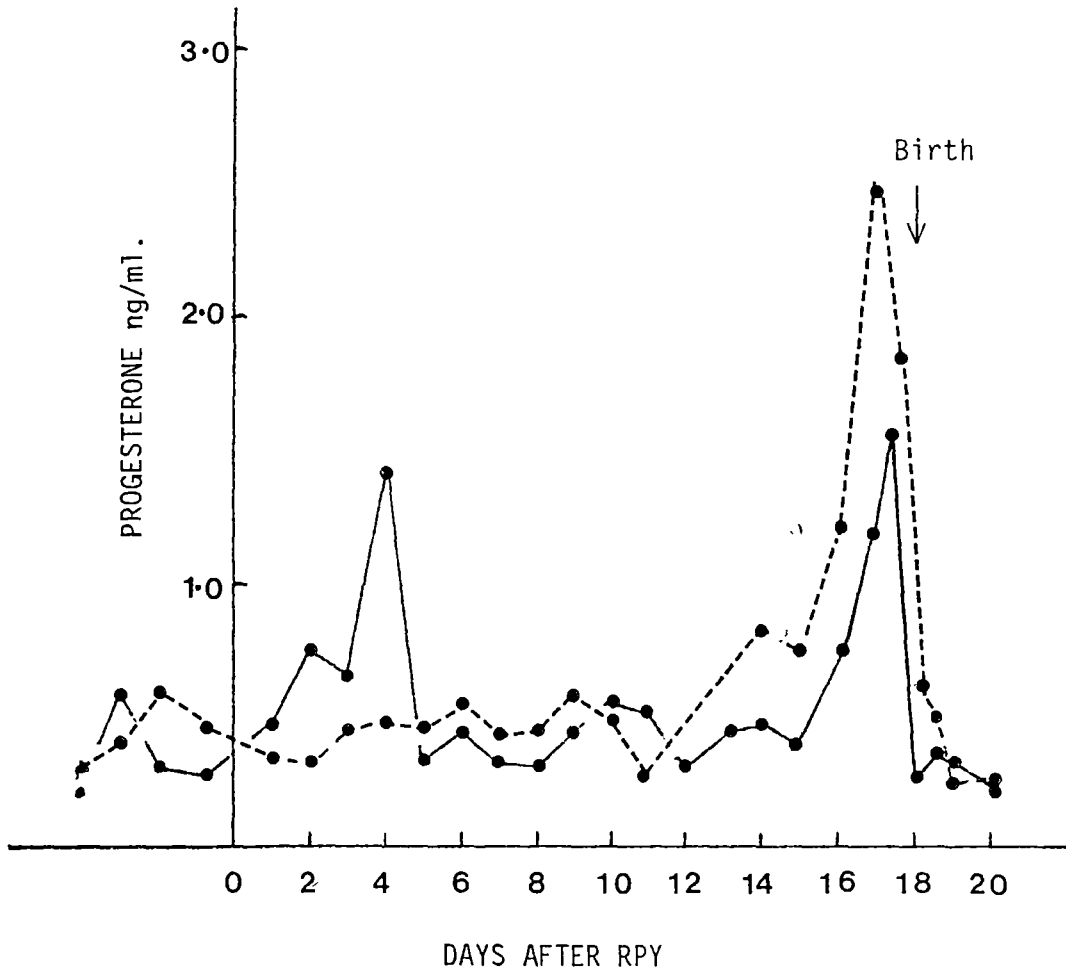
Progesterone ng/ml

Days Before	Bettong 1	Bettong 2
1	0.235	0.314
3	0.600	0.440
2	0.335	0.615
1	0.345	0.440
RPY	Removed at 2.00 p.m. Sat. 16 October 1982	
1	0.475	0.350
2	0.770	0.320
3	0.650	0.455
4	1.415	0.480
5	0.325	0.450
6	0.455	0.570
7	0.335	0.440
8	0.309	0.475
9	0.440	0.600
10	0.575	0.515
11	0.520	0.287
12	0.300	-
13	0.445	-
14	0.500	0.850
15	0.380	0.760
16	0.785	1.220
17 (1) 1.00 a.m.	1.200	1.870
(2) 4.00 p.m.	1.580	1.870
BIRTH	on the Wed. 3 November 1982 - young present at 10.00 am	
18 (1) 10.00 a.m.	0.270	0.610
(2) 4.00 p.m.	0.370	0.495
19	0.345	0.250
20	0.230	0.247

Blood samples obtained by M. Walker and B. Gemmell and analysed in their laboratory in the Anatomy Department of the University of Queensland.

APPENDIX FIGURE 1

PROGESTERONE PROFILE IN TWO BETTONGS



Samples obtained and analysed by R.Gemmell and M.Walker.