

The marsupial pouch: implications for reproductive success and mammalian evolution

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Abstract. Extant mammals are divided into sub- and infraclasses that are distinguished by their mode of reproduction. The monotremes lay eggs, the marsupials give birth to altricial young that typically develop in a pouch, and the eutherians have prolonged *in utero* development, resulting in well developed young at birth. The three groups exhibit what appears to be a nice progression of evolution towards the well developed newborn young of eutherian mammals. However, marsupials do not represent a step in the progression of producing well developed young, but maintain a reproductive strategy that has evolved to prosper in their specific niche. The production of undeveloped young with increased development in the pouch (or counterpart) provides specific advantages to those species living in diverse environments. The evolution of this reproductive strategy provides a clever solution to the uncertain and often adverse conditions encountered by many species, and the survival of the developing young in a pouch containing potentially harmful microorganisms is truly remarkable. In this review, we explore the unique features of the pouch, highlight the research questions that remain unanswered regarding this unique marsupial attribute and discuss the advantages of the marsupial reproductive strategy and the potential role of the pouch in mammalian diversification.

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Introduction

Many characteristics of reproduction are shared amongst the vertebrates. However, there are also a countless number of differences, demonstrating that different solutions have evolved independently to solve the various problems of reproduction (Lombardi 1998). The vertebrates not only display oviparous, viviparous and ovoviviparous reproductive strategies, but also exhibit extremely diverse developmental stages at birth, including the production of precocial, altricial and highly altricial offspring. The former two terms describe ‘well developed’, or sighted, covered young and ‘undeveloped’, or blind, naked young, respectively, while the latter term describes the most extreme form of altriciality. Furthermore, each strategy is not necessarily limited to a lineage, but is established in many lineages throughout the subphylum. For example, the snakes (suborder: Serpentes) include lineages of species that reproduce by the various ‘viparous’ or embryonic developmental strategies (Pope 1956) and the birds (class: Aves), although all oviparous, produce offspring that display a spectrum of altricial and precocial developmental stages (Starck and Ricklefs 1998).

Not surprisingly, there are similar variances in the reproductive strategies of mammals. All animals that produce milk to meet the nutritional requirements of their young are grouped into the class Mammalia and it is thought that lactation

provides a specific advantage to mammals, enabling them to support the nutritional requirements of the young in any environment where adults are able to survive (Pond 1984). The extant mammals are divided into three major lineages that are distinguished by their modes of reproduction. The monotremes (or Prototheria – meaning ‘first beasts’) display a mixture of reptilian and mammalian features, as they lay eggs yet feed their young milk produced by mammary glands. Monotreme hatchlings are highly altricial. In contrast, ‘placental’ mammals (or Eutheria – meaning ‘true beasts’) produce developed young after a relatively long gestation time in which the developing foetus relies on a placenta for the exchange of factors between mother and foetus that are critical for survival, such as nutrients, gases and immune compounds. The eutherian mammals produce offspring varying from altricial to precocial; for example, even within the Leporidae, there are altricial rabbits and precocial hares (Trevathan 1987). The marsupials (or Metatheria – meaning ‘behind true beasts’) do not lay eggs but, like the monotremes, they produce highly altricial young that complete most of their development during a complex lactation phase rather than throughout gestation. Marsupials are born at a stage of development comparable to an 8–10-week-old human embryo or an 11–12-day-old mouse embryo (Block 1960; Smith 2001). Hence, much of the development that occurs in a mostly

pathogen-free environment in eutherian mammals takes place *ex utero* in marsupials and typically while the young are permanently attached to a teat within a pouch.

The unfortunate naming of these three groups of mammals – Prototheria, Metatheria and Eutheria – may suggest that monotremes and marsupials are evolutionary steps in the progression to eutherians, but a closer examination of the unique reproductive strategies adopted by each lineage shows that their modes of reproduction simply provide alternative solutions that have adapted under different conditions. The survival of the highly altricial young of marsupials is truly remarkable. Des Cooper demonstrated an interest in the uniqueness of the marsupial reproductive strategy, and was particularly interested in the survival of the altricial young in the pouch and even in the development of the pouch itself. Without Des Cooper's contribution, we would know little about the marsupial pouch, as the pouch has been largely overshadowed by another marsupial reproductive trait – the production of highly altricial pouch young. In this review we link highly altricial young to the pouch and make a case for the pouch in the role of marsupial reproductive success and mammalian evolution.

Highly altricial young

In comparison to the broad altricial to precocial spectrum observed amongst different eutherian mammals at birth, marsupials are born at a similar developmental stage, which marks the minimum onset of functionality for specific tissues (Hughes and Hall 1988). This stage of development can be visually compared with the developmental stage of a bird embryo, well before hatching, or to a eutherian mammal embryo *in utero*. Particularly, the most visually noticeable characteristics are the forelimbs, which the young uses to take hold of the mother's fur to propel itself from the mother's urogenital opening to a teat. The mouth parts are also well developed and have a very important role in fastening onto the mother's teat so the young can remain attached to the mother and attain colostrum and milk. Remarkably, the undeveloped characteristics of marsupials outweigh the comparatively developed characteristics several-fold at birth, with the undeveloped characteristics considered unnecessary for survival at this time of development.

Not only is the marsupial young visually undeveloped, but many general physiological processes essential for adult survival also remain immature until sometime after parturition. For example, the lungs are partly developed with partial gas exchange occurring through the skin. The degree of development differs between species; Julia Creek dunnarts (*Sminthopsis douglasi*), under approximately seven days, exhibit gas exchange through the skin which exceeds that through the lungs (Mortola *et al.* 1999), while the integument of the tammar wallaby (*Macropus eugenii*) is responsible for ~33% of gas exchange at birth, decreasing to 14% at six days post partum (MacFarlane *et al.* 2002).

Marsupial neonates are also ectothermic and exhibit a large surface area to weight ratio, which can cause rapid heat loss. However, the rate of heat loss reduces as the young develops. When the thermogenic response is initiated, the response is small and it is not until they are covered in body hair that the development of thermogenesis is complete (Hulbert 1988).

Additionally, young are born with undeveloped immune tissue (Deane and Cooper 1988). For example, tammar wallaby young are not able to mount an adaptive immune response until 35 days post partum (Old and Deane 2003) and the maturation of their lymphoid tissue is determined to occur at ~90 days post partum (Basden *et al.* 1997).

The survival of highly altricial young appears to be a phenomenon when compared with the reduced survival rates of human neonates that are born prematurely. However, marsupials have evolved to produce young at a highly altricial developmental stage and exhibit specific traits that undoubtedly aid in the rearing of such undeveloped young. In particular, the pouch resolves many of the problems encountered by the production of highly altricial newborn young.

The pouch

Pouches are located ventrally but vary markedly between marsupial species; they can be shallow or deep and contain varying numbers of teats between species (Tyndale-Biscoe 2005). Russell (1982b) described six different pouch types. Figure 1 shows a phylogeny of the pouch and the pouch counterpart types for the major orders and suborders of marsupials. In Type 1 the mammary area is not covered, but folds of skin can develop in the breeding season; in Type 2 the mammary area is partially covered; in Type 3 the mammary area is covered and the teats are displayed in a circular arrangement with the pouch opening in the centre; in Type 4 the mammary area is covered and the teats are located in two pockets; in Type 5 the mammary area is covered and the pouch opens to the anterior; and in Type 6 the mammary area is covered and the pouch opens

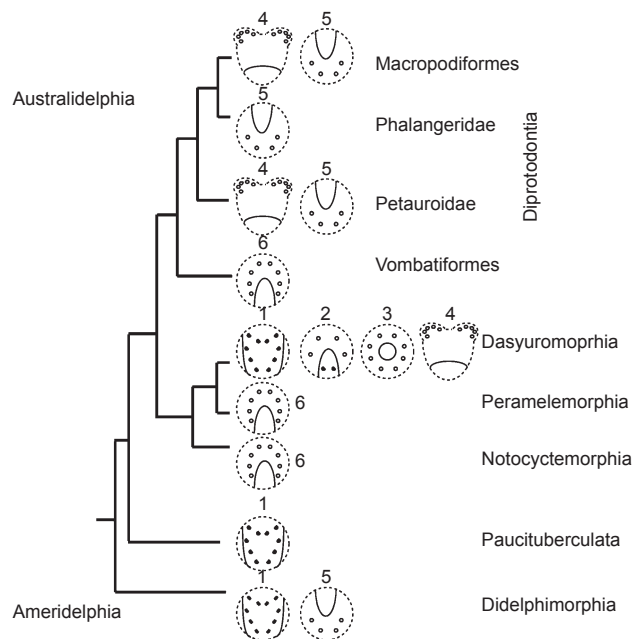


Fig. 1. A phylogeny showing different pouch types and pouch counterparts. Dark and light circles represent teats that are located exteriorly and interiorly, respectively. Solid and dotted lines represent the pouch opening and covered areas, respectively. Pouch types and diagrams are reported from Russell (1982b) and Tyndale-Biscoe and Renfree (1987).

to the posterior. Figure 2 shows external and internal images of a tammar wallaby pouch (Type 5), with and without a pouch young and with different degrees of pouch cleanliness (also known as ‘pouch grot’), as described by Sharman and Calaby (1964) as brown to black scale for a ‘dirty’ pouch or clean and pinkish for a ‘clean’ pouch.

Russell (1982b) also identified three different patterns of parental care that are associated with different pouch types. Pattern A describes species with small pouches and large litters, whereby the mother leaves the young at an early developmental stage (with little fur, their eyes closed and no thermoregulation) in a nest after a period of teat attachment. Pattern B describes species with well developed pouches and fewer young, whereby the young remain in the pouch and are then left in a nest at a later developmental stage (when they are well furred, their eyes are open and they can thermoregulate). Pattern C describes species with large pouches and typically only one young, whereby the young remain in the pouch, as in Pattern B, and then leave the pouch but continue to follow the mother at foot. Gemmell *et al.* (2002) describes different methods, correlating to different pouch types, for newborn young to travel from the urogenital opening to the pouch; young may either climb upward to the pouch (e.g. the forward-facing pouch (Type 5) of the brushtail possum (*Trichosurus vulpecula*)) or the mother may place her urogenital opening above the pouch so the newborn can move down to the pouch (e.g. the backward-facing pouch (Type 6) of the bandicoot (*Isodon macrourus*)).

Remarkably, there are still so many questions about the pouch that remain unanswered. Foremost of these is the trigger for pouch development. The pouch is not under hormonal control, but is thought to be under the control of a locus on the X chromosome, along with the scrotum and mammary glands (Shaw *et al.* 1989; Watson and Cooper 1995; Watson *et al.* 1997).

The role of chemical protection in the pouch is also relatively unknown, although four genes for the antimicrobial peptide cathelicidin are expressed in tammar wallaby pouch skin (Wang *et al.* 2011). A haematoxylin and eosin stain of the pouch skin taken from within the tammar wallaby pouch also shows a very large active apocrine gland (Fig. 3); these are usually found in the axillary and genital areas (Morimoto and Saga 1995). Large sweat glands have also been identified in the red kangaroo (*Macropus rufus*) and brushtail possum pouches, suggesting that there may be active secretions into the pouch (Kubota *et al.* 1989; Old *et al.* 2005). Further work, possibly linking the glands to antimicrobial or mucosal activity needs to be completed (Edwards *et al.* 2011).

Although the pouch or marsupium gives the marsupials their name, not all marsupials have pouches. Instead, the position of their reproductive and excretory organs distinguishes them from the monotremes and the eutherians (Cooper and Hope 1989; Tyndale-Biscoe 2005). Species without a pouch instead form a tissue between the teat and the young so the young stay attached and do not become separated from the mother. To support the young the *ilio marsupialis* muscle passes through the mammary gland and up each teat (Griffiths and Slater 1988). It is surprising that a pouch is not associated with all marsupials as many challenges that are presented by producing highly altricial young appear to be overcome by the pouch. As a pouch is not necessary for the production of highly altricial young, below we examine the role of the pouch in reproduction and ultimately in mammalian evolution.

Implications of the pouch for reproductive success and mammalian evolution

The production of highly altricial young, and not the pouch, is usually the focus of discussion for marsupial reproduction. In the

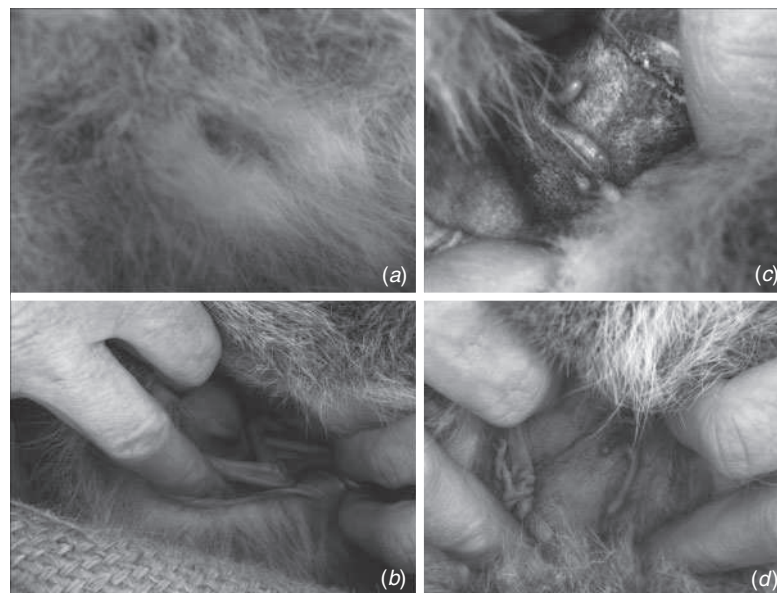


Fig. 2. An external view of a closed tammar wallaby (*Macropus eugenii*) pouch (a), a pouch young inside the pouch (b), and two images of an internal view of a tammar wallaby pouch without a pouch young (c and d). An elongated teat is evident in (c) and (d) and one pouch displays a large amount of ‘pouch grot’ (c).

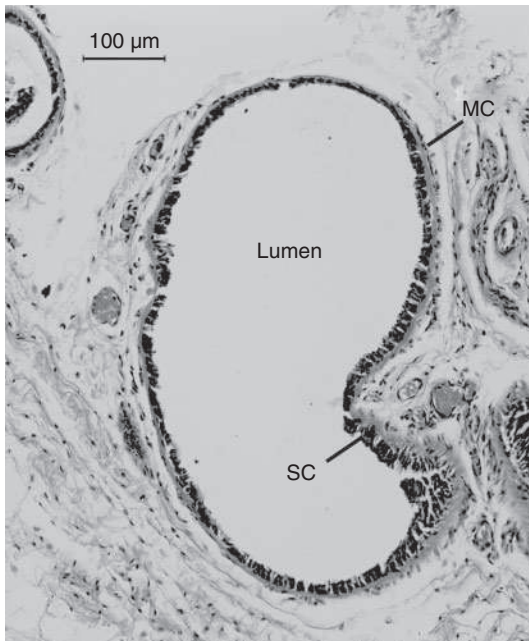


Fig. 3. An 8- μm section of a pouch skin biopsy stained with haematoxylin and eosin, taken from within the tamar wallaby (*Macropus eugenii*) pouch with a 2–3-day-old pouch young. An apocrine gland is evident with an extremely large lumen surrounded by secretory cells (SC) and myoepithelial cells (MC).

past, the notion that eutherian reproduction has a fundamental evolutionary advantage over marsupial reproduction was explored (Müller from Lillegraven 1975); however, Kirsch (1977a) highlighted that we should not be asking why marsupials lack a complex gestation, but asking whether a long gestation is necessary? Kirsch (1977a, 1977b), Parker (1977) and Low (1978) discussed that the difference between the reproductive strategies of marsupials and eutherians amounted from different selective environments. The support for the production of highly altricial young included that marsupials could terminate their investment in reproduction in response to unfavourable conditions (such as irregular drought episodes: Kirsch 1977a, 1977b; Parker 1977; Low 1978). Specifically, advantages were identified in terms of reproductive value and effort, and for marsupials these included: rapid birth (which reduced vigilance time), low cost associated with the foetus in terms of risk and energy (such as reduced vulnerability to predation and increased ability to forage), ability to resorb reproductive material, and low cost of the uterus when compared with finding or building a nest (Kirsch 1977a, 1977b; Parker 1977; Low 1978). However, Russell (1982b, 1982a), Lee and Cockburn (1985) and Cockburn (1989) examined the marsupial strategy further using additional examples of marsupial species and provided a comprehensive discussion that pointed to discrepancies in the ‘unfavourable conditions’ hypothesis.

Hopson (1973) proposed that the fundamental components of mammalian reproduction are selection for endothermy and small body size. As metabolic rate increases as body size decreases (creating an energetic dilemma), Hopson (1973) suggested that there is selection for the production of ectothermic young with low metabolic rate and increased parental care,

including adaptations for creating warmth. Case (1978) presented a slightly different hypothesis to Hopson, and proposed that smaller young have a lower energetic requirement than larger young. Thus, parental costs are greater after birth and can be shared between parents, particularly when brooding and foraging cannot co-occur. Furthermore, the mammary gland and attachment to the teat increased maternal care and reduced the need for paternal care. Case (1978) also suggested that reproductive characteristics are shaped by aspects of a species’ niche and habitat. For example, it may be beneficial for those species that spend a large amount of time and energy searching for food to produce small embryos as larger embryos may hinder their ability to forage.

Mostly, the pouch is disregarded in discussions on the marsupial reproduction strategy, although Hopson (1973) stated that pouches helped with trends to produce altricial young. It is likely that researchers question the role that the pouch has in the discussion of marsupial reproduction, as some marsupial species lack a pouch. However, when present, the pouch plays a major role in reproduction, thus it is necessary to examine the pouch explicitly.

The pouch has more than one role in the reproduction of many marsupial species and the large variation of pouches seen within the marsupials is likely to correspond to the varying levels of importance of the role of the pouch. First, direct physical protection from the external environment is provided by the pouch (Russell 1982b). In birds and monotremes, physical protection comes from the egg, while protection for eutherian mammals, at a comparable developmental stage, comes from the uterus and integument of the mother. The pouch may also provide protection on a chemical level, as pouch washes (obtained by flushing pouches with sterile water) contain proteins with antimicrobial activity and display antimicrobial activity when they are subjected to bacteria (Bobek and Deane 2001; Ambatipudi *et al.* 2007, 2008; Edwards *et al.* 2012). Second, the pouch may influence the humidity of the direct environment of the young (Kubota *et al.* 1989), which may aid in integumental gas exchange. Chemical protection and humidity control could come from secretions within the pouch (Kubota *et al.* 1989), or from the mother licking the pouch and depositing saliva (Charlick *et al.* 1981). Third, as the pouch is associated with the mother, direct contact between the mother and young allows the young to develop at a constant temperature that is comparable to that of the adult, thus resolving the issues of ectothermy in the developing young (Hulbert 1988). The ability to open and close the pouch may also provide an air-conditioning effect (Kubota *et al.* 1989). If a pouch provides a constant environment specific to the requirements of the young for protection, humidity, and warmth, it may have an increased chance of surviving and reproducing. For example, if the mother moves into or even through an environment in which conditions are unfavourable to the young, the pouch will keep the direct environment of the young constant.

Fourth, the pouch may make young inconspicuous to predators so that mothers may not be targeted while foraging, and, finally, the pouch evolved in unison with mammary glands. Although the pouch is always associated with mammary glands, the mammary glands are not always associated with a pouch (Shaw *et al.* 1989). Mammary glands provide the young with

essential nutrients and also pass on immune compounds to the developing animal (Deane *et al.* 1990; Young *et al.* 1997). In contrast to eutherian mammals, the supply of marsupial milk is multifaceted, as milk production occurs over three or four phases, which are associated with major periods of growth (Nicholas 1988; Joss *et al.* 2009).

It is clear that the pouch supports the undeveloped marsupial; however, the pouch also presents potential challenges. For example, the young must travel from the urogenital opening to the pouch before latching onto a teat. Additionally, the pouch is a non-sterile environment containing a range of bacteria that have the potential to harm the young (Yadav *et al.* 1972; Charlick *et al.* 1981; Old and Deane 1998; Deakin and Cooper 2004; Chhour *et al.* 2010).

The pouch not only provides insight into marsupial reproduction but may also shed light on mammalian diversification and evolution. Currently, the mammalian lineages are dominated by eutherian mammals, even though marsupials have been evolving for the same period (Bininda-Emonds *et al.* 2007). Müller (from Lillegraven 1975), Cooper and Stepan (2010), and Kelly and Sears (2011) suggested that the reproductive strategy of marsupials has contributed to limiting the marsupial's evolutionary potential when compared with eutherian mammals. The limitation comes from the need for developed forelimbs to travel from the urogenital opening to the teat, which may pose a functional constraint on the forelimb, limiting its morphological evolution. In other words, the marsupials must retain their forelimbs and cannot evolve wings, flippers or hooves, which have evolved in the eutherian clade. Kirsch (1977b) found the hypothesis unconvincing as claws in some marsupial species are deciduous.

Whether or not the forelimb of the marsupial has limited the evolutionary potential of the marsupials, we suggest that the pouch has positively influenced the range of extant marsupial species. The pouch provides a practical explanation that supports the movement of marsupials into niches that are not supported by free-hanging highly altricial young. For example, the water opossum (*Chironectes minimus*) has a strongly developed pouch muscle, the *pars pudenda*, which allows the pouch to close effectively enough to help protect the young from water (Enders 1937). The marsupial mole (*Notoryctes typhlops*) and other burrowing species have posterior-opening pouches that protect the young from sand and soil entering the pouch while they are burrowing (Johnson 1995). It is also likely that the pouch provides increased protection in jumping and gliding species (genus: *Macropus* and *Petaurus*) when the mother lands on successive trees or hops through scrubland, as it is difficult to see how free-hanging altricial young would survive in these types of situations.

The pouch, as a reproductive feature, fits Case's (1978) hypothesis, as it may be shaped by attributes of niche and habitat. Although we only provide examples of how a pouch might promote reproductive success in clearly different environments, it is likely that the presence or type of pouch is also going to be influenced by specific ecological traits such as mode of foraging or predator avoidance. Researchers are frequently fixed on explaining why there are not as many marsupials as there are eutherian mammals; perhaps turning the question around to ask why are there as many marsupials as there are, is just as interesting in evolutionary terms.

Conclusion

The marsupials, often referred to as Metatheria (or halfway mammals), have sometimes been thought of as being an intermediate group between the egg-laying monotremes, often referred to as Prototheria (or first mammals), and the comparatively more highly developed eutherians (or true mammals). However, it is important to remember that marsupials do not represent a step in the evolution of producing well developed young, but maintain an alternative mode of reproduction compared with the eutherian strategy that has evolved to prosper in their specific niche (Graves *et al.* 1989).

Much emphasis has been placed on the production of highly altricial young in the marsupial reproduction strategy, while the pouch is often disregarded. However, the pouch plays an extremely important role in the reproductive success of those species that have them. Additionally, the pouch may have played an important role in the diversification and evolution of mammals by supporting the movement of marsupials into niches that are not supported by free-hanging highly altricial young.

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