ABSTRACT

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ABSTRACT

This dissertation developed and assessed postcranial age estimation methods in the Macropodidae. Data was collected from museum specimens of nine macropodid genera. Collected data included both postcranial measurements of size, shape, and epiphysial fusion and cheek tooth observations of morphology and eruption. The objectives of this study were: 1) to describe cheek tooth morphology for species absent in the literature, 2) develop a system for scoring molar eruption, 3) describe molar eruption patterns across the family, 4) develop a method for estimating age using degree of fusion at the epiphysis of the forelimb, 5) describe patterns of epiphyseal fusion in the forelimb across the family, 6) use epiphyseal fusion scores to assign specimens to age categories, 7) assess whether any specimens with partly unfused epiphyses can be placed in the same morphological group as those with totally fused epiphyses, and 8) to compare potential postcranial age estimation methods.

The results of this study show that of the four postcranial age estimation methods (total fusion, humerus fusion, ulna fusion, and radius fusion), that of humerus epiphyseal fusion is the most significant when regressed on and correlated with molar eruption scores and as such is the best indicator of age. The other three postcranial fusion scores also are significant (though less so) when regressed on and

correlated with molar eruption scores and can therefore also be used in age estimation.

Results for age categories and assessing which specimens group together morphologically were less clear. Discriminant function analysis using the long bones did clearly show three age categories: adult (fusion scores of 5), subadult (fusion scores of 3 and 4), and juvenile (fusion scores of 1 and 2). However, these analyses also showed that on some of the functions generated by the analyses (especially those where measures of the trochlea and capitulum were influential) the highest three scores were indistinguishable, indicating that these specimens grouped together and could be included in the same morphological study. Discriminant function analysis using total fusion scores did not produce meaningful plots.

NORTHERN ILLINOIS UNIVERSITY

# USE OF EPIPHYSEAL AND TOTAL FUSION SCORES AS METHODS OF AGE ESTIMATION AND EVALUATION OF MORPHOLOGICAL INDICES IN THE MACROPODIDAE

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# DEDICATION

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# CHAPTER I

# INTRODUCTION

The majority of marsupial studies have focused on phylogeny (Ride, 1964; Kirsch, 1977; Baverstock *et al.*, 1982; Groves, 1982; Flannery, 1989), reproductive physiology and anatomy (Burns, 1939; Bolliger, 1946; Tyndale-Biscoe, 1955, 1966; Clark and Poole, 1967), embryonic and pouch development (Caldwell, 1884; Sharman *et al.*, 1964; Clark, 1968; Maynes, 1976), and dentition (Flower, 1867; Thomas, 1887; Kirkpatrick, 1964; Bartholomai, 1971; Sanchez-Villagra and Kay, 1996). These areas are those in which marsupials differ significantly from placental mammals. Less work has been done on behavioral and morphological differences among marsupials, especially in broad studies across and within families. Outside of the Macropodidae, most morphological studies to date have largely concentrated on the American marsupial, *Didelphis* (Coues, 1872; Haines, 1941; Washburn, 1946; Jenkins and Weijs, 1979; Hamrick, 1999). In the Macropodidae, most morphological studies focus on the unique bipedal locomotion form of saltoriality (bipedal hopping) (Badoux, 1965; Hopwood, 1974; Alexander and Vernon, 1975; Griffiths, 1989; Hopwood and Butterfield, 1990) or the dental adaptations to a grazing diet (Kirkpatrick, 1964, 1969; Bartholomai, 1971; Newsome *et al.*, 1977; Sanson, 1982). However, there are many more potentially interesting evolutionary

and morphological questions that can be investigated by using the Macropodidae as a study model. One hindrance to such studies in this family is the difficulty in correctly assigning museum specimens (especially postcranial specimens) to adult, subadult and juvenile age classes. The difficulty arises from the unique cheek tooth adaptations and prolonged growth patterns exhibited in the majority of the species. The following study addresses this problem by designing an age scoring system that allows any researcher to assign specimens to one of the three age groupings. Prior to investigating age estimation methods in the Macropodidae, it is important to understand marsupial phylogeny and the key differences distinguishing marsupials from placentals. It is also important to understand macropodid biology.

## Marsupial Phylogeny

### Phylogeny and Evolutionary History of Marsupialia Relative to Mammalia

Marsupials, placentals, and monotremes comprise three groups of extant mammals. Long known to scientists, the first marsupial was collected in 1499 in Brazil by the Spanish explorer Vicente Pinzón, who had commanded Christopher Columbus's flagship the Niña in 1492. Pinzón described this monstrous animal as having a face like a fox, a tail like a monkey, feet like a man, and a great bag hanging from its belly for carrying its young (Nickens, 2003). Pinzón returned to Spain and delivered his strange prize (an opossum) to Queen Isabella and King Ferdinand. He was not the only European explorer to notice similarities between the newly discovered marsupials and their placental counterparts. Naturalists who first

discovered and described these marsupials incorporated their likenesses in their scientific names, e.g., *Phascol* (pouched) - *arctos* (bear) or *Thyla* (pouched) - *cinus* (dog).

The first biologist to explore the relationships between the three mammalian groups and to devise a classification scheme was de Blainville (1816). He based his classification on female reproductive anatomy and coined the terms Didelphia (Greek for two uteri), Monodelphia (Greek for one uterus) and Ornithodelphia (Greek for bird uterus). Though the animals encompassed by each of these three taxa remain associated, the names of the groupings were soon changed. Richard Owen (1839), the first to study the monotremes, devised the terms Placentalia, Marsupialia and Monotremata to refer to the three groups of mammals. He based these names on their most obvious morphological characteristics (described below).

In 1880, Huxley devised a new classification terminology based on evolutionary relationships. This classification incorporated the terms Prototheria (first beasts), Metatheria (halfway beasts) and Eutheria (true beasts). Whereas Owen was a staunch anti-evolutionist, Huxley was strongly influenced by the work of Charles Darwin and believed that organisms did not arise independently, but must be the result of gradual modification. Furthermore, he believed that evolutionary lineages represent a *scala naturae* and as such could be ordered based on a ranking hierarchy of increasing deviation from earlier stages of evolution. Huxley therefore named the monotremes the Prototheria and placed them as a basal group closest to non-mammalian vertebrates. He based his conclusion on the following mammalian

characteristics: the presence of milk (though without teats, the milk is secreted onto the abdominal fur or into a temporary pouch), a four-chambered heart, a single dentary bone in the mandible, and three ear ossicles and the following reptilian characteristics: a splayed pelvic girdle and epipubic bones, incubated eggs for development, a cloaca, and a vaginal canal undifferentiated from the urethra.

Next he concluded that marsupials must be the intermediary between monotremes and placentals and so named them Metatheria. Like the Prototheria, metatheria have epipubic bones, they have a small corpus callosum connecting the cerebral hemispheres, and finally, they have a chorio-vitelline, or yolk-type, placenta (note, however, that reproductive studies have revealed a more advanced chorio-allantoic placenta attached to the uterine wall in bandicoots; see Padykula and Taylor, 1982). Though he considered Metatheria an intermediary evolutionary stage, he did recognize that they were closer to placentals with whom they shared more traits: release of milk through teats rather than into the fur, a separate vagina and urethra, and limbs brought under the body.

Finally, the placentals were the most dissimilar to non-mammals and were named the Eutheria. As such, they represented the highest stage of evolution in the *scala naturae*. Though the modern synthesis does not view evolution as progressive, arguments do exist for a view of marsupials as either more primitive than placentals or as actually more closely related to monotremes in a grouping referred to as the Marsupionta (but see counterarguments in Kirsch *et al.*, 1997; Belov *et al.*, 2002). In point of fact, Metatheria are almost as dissimilar to non-mammals as they are the

Eutheria. They usually have pouches, possess a largely deciduous dentition, have a bifurcated vagina and glans penis, and have very short gestations with long lactation periods. Huxley (1880) sidestepped this problem by hypothesizing that the Metatheria were a modified group of the "Metatherial type," a representative of which did not currently exist but would surely be forthcoming with more Mesozoic fossil discoveries.

Current fossil research (Janis, 1993) and research into reproductive physiology (Tyndale-Biscoe, 2005) indicate that only with the climatic and vegetative changes of the Cretaceous (with the radiation of the angiosperms) does the body size of ancestral therians significantly increase. To accommodate the changing developmental needs, increased gestation evolved in eutherians with a true placental system. On the other hand, metatherians evolved a significantly shortened gestation and an increased period of lactation. The development of the lactation system in metatherians is as advanced and adaptive as the placental system in eutherians. In short, though eutherians form a monophyletic group with metatherians, they did not evolve from metatherians. Both reproductive conditions represent equally derived evolutionary trajectories. Having a lactational/pouch system allowed metatherians to take advantage of extreme and variable conditions present in the Miocene/Pliocene in Australia. This reproductive system gave the female flexibility to terminate development if environmental conditions demanded it, and in some species it even allowed the female to keep developing young in two or three different stages. Embryonic diapause is so successful a strategy that it appears

independently in many placental taxa as well (e.g., armadillos, mustelids, and rodents; Nowak, 1999). In short, the differences between metatheria and eutheria are not evidence of a "halfway beast" but are instead evidence of equally competitive reproductive strategies in response to new environmental stressors (Tyndale-Biscoe, 2005).

Current research does not support Huxley's (1880) terminology indicating an evolutionary progression. This study will use the older and more neutral terminology of Owen (1839): monotremes, marsupials and placentals. It is important to note, however, that there are flaws with this nomenclature system as well. Monotreme refers to "one hole," or the single opening for urinary, digestive, and reproductive tracts. The cloaca in marsupials also serves as a single opening for all three organ systems. Marsupial refers to a pocket or pouch; however, not all marsupial females possess a permanent pouch, and some (e.g., the numbat, *Myrmecobius*, and shrew opossums, *Caenolestes*) do not possess a pouch at all (Nowak, 1999). Conversely, during lactation the female echidna, *Tachyglossus*, displays a temporary pouch. Placental refers to the support of the fetus by an allantoic placenta attached strongly to the uterine wall. During the stage of development when the marsupial fetus is in the uterus, there does exist a chorio-vitilline placenta, but, as mentioned earlier, in the bandicoot this placenta is chorio-allantoic and firmly attached to the uterine wall (though it is smaller than that seen in placental mammals).

Aside from debates on terminology, there are also debates on historical biogeography and marsupial phylogeny. With the recent discovery of marsupial fossils in Africa and Asia (Bown and Simons, 1984; Luo *et al.*, 2003), marsupial fossils are known to occur on every continent, though extant species only remain in the Americas and Australasia. For a long time, this has led to the postulation of an "American" and "Australasian" grouping of marsupials (Simpson, 1930, 1945; Ride, 1964; Szalay, 1982; Archer, 1984). Previously, paleontologists proposed that the last common ancestor of the marsupials and placentals existed in North America prior to its separation from Gondwana and that the marsupials then spread throughout the entire landmass, but eventually were outcompeted and went extinct in Laurasia after the Pangean supercontinent broke up (Cifelli, 1993). However, a 125-million-year-old fossil from China that is more closely related to marsupials than placentals (Luo *et al.*, 2003) lends support to Asia being the center of diversification for these groups. Much work remains to be done concerning these questions, but answers are dependent upon future fossil discoveries. In either case, the introduction of marsupials back into North America and placentals into South America did not occur until the formation of the Isthmus of Panama during the Pliocene-Pleistocene, when the current distribution of marsupials arose.

Early studies of marsupial phylogeny based relationships on dentition (i.e., the numbers of incisors, canines and premolars and on molar morphology) or on the number of digits in the pes (Bensley, 1903; Gregory, 1910; Simpson, 1930; Ride, 1964; Archer, 1976). The past thirty years of molecular studies have yielded

modifications to these early phylogenies by comparing protein amino acid sequences and nucleic acid base sequences (see reviews in Baverstock *et al.*, 1990; Hope *et al.*, 1990; Kirsch *et al.* 1997; LaPointe and Kirsch, 2001).

The most recent biochemical and DNA hybridization work of Kirsch and others (Kirsch, 1977; Edwards and Westerman, 1995; Springer, 1995; Kirsch *et al.*, 1997) places seven extant monophyletic orders within the infraclass Marsupialia (sensu McKenna and Bell, 1997, but see Simpson, 1945, for ordinal designation and Kirsch, 1977, for superorder designation). Four of these orders are indigenous to Australasia: Peramelemorphia (bandicoots), Dasyuromorphia (numbats, quolls and dunnarts), Notoryctemorphia (marsupial mole) and Diprotodontia (kangaroos). Of interesting note is the South American order Microbiotheria. Recent molecular data (e.g., Edwards and Westerman, 1995; Springer, 1995; Kirsch *et al.*, 1997; Nilsson *et al.*, 2004), as well as evidence such as Szalay's (1982) arguments based on relationships in the pes, place the South American taxon *Dromiciops* as the sister group to the Australian diprotodonts. The split appears to have occurred 63 mya (Bininda-Emonds *et al.*, 2007). A fossil microbiotherid from Queensland dating to 55 mya places the exact origin and migration in question (Woodburne and Case, 1996). The timing is right to take advantage of the connections between the three southern Gondwanan continents, and conceivably, this order could have arisen in Antarctica and dispersed as one population to South America where it remained a monotypic species (or a sole surviving relict) and another to Australia. Alternately, the stem microbiotherid could have arisen in Australia (a population having

dispersed from South America and through Antarctica without leaving fossils) while a basal group quickly left and entered South America.

## Phylogeny and Evolutionary History of Macropodidae Relative to Diprotodontia

Classification within the order Diprotodontia has as complex a history as classification in the Marsupialia. The term "diprotodont" refers to the large pair of procumbent lower incisors that characterize this order. The order also shows syndactyly, in which the second and third digits of the pes fuse at the base of the claws (leaving separate, distinct claws). Many species show a further-modified pes with a greatly enlarged fourth digit and lack of a hallux. Prior to the work of Kirsch (1977), the order was divided into two suborders: Vombatiformes, which included the wombats, and Phalangeriformes, which included all other taxa.

The last thirty years of morphological, cytological, biochemical and molecular research (Kirsch, 1977; Archer, 1984; Baverstock, 1984; Aplin and Archer, 1987; Flannery, 1987; Edwards and Westerman, 1995; Springer, 1995; Kirsch *et al.*, 1997) has led to the subdivision of the Diprotodontia into three suborders: Vombatiformes, Phalangeriformes and Macropodiformes. However, the exact taxonomic composition of the Macropodiformes has been an issue of debate. Kirsch *et al.* (1997), using data from DNA studies, divided the Macropodiformes, which contains over 60 extant species, into two families: Macropodidae and Hypsiprymnodontidae (the only extant species of which is the musky rat-kangaroo). They further divided the Macropodidae into two subfamilies: Macropodinae

(kangaroos, wallabies, pademelons and tree-kangaroos) and Potoroinae (potoroos, bettongs and rat-kangaroos). Previously, the Hypsiprymnodontidae were included as a subgroup of the potoroines, but Kirsch *et al.* (1997) elevated them to the level of family based on both their results and the gene sequencing results of Burk *et al.* (1998). Such a familial level designation is supported by morphological data also, as unlike the rest of the suborder, the musky rat-kangaroo retains a prehensile tail like possums, bounds rather than hops, and has not lost its hallux. It further lacks the complex stomach and molar specializations characteristic of the grazing macropodids.

Whereas the familial status of Hypsiprymnodontidae is supported by both the biochemical and morphological data, the subfamily level of Potoroinae is not. Biochemical studies (Kirsch *et al.*, 1997; Burke and Springer, 2000) maintain the potoroines and macropodines within the same family based on their short DNA hybridization distances between the species tested. However, studies based on morphology (Flannery, 1989; Ride, 1993; Groves, 2005) agree that these taxa should be considered distinct families. Whereas such a familial level designation does acknowledge the morphological differences present between the two taxa (described below), it also indicates that the morphological characteristics specific to kangaroos and bettongs, but absent in musky rat-kangaroos (e.g., complex foregut adaptations, molar and mandibular morphology), arose independently in the two groups. One piece of supporting evidence for such an independent evolution is the presence of a rat-kangaroo, which is not a potoroine, in the fossil record with the molar and

mandibular morphology of the two extant groups (Kirsch *et al.*, 1997). This study uses the most recent and most supported taxonomy that places both Macropodidae and Potoroidae in separate families (Groves, 2005).

The earliest known fossil Macropodiformes is from the mid-Miocene of Australia, about 30 mya (Flannery and Rich, 1986). Flannery (1989) hypothesizes that this group is more ancient in origin. In support of such an hypothesis are mitochondrial and nuclear gene studies done by Springer and Kirsch (1991) and Burke and Springer (2000), which place the Macropodiformes split from its possum-like Phalengeriformes ancestor some 38-44 mya and the split between the Hypsiprymnodontidae and the Macropodidae at 34-38 mya. This period witnessed an increase in ice levels in Antarctica and a corresponding drop in sea level, a geological change that connected Australia and Papua New Guinea and a connection which would be repeated several times in the Pleistocene and late Tertiary as sea levels fluctuated (Crook, 1981; Galloway and Kemp, 1981). There were also significant climatic and vegetative changes during this period. Many early researchers (Huxley, 1880; Dollo, 1899) hypothesized that kangaroos evolved during these shifts from a small arboreal species similar to the extant possum *Phalanger*. Current biochemical and molecular work supports such a sister-taxon status of Phalangeriformes to Macropodiformes (Springer and Kirsch, 1991; Burke and Springer, 2000). The rainforest of Australia became restricted to the eastern and southeastern coastal regions while the rest of Australia evolved more arid-adapted

vegetation. At the same time, the rainforest of New Guinea remained. The changes in the macropodid faunas tracked these environmental changes.

Although at their appearance in the fossil record 30 mya the macropodids were still small and adapted to rain forest habitat, by 4-5 mya most of the modern macropodid genera appeared, suggesting a very rapid explosion of species in the late Miocene (Ride, 1964; Flannery, 1989). It is believed that the macropodids then arrived in New Guinea at the end of the Miocene or during the early Pliocene. Three species, *Dendrolagus*, *Dorcopsis* and *Thylogale*, underwent relatively broad radiations upon arrival (Ride, 1964; Flannery, 1989). Overall, the macropodid radiation is as extensive, if not more so, as that of any placental group except perhaps the muroids (Flannery, 1989). They are found in habitats ranging from semi-arid rocky terrain (*Petrogale* and *Macropus rufus*) to grassy plains (most species of *Macropus*) to tropical rainforests (*Dendrolagus*). Macropodids are increasingly being viewed as a highly successful group, well adapted to flourishing in the harsh environment in which they evolved, rather than a primitive, early offshoot of placental mammals (Gilmore, 1977).

Flannery (1989) notes the remarkable number of convergences to placental mammals within the Macropodiformes. More specifically within the macropodids, there is strong convergence with placental grazers as evidenced by many independent appearances of odd, specialized dental characters (e.g., a trend toward adding transverse cutting ridges to the anterior portion of cheek teeth while simultaneously losing, or dramatically reducing, the longitudinal cutting ridges).

Along with others (Raven and Gregory 1946), Flannery (1989) suggests that much of this convergence can be attributed to the new and unexploited habitats opened in the late Tertiary. As a result of the number of dental and cranial convergences, Flannery (1989) speculates that convergent characters will be found in other regions of the skeleton as well. Such possibilities remain to be tested.

## Anatomy of the Macropodidae

### Overall Anatomy

Body size in this taxon varies widely, from the 340 g musky rat-kangaroo (Johnson and Strahan, 1982) to the 85 kg red kangaroo (Jarman, 1989). The hindlimbs are longer and stronger than the forelimbs, and the hind foot is long and narrow. The nonprehensile tail is thickened at the base and is used as a prop (a balancing organ) or for thrust in locomotion (Windsor and Dagg, 1971). The first digit of the pes is absent, the second and third are extremely narrow and united by skin, and the fourth digit is long and strong. The fifth digit is moderately long and thickened (Hopwood and Butterfield, 1990). The exceptions to these general characterizations are seen in three genera. *Dendrolagus*, an arboreal species, has fore- and hindlimbs of nearly equal lengths, nails that curve, a longer tail of even thickness, and in all species except those of the more primitive group (*D. lumholtzi*, *D. bennettianus* and *D. inustus*; Groves, 1982), feet that are shorter. In *Dorcopsis* the feet and hindlimbs are smaller than in the other species, whereas the forelimb remains relatively large (Nowak, 1999). The genus most uncharacteristic of the

group is the more primitive *Hypsiprymnodon*. In this taxon the limbs are of more equal length and the first digit is present and large, although it is not opposable as is seen in the Phalangeriformes. The tail in Phalangeriformes is also markedly different, as it is naked, scaly except at the base, and prehensile (Flannery, 1994).

Reproductive Physiology and Anatomy

Marsupials are distinguished from other mammals by their unique reproductive physiology and anatomy, the extremely small size of their neonates, and their dentition (to be covered in Chapter Three). During fetal development in the marsupial, the reproductive tract of females exhibits two lateral vaginae, two cervices, and two uteri connected to the two ovaries by two separate fallopian tubes. In the male, there is correspondingly a bifurcation of the glans penis. Moreover, just prior to parturition the corpus luteum induces the tissue of the cervix to soften, and as the fetus is birthed, the tissue tears, forming a temporary canal fusing the two lateral vaginae (Tyndale-Biscoe, 1969). Within a day the tear heals so that this canal must be reformed during every birth. The exception is found in the genera *Macropus* and *Tarsipes* (the honey possum). In these two genera the birth canal remains open after the first birth, not needing to be reformed subsequently, and as such is counted as a third or median vagina (Tyndale-Biscoe, 2005).

Marsupial neonates are birthed at a significantly smaller size and more altricial stage of development than are placental mammals. The smallest neonate marsupial is the honey possum (*Tarsepes*), born at a mere 4 mg. It is the smallest

neonate of any known mammal (Nowak, 1999). Even the largest extant marsupial, the red kangaroo (*Macropus rufus*), which weighs in at 85 kg for the male and 35 kg for the female, has a neonate that weighs only 0.750 g at birth (Sharman and Pilton, 1964). By comparison, the female wolf, which as an adult is of comparable size to a female red kangaroo, has a neonate weighing 454 g (Nowak, 1999). Almost all growth and development of the marsupial young occurs while it is attached to the mother's teat. This teat is most often located in a pouch, although not always. In the macropodids this reproductive system is so specialized that the female can be nursing a joey on one teat which is lactating milk of one nutritional composition, nursing a neonate on a second teat which is lactating another nutritional composition, and have an embryo arrested as a blastocyst and held in embryonic diapause in the uterus (Clark and Poole, 1967).

## Behavior of the Macropodidae

### Locomotion and Positional Behavior

The majority of locomotor studies within the macropodids have been conducted on the genus *Macropus*, although a few studies have compared the gaits of a wide range of species within the macropodids (Windsor and Dagg, 1971; Buchmann and Guiler, 1974; Baudenette, 1994). Locomotor studies of the kangaroo generally fall into three categories: gait analysis, biomechanics, and morphology. When moving slowly, kangaroos often utilize a pentapedal gait in which the tail touches the ground during the last few centimeters of the stride. This stride is

referred to as the slow progression (Windsor and Dagg, 1971) or the quadrupedal crawl (Buchmann and Guiler, 1974). This gait is exhibited by all species in the group except for *Dorcopsis* (Table 1), although in *Setonix* the tail is not used during this gait. With an increase in speed, the animals begin to display the bipedal hop in which the two hindfeet land simultaneously, and the center gravity is near the rear of the animal (Windsor and Dagg, 1971). Windsor and Dagg note that in their study the percent time that the hindfeet are in contact with the ground per stride length corresponds more with the animal's habitat (Table 2) than with its size. Animals living in a more rocky terrain requiring the clearance of high objects and those living in more open habitats that allow quick changes in direction are suspended for a greater proportion of the stride (Tables 1 and 2).

Several other specialized gait patterns have also been described (Dagg, 1973; Baudinette, 1994): the walk and the quadrupedal bound. In the walk, which is seen only in the genus *Dendrolagus* (Table 1), both the forelimb and hindlimb support the animal for approximately 70 % of the stride. This gait is usually exhibited when the animals are on horizontal tree trunks. In the quadrupedal bound, which is observed only in the genera *Dendrolagus* and *Setonix* (Table 1), both the hindfeet and forefeet are on the substrate for approximately 50 % of the stride. For both of these gaits there is no period of suspension. Because of their unique anatomy, kangaroos generally do not "walk" backwards as four-legged mammals can (said to be one of the reasons kangaroos are symbolically represented on the Australian coat of arms).

Similarly, they do not generally move their legs independently, except when swimming or lying down (Strahan, 1995).

As an arboreal group, the tree-kangaroos have several other specialized gaits (Proctor-Gray and Ganslosser, 1986). In the arboreal hop, the forelimbs are extended out to grasp the branch simultaneously and then the feet hop together. In the quadrupedal walk, all four limbs are placed slowly and separately when walking on thin branches or descending from a tree. This species also exhibits downward leaps to the ground from heights of 15 – 20 m.

Table 1

Locomotion Types in Macropodidae

| | *Bipedal Hop* | *Quad. Crawl* | *Quad Bound* | *Quad Run* | *Quad Walk* | *Arboreal Hop* |
|---|---|---|---|---|---|---|
| *Dorcopsis* | X | | | | | |
| *Dendrolagus* | X | | X | | X | X |
| *Macropus* | X | X | | | | |
| *Onychogalea* | X | | | | | |
| *Petrogale* | X | | | | | |
| *Setonix* | X | | X | | | |
| *Thylogale* | X | | | | | |
| *Wallabia* | X | | | | | |

Table 2

Habitats of the Macropodidae

| | *Woody Grassland* | *Rocks* | *Swamp Marsh* | *Scrub* | *Grass* | *Tropical Rainforest* | *Sclerophyll Forest* |
|---|---|---|---|---|---|---|---|
| *Dorcopsis* | X | | | | | X | |
| *Dendrolagus* | | | | | | X | |
| *Macropus* | X | | | X | X | | |
| *Onychogalea* | X | | | X | | | |
| *Petrogale* | X | X | | | X | | |
| *Setonix* | | | X | | | | X |
| *Thylogale* | | | | X | X | X | X |
| *Wallabia* | X | | X | | | | |

Diet

All extant macropodids are herbivorous (although the extinct genus *Propleopus* is believed to have been carnivorous). All macropodids are adapted to browsing diets of dicotylydenous leaves and fruits or grazing diets of grasses (Table 3) (Sanson, 1982; Dawson, 1989). The first pair of incisors are long and robust (from which arises the designation "diprotodont"), the lower canines are absent, and the upper canines are small or absent. The premolars are bladelike and narrow, whereas the molars stress either shearing abilities in the grazers or grinding abilities

in the browsers. More specific specializations of dentition will be covered in Chapter Three. As in placental ruminants, the macropodids are foregut fermenters (Freudenberger *et al.*, 1989). The foregut is expanded and populated by bacteria that ferment and digest the high cellulose content of the plant material.

Table 3

Diets of the Macropodidae

| | *Fruit* | *Grass* | *Roots/Tubers* | *Leaves/Shrubs* |
|---|---|---|---|---|
| *Dorcopsis* | X | X | X | X |
| *Dendrolagus* | X | | | X |
| *Macropus* | | X | | |
| *Onychogalea* | | | X | X |
| *Petrogale* | | X | | |
| *Setonix* | | X | X | X |
| *Thylogale* | | X | | X |
| *Wallabia* | | X | | |

## Overall Perspective and Implications for This Study

Clearly there is still much to be done in answering questions of evolutionary relationship and history both within the marsupials and in their comparison with the

placentals. Although comparative biochemical and molecular data have been provided in the last three decades, there are relatively few more traditional comparative morphological studies (Aplin and Archer, 1987). Within the order Diprotodontia, many interesting questions exist. For example, how do the closely related families Potoroidae and Macropodidae relate to each other. Even more specifically, what are the relationships within the genus *Macropus*? (For a review of the difficulties in distinguishing phylogenetic relationships, see Peacock *et al.*, 1981.) Considering the genus *Dendrolagus* or tree-kangaroo, are the adaptations for arboreal locomotion secondary adaptations constrained by their highly specialized terrestrial ancestor, or are they reversions to an earlier and more primitive possum-like morphology? What are the ontogenetic differences in morphology between the specialized Macropodiformes and Vombatiformes as compared to the more generalized Phalangeriformes? What are the ontogenetic differences in morphology between the Macropodidae and the Potoroidae?

The answer to any of these questions necessitates appropriate sampling of museum specimens. Any ontogenetic study must be able to place specimens in different age categories of known order. Any study of morphological differences should be based on samples of adult specimens for comparison with other studies. In most research of mammal taxa the assignment of specimens to adult, subadult or juvenile categories is straightforward based on molar eruptions, basisphenoid sutural closure and long bone epiphyseal closure. In macropodids such an assignment is complicated by growth patterns and molar eruption and progression patterns (as

discussed in the following chapters). Therefore, prior to any large sampling of specimens to address the questions previously noted, there must first be a method available for assigning specimens to appropriate age categories. That is one purpose of this study.

# CHAPTER II

# CREATION OF AGE ESTIMATION SCORES BASED ON PATTERNS OF EPIPHYSEAL CLOSURE IN THE MACROPODIDAE

## Introduction

Adult specimen age is traditionally determined from one of two sources in osteological studies. The first uses the cranium and requires either complete molar eruption, basisphenoid sutural closure, or a combination of the two. The second uses the postcranial skeleton and requires complete fusion between the epiphysis and diaphysis of the major long bones. However, within the Macropodidae these traditional methods are problematic due to continuous growth patterns in many of the *Macropus* species, variability in tooth morphology among the species, and forward movement of the tooth row. At the onset of this project it was quickly determined that traditional age estimation methods would result in the omission of many specimens that appeared to be adult (e.g., fused skull sutures, well-developed sites of muscle attachment and maximum bone length) but were in fact subadult (e.g., partly unfused epiphyses).

This study investigates whether a significant number of traditionally nonadult specimens determined by epiphyseal fusion can be included in an adult

morphological study of the Macropodidae postcrania. It also investigates a reliable criterion for determining age classifications. This project develops both an age estimation based on epiphyseal closure in individual bones and a total fusion score based on closure across all bones of the study. Three main hypotheses are then tested: 1) are there significant differences between proximal and distal epiphyses of a bone; 2) are there significant differences in patterns of fusion between the three long bones; 3) are there regions of epiphyseal fusion or does each long bone fuse independently of each other?

## Patterns of Sexual Dimorphism in the Macropodidae

Outside of the unique reproductive and metabolic differences between marsupial and placental mammals, there is a comparatively small body of knowledge available concerning the Marsupialia. One major deficiency concerns sexual dimorphism, both within the Macropodiformes and within the family Macropodidae. Given its importance in teasing apart explanatory factors to evolutionary, behavioral and functional questions, it is surprising that so little comparative work has been done to describe and explain the extreme sexual dimorphism seen in this family (Jarman, 1989). Whereas there are limited comprehensive or comparative studies that explore patterns, there are numerous individual species studies that report growth rates, pouch young size, and adult size (Dunnet, 1962; Johnston and Sharman, 1976; Johnson and Strahan, 1982; Poole *et al.*, 1982a, 1982b; Sinclair, 1998). These data are important to this study and are summarized below. The wide

range of individual species data, especially when viewed in relation to the lack of comparable data relative to the rest of the Marsupialia, most likely results from a keen interest in the unique locomotion adaptation (bipedal hopping) of the macropodids and/or in their convergent evolution to placental herbivores (a grazing diet). These two areas of study make this group especially interesting.

Species studies reveal that although there are surprisingly no differences in size when the joey first exits the marsupium, or pouch, for any species within the Macropodidae (Tyndale-Biscoe, 1955; Shield and Woolley, 1961; Sadlier, 1963; Sharman *et al.*, 1964; Murphy and Smith, 1970; Maynes, 1972; Rose, 1989), there are many species that reveal medium to large degrees of sexual dimorphism in body size at or before reaching adult size. These include: *Macropus giganteus* and *M. fuliginosus* (Poole *et al.*, 1982a, 1982b); *M. rufus* (Sharman *et al.*, 1964; Kirkpatrick, 1970); *M. robustus*, *M. rufogriseus*, and M. *dorsalis* (Jarman, 1989); and *M. agilis* (Kirkpatrick and Johnson, 1969; Newsome *et al.*, 1977). There are also a few macropodids that exhibit little to no sexual dimorphism: *M. eugenii* (Jarman, 1989), *M. parma* (Maynes, 1976), *Setonix* (Dunnet, 1962) and *Petrogale* (Poole *et al.*, 1985).

## Patterns of Growth in the Macropodidae

One of the few comparative studies of dimorphism in the Macropodidae looked not only at dimorphism in body size but also at heteromorphic growth patterns (Jarman, 1989). In the smaller macropodids (those weighing less than 3 to 4

kg), both males and females stop growing relatively early in adult life. In these species the growth curve plateaus at adult weight, with fluctuations around that weight (Jarman, 1989). In medium- to large-sized macropodid species, the males and sometimes the females exhibit continuous growth. Jarman however, notes that no studies exist for captive animals beyond eight years of age. It is possible that these species exhibit a growth plateau similar to the smaller species but that this plateau occurs late in life. A heteromorphic growth pattern (as determined by skull measurements) is exhibited in *Macropus agilis*, *M. dorsalis*, *M. parryi* and *M. rufogriseus* and raises the possibility that the male growth pattern also decelerates and eventually tapers off; however, these studies were not long enough to provide these data. In other sexually dimorphic species (i.e., *Wallabia* and *Thylogale*), the sexes are essentially homomorphic in their continuous growth rates, so heteromorphic patterns are not linked to sexual dimorphism.

## Other Patterns of Dimorphism in the Macropodidae

Some researchers have noted differences in forelimb length between males and females in many macropodid species (Maynes, 1976; Johnson, 1977; Jarman, 1983). The forelimb (especially the manus) is both longer and carries heavier musculature in males than in females. This holds true for many species even outside the larger *Macropus* species (e.g., *Thylogale thetis*). While studying *Thylogale thetis,* Johnson (1977) discovered that not only is the forelimb longer in males and/or more heavily muscled, but it also grows at an accelerated rate when compared to the

pes and other parts of the body. Other researchers have shown similar results for other macropodid species (Jarman, 1989 for *M. giganteus* and *M. robustus*; Poole *et al.*, 1982b for *M. fuliginosus*). In sum, the length of the forelimb is much greater in males than in females in the large *Macropus* species. Within medium-sized *Macropus* spp., only small differences are observed, whereas the smallest macropodids show no sexual dimorphism in forelimb length. Furthermore, among the macropodids that do show forelimb sexual dimorphism, some show exaggerated musculature, whereas others show exaggerated forearm length. Jarman (1989) conjectures that elongated forelimbs result from an elongation of the manus, whereas a less prominent manus occurs in stockier species.

Agonistic studies within the macropodids reveal two fighting styles that could form the basis influencing male forearm morphology (Ganslosser, 1989). One is a close-in, biting style requiring short stocky arms. This style is exhibited by wallaroos. In other species, such as *M. giganteus*, the males hold each other at arms length to position themselves for a kick or to push their opponent's head back. These species tend to have longer arms. Although all species use the hindlimb to kick when fighting, these limbs are most likely committed to the unique specialization of bipedal hopping and are not as available for evolutionary modification. However, forelimbs are not similarly committed, and they are able to exhibit more morphological plasticity between the sexes and among different species. Jarman (1989) suggests that this is an explanation that contributes to the differences in forelimb growth patterns. This has also yet to be investigated.

## The Epiphysis in Mammals

In the mammalian long bone, the epiphysis is separated from the metaphysis and diaphysis by a cartilaginous disk, or growth plate. Recent studies of human growth indicate that the genetic control of growth occurs at the growth plate as the rates of chondrocyte proliferation at the diaphysis end outpaces or is outpaced by chondrocyte death and osteoblast proliferation, at the epiphysis end (Parfitt, 2002; Nilsson and Baron, 2004). Nilsson and Baron (2004) state that chondrocytes in the growth plate have a finite number of proliferations and as the proliferative capacity ends, growth slows and stops. They state that the cessation of growth intrinsic to the growth plate itself is not directly under the hormonal control of estrogen. In light of the work on telomeres and the Hayflick limit (Hayflick and Moorhead, 1961; Olovnikov, 1996), such an intrinsic method of cell division control appears feasible and intriguing. Both Nilsson and Baron (2004) and Parfitt (2002) agree that unlike the traditional view that cessation of growth is caused by fusion of the growth plate, in actuality, growth stops prior to fusion as chondrocytes cease to proliferate. Fusion then, under its own hormonal controls (in which estrogen plays a large role), follows but is not tied to the cessation of growth. This is perhaps analogous to how cytokinesis occurs during telophase of karyokinesis and yet is not tied to it.

Mechanical loadings experienced at the articular surface via locomotion and postural behaviors stimulate osteoblast activity so as to determine the adult form of the joint (Haines, 1947; Drachman and Sokoloff, 1966; Carter and Wong, 1988; Herring, 1994; Hunziker, 1994). Results from a study of epiphyseal development in

*Didelphis virginiana* (Hamrick, 1999) indicate that the development of positional behaviors in growing animals correlates with both the formation of epiphyseal cartilage and with osteoblast activation and may act as a stimulator to the development of both.

One of the few studies to describe patterns of epiphyseal closure in marsupials was Washburn's (1946) study of the opossums *Didelphis, Philander,* and *Metachirus*. In this study (and in Washburn, 1943) he discusses grouping epiphyses into regions. In each region all the bones involved fuse at a similar time and change their rates of fusion simultaneously. For example, the elbow will have the distal humerus, proximal radius, and proximal ulna fuse simultaneously or very close in time. He then postulates that growth patterns and rates are controlled one region at a time in contrast to one bone at a time. Each region is decoupled from the rest. Evolutionarily, this allows for greater flexibility in achieving morphological change. For example, a change at the elbow would require a mutation in only one developmental gene, rather than simultaneous mutation in three developmental genes.

Washburn (1946) used 30 animals of known age and scored each epiphysis/diaphysis union as either open or completed in union. He then concluded that complete skeletal fusion did not occur in any of the animals, even those with greatly worn dentitions. Washburn (1946) mentions work on the rat (Dawson, 1925, 1927) and the guinea pig (Zuck, 1938), placental mammals which also show failure of the epiphyses to completely fuse. He links these observations to a primitive

pattern seen in reptiles which show continuous growth. In support of this connection is a study by Haines (1941) that examines the epiphyseal structure in lizards and marsupials. Haines concludes that marsupials have a primitive epiphyseal structure similar to reptiles and unlike that seen in placental mammals. Placental mammals exhibit perichondral tissue in the epiphysis that forms cartilage canals branching in a dendritic pattern and terminating near the articular surface. The canals form new chondrocytes at the periphery and allow for the invasion of blood vessels into the cartilage of the epiphysis. These canals are absent in most reptiles (present only in the genus *Varanus*; Haines, 1941) and marsupials and have been secondarily lost in the rat and the guinea pig.

Not only did Washburn's (1946) study reveal mature specimens with still-open epiphyses, it also noted that there was more variability in marsupial closure patterns than in placental mammals. Later work by Sharman *et al.* (1964) proposed that such variability would prove problematic in using epiphyseal fusion as an age estimation method. In part, this current research investigates that proposal. In Washburn's (1946) study, he also compared opossums to several placental mammals (lemurs in Todd, 1930; monkeys in Washburn, 1943; rats in Dawson, 1925; gorillas in Randall, 1944; and bison in Koch, 1935) and noted the same groupings of regional epiphyses across all taxa. His conclusion was that regional epiphyseal groupings were a primitive mammalian character. He also concluded that whereas the sequence of regional fusion could vary between species, timing of fusion at the elbow was consistently first in all mammals.

In a study of *Trichosurus vulpecula*, Tyndale-Biscoe (1955) observed 125 specimens for degree of epiphyseal fusion in the tibia and noted 63 specimens exhibiting complete tibial fusion. His conclusion was that these results differed from those of Washburn (1946). However, this is not necessarily a contradiction as Tyndale-Biscoe's (1955) study only considered the tibia. In contrast, Washburn (1946) considered the entire skeleton. Tyndale-Biscoe's (1955) data table showed specimens in the range of 11 to 32 months. In the oldest of these, the proximal tibia was still open, but the data table does not extend beyond animals of 32 months of age. In contrast, Washburn's (1946) paper describes two animals with extremely worn dentitions (indicating age well beyond 32 months) and states that they still have open epiphyses at the proximal femur, the girdles, and vertebrae. It remains possible that Washburn's (1946) conclusion stems from these two specimens in which the process of fusion is not complete even in the oldest of opossums. Since Tyndale-Biscoe (1955) referred only to the tibia, it is also possible that some or all of his specimens also showed lack of fusion in these areas. This would make their ossification pattern similar too the more primitive opossums and reptiles, not like the placental mammals as he suggested.

Use of Epiphyseal Fusion as an Age Estimator in the Macropodidae

Tyndale-Biscoe's (1955) study goes beyond patterns of epiphyseal fusion to explore the possibility of using the degree of epiphyseal closure to create age categories. Although this study was large (over 125 specimens), the animals were

captured in the field and, thus, no known age was available with which to assess the accuracy of his estimated age classifications. Tyndale-Biscoe (1955) divided the sample into three groups based on no, part, or full fusion of the epiphyses of the tibia. He then recorded the mean tibial length and mean specimen weight for each of the three groups. From this he concluded that sexually immature animals are represented in tibial group three (i.e., less than 1 year of age) and that tibial ossification could be used to divide the animals into two groups: sexually immature and mature. However, since juvenile and subadult growth extends beyond the age of sexual maturity, this is not a very accurate technique.

Kingsmill (1962) attempted to assign actual ages to the corresponding three tibial groups created by Tyndale-Biscoe using 17 skulls and seven skulls plus postcrania of *T. vulpecula* and seven skulls and postcrania of *Perameles nasuta.* However, none of the *P. nasuta* specimens were older than 592 days of age, making these data of limited use for age estimation by epiphyseal fusion. Data was gathered from radiographs of the knee, ankle and wrist. Similar to the Tyndale-Biscoe (1955) study, Kingsmill (1962) divided her sample into three groups; however, as the animals were of known age, she was able to place definitive age limits on each group. Group one had a broad cartilage disk and ranged in age from 177-488 days. Group two had either a narrow cartilage disk or an indistinctly discernable suture line and ranged in age from 488-1519 days. Group three would have included animals with complete fusion, but none were present in this study.

This study seeks to broaden the work by Washburn (1946), Tyndale-Biscoe (1955), and Kingsmill (1962). It uses a large sample size across an entire family (the Macropodidae) to examine the entire forelimb, comparing the epiphyseal fusion groups to an external age criteria (molar eruption scores) and determining patterns of fusion.

## Materials and Methods

### Specimens

Macropodidae skeletal specimens were examined at the following museums: Field Museum of Natural History (FMNH), Chicago; American Museum of Natural History (AMNH), New York; National Museum of Natural History (NMNH), Washington, D.C.; and Museum of Vertebrate Zoology (MVZ), Berkeley. Specimens were included for data collection if they met two criteria: complete fusion of the three bones of the os coxa and humeral and femoral epiphyses that were whole (although not necessarily fused). A summary of the number of Macropodidae specimens measured is shown in Table 4. Included in this table is a breakdown for each genus by sex and by specimen type.

Table 4

Specimens Included in Epiphyseal Study

| | Genus | Total | Male | Female | Unknown | Wild | Zoo |
|---|---|---|---|---|---|---|---|
| | *Dendrolagus* | 38 | 8 | 22 | 8 | 17 | 21 |
| | *Dorcopsis* | 2 | 1 | 1 | 0 | 2 | 0 |
| | *Dorcopsulus* | 4 | 2 | 2 | 0 | 4 | 0 |
| | *Macropus* | 50 | 18 | 25 | 7 | 23 | 27 |
| | *Onychogalea* | 6 | 3 | 3 | 0 | 4 | 2 |
| | *Petrogale* | 18 | 7 | 7 | 4 | 11 | 7 |
| | *Setonix* | 13 | 4 | 6 | 3 | 9 | 4 |
| | *Thylogale* | 22 | 4 | 13 | 5 | 13 | 9 |
| | *Wallabia* | 4 | 2 | 2 | 0 | 3 | 1 |
| **TOTALS** | | **157** | **49** | **81** | **27** | **86** | **71** |

## Creation of Age Estimation Scores for Epiphysis

### Long Bone Fusion Scores

Degrees of closure were recorded for each specimen for the proximal and distal epiphyses of the humerus, radius, and ulna. Each epiphysis was given a score of one for not fused, two for partly fused, and three for completely fused. Fusion was determined by the absence of cartilage between the epiphysis and diaphysis. An epiphysis was determined to be partly fused if fusion had begun in any area. An epiphysis was scored as not fused if either the epiphysis was separated from the diaphysis or if it was attached with cartilage completely surrounding it where it met the diaphysis. Once the degree of fusion at the proximal and distal end was

determined, a numerical progression was devised to represent and track patterns of closure in each of the three bones (Table 5). The progression ranged from one (both epiphyses unfused) to nine (both epiphyses fully fused). In between those two values were ones representing one partly fused epiphysis with an unfused epiphysis (two and three), both epiphyses partly fused (four), one fully fused epiphysis with an unfused epiphysis (five and six), and one fully fused epiphysis with a partly fused epiphysis (seven and eight).

Table 5

Scoring Progression for Epiphyseal Closure

| | Proximal Not Fused | Proximal Partly Fused | Proximal Fully Fused |
|---|---|---|---|
| Distal Not Fused | 1 | 2 | 5 |
| Distal Partly Fused | 3 | 4 | 7 |
| Distal Fully Fused | 6 | 8 | 9 |

Total Fusion Score

Besides the creation of long bone fusion scores, total fusion scores (TFS) were also created as potential estimators of age. The total fusion score consists of the summation of the epiphyseal score for both the proximal and distal epiphyses of all three bones. Possible score values ranged from 6-18. Only those specimens with all three long bones present were used for this portion of the analysis.

## Analysis

To assess differences in fusion patterns among the three forelimb bones, contingency tables were created and analyzed using Bowker's test for symmetry (BTS). This test is a k x k extension of the McNemar test for square contingency tables (May and Johnson, 2001). The null hypothesis in this test is that the probabilities in each cell are symmetrical ($p < .05$). The first hypothesis tested in this section of the study was that there would be no differences between the degree of fusion in the proximal and distal epiphyses in each of the three bones. The second hypothesis tested was that there would be no differences in the patterns of fusion found in each of the three bones.

# Results

## Comparison of Proximal to Distal Epiphyses

The results in Tables 6 – 8 show that the epiphyses forming the elbow fused completely prior to the epiphyses at the shoulder and wrist. In the humerus, 26.5% of the specimens retained a partly fused epiphysis (Table 6), whereas the distal epiphysis was fully fused. Conversely, in the radius (Table 7) and ulna (Table 8), 26.5% and 27.5%, respectively, retained a partly fused distal epiphysis and fully fused proximal epiphysis. All three bones had similar percentages of full fusion at

Table 6

Comparison of Epiphyses of Humerus

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 56 (37%) | 40 (26.5%) | 9 (6%) |
| Distal Partly Fused | 0 | 28 (18%) | 8 (5%) |
| Distal Not Fused | 0 | 1 (0.5%) | 10 (7%) |

Significant at $P < .05$

Table 7

Comparison of Epiphyses of Radius

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 56 (36.5%) | 0 | 0 |
| Distal Partly Fused | 41 (26.5%) | 26 (17%) | 0 |
| Distal Not Fused | 11 (7%) | 1 (0.5%) | 18 (11.5%) |

Significant at $P < .05$

Table 8

Comparison of Epiphyses of Ulna

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 57 (37.5%) | 0 | 0 |
| Distal Partly Fused | 42 (27.5%) | 22 (14.5%) | 0 |
| Distal Not Fused | 17 (11%) | 0 | 14 (9.5%) |

Significant at $P < .05$

both epiphyses, with ranges of 36.5% (radius) to 37.5% (ulna). There was a little more variability in the percentage of fully unfused epiphyses for both ends of the bone: humerus 7% (Table 6), radius 11.5% (Table 7), and ulna 9.5% (Table 8).

## Comparison of Like Epiphyses

In the first part of this section, the radius and ulna were compared relative to each other (Tables 9 and 10). Table 9 reveals 94% of the observations lying along the diagonal of the table, indicating that almost all of the proximal radial and ulnar epiphyses in the study matched. Similarly, Table 10 for the distal radial and ulnar epiphyses reveals 99.5% of the observations lying along the diagonal. Such high percentages suggest no significant differences in fusion between the radius and ulna. For this reason, the final comparisons with the humerus in this chapter were made only in relation to the ulna. In part, the ulna was chosen over the radius for all further analysis because it is represented more frequently in fossil collections (personal observations from MVZ and AMNH). There were a significant number of differences between the proximal ends of the humerus and ulna and between their distal ends. Table 11 reveals that when comparing the proximal humerus and ulna, only 54.4% of the observations lay along the diagonal. Table 12 shows a similar 50.5% of the observations along the diagonal for the distal humerus and ulna.

Table 9

Comparison of Proximal Epiphyses of Radius (top) and Ulna (side)

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 108 (69.5%) | 0 | 0 |
| Distal Partly Fused | 5 (3%) | 23 (15%) | 0 |
| Distal Not Fused | 5 (3%) | 0 | 14 (9%) |

BTS Not Significant

Table 10

Comparison of Distal Epiphyses of Radius (top) and Ulna (side)

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 56 (36.5%) | 0 | 0 |
| Distal Partly Fused | 1 (0.5%) | 65 (42.5%) | 0 |
| Distal Not Fused | 0 | 0 | 31 (20.5%) |

BTS Not Significant

Table 11

Comparison of Proximal Epiphyses of Humerus (top) and Ulna (side)

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 56 (36.5%) | 49 (32%) | 10 (6.5%) |
| Distal Partly Fused | 0 | 19 (9.5%) | 4 (2.5% |
| Distal Not Fused | 0 | 1 (0.5%) | 13 (8.5%) |

Significant at $P < .05$

Table 12

Comparison of Distal Epiphyses of Humerus (top) and Ulna (side)

| | Proximal Fully Fused | Proximal Partly Fused | Proximal Not Fused |
|---|---|---|---|
| Distal Fully Fused | 57 (35.5%) | 0 | 0 |
| Distal Partly Fused | 39 (24%0 | 24 (15%) | 0 |
| Distal Not Fused | 10 (6.5%) | 9 (5.5%) | 11 (6.5%) |

Significant at $P < .05$

## Pattern of Epiphyseal Closure and Creation of Long Bone Fusion Scores

After determining the degree of individual fusion at each epiphysis, the next step in the study was to develop a fusion scoring system for each bone. The system needed to represent age and also be comparable between the three long bones of the forelimb. To arrive at such a fusion scoring system, the pattern of epiphyseal closure for each bone had to be determined and then scores assigned to each step in the pattern. Using the scoring progression for epiphyseal closure from Table 5, the majority of macropodids show a humeral pattern of no fusion at either epiphysis and then part to full fusion with the proximal joint trailing the distal (i.e., a score progression of 1 – 3 – 4 – 8 – 9, Table 13). However, some animals showed no fusion proximally with full fusion distally. This results in a modified score progression of 1 – 3 – 6. Similarly, the ulna and radius in the macropodids also showed two patterns. These patterns were the inverse of those in the humerus as the proximal epiphysis preceded the distal in fusion. The main ulnar and radial score progression was 1 – 2 – 4 – 7 – 9 (Table 13), with a small subset exhibiting a

modified pattern of 1 – 2 – 5, where 5 indicates that the proximal epiphysis was not fused whereas the distal end was fully fused. Under the likelihood that the modified pattern was an artifact (see discussion for explanation), a fusion score was created for all three long bones, with a score range from 1 to 5. In Chapter Three, these scores are used to estimate age in the Macropodidae. Table 14 gives a summary of the fusion scores for each of the three long bones.

Table 13

Patterns of Epiphyseal Closure in the Long Bones

| Bone | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Humerus | 10 | 1 | 8 | 28 | 0 | 9 | 0 | 40 | 56 |
| Ulna | 14 | 0 | 0 | 22 | 17 | 0 | 42 | 0 | 57 |
| Radius | 18 | 0 | 1 | 26 | 15 | 0 | 41 | 0 | 55 |

Table 14

Long Bone Fusion Scores

| Long Bone | 1 | 2 | 3 | 4 | 5 | No Score |
|---|---|---|---|---|---|---|
| Humerus | 10 | 8 | 28 | 42 | 63 | 6 |
| Ulna | 14 | 0 | 22 | 42 | 57 | 17 |
| Radius | 18 | 1 | 26 | 41 | 55 | 15 |

## Analysis of Continuous Growth

As mentioned in the introduction of this chapter, in the medium- to large-sized macropodids the males are reported to exhibit continuous growth. Table 15 reports on the genera (breaking *Macropus* down into species) and sexes encompassed in the 37% of the study specimens in which full epiphyseal fusion was observed. This table will be used to assess the presence and patterns of potential continuous growth in this study.

Table 15

Species and Sex Breakdown for Specimens Exhibiting Complete Fusion

| Species | Body Size | Males | Females |
|---|---|---|---|
| *Dorcopsulus* | Small | 2 | 0 |
| *Petrogale* | Small | 5 | 2 |
| *Setonix* | Small | 1 | 3 |
| *Dendrolagus* | Medium | 7 | 17 |
| *Onychogalea* | Medium | 1 | 0 |
| *Thylogale* | Medium | 1 | 3 |
| *M. eugenii* | Medium | 0 | 1 |
| *M. agilis* | Large | 1 | 1 |
| *M. antilopinus* | Large | 0 | 2 |
| *M. fuliginosus* | Large | 0 | 3 |
| *M. giganteus* | Large | 0 | 1 |
| *M. robustus* | Large | 0 | 1 |
| *M. rufus* | Large | 0 | 1 |

# Discussion

All three bones involved in forming the elbow joint fused at a similar rate as indicated by the high match among a specimen's epiphyseal fusion scores on the distal humerus, proximal radius, and proximal ulna (Tables 6-8). Similarly, the distal radius and distal ulna scores matched for all specimens except one (Table 10). Although data were not collected on the full postcranial skeleton as in the study by Washburn (1946), these data do support his conclusion. Epiphyseal regions of fusion exist in comparison to independently fusing bones. But these data do agree with Washburn's (1946) conclusion that fusion of the elbow occurs first and that this can be viewed as a primitive mammalian characteristic. If morphological change occurs in functional units determined by epiphyseal regions, then it is easy to see how the decoupling between the shoulder, elbow, and wrist allows for greater evolutionary flexibility. One possible example may be a change in the morphology of the wrist and manus in response to the selective pressure of the different fighting styles reported by Ganslosser (1989). A change in functional units also can account for the morphological plasticity in growth patterns discussed in Jarman's (1989) study.

Although this present study only involved the forelimb, it would be interesting to compare the sequence of epiphyseal fusion throughout the body relative to opossums. Washburn (1946) reports that the hip, ankle and knee fuse last (excluding the axial skeleton from his sequencing) in these animals. Given the presence of bipedal hopping locomotion in macropodids after they leave the

marsupium, it would seem logical to predict that these epiphyseal regions would fuse earlier in this family.

Examination of Tables 11 and 12 reveals that for the macropodids, in 34% of the specimens the proximal humerus lags behind the proximal ulna and radius with respect to its epiphyseal fusion score, whereas in 37% of the specimens the two areas match. As the proximal ulna and radius are the parts of the elbow which fuse first, this difference suggests a trend in the macropodids to maintain a zone of growth at the shoulder. However, the specifics of timing cannot be ascertained without comparisons to ages determined by molar eruption scores (to be covered in Chapter Three).

As noted earlier, Jarman (1989) proposed continuous growth for medium to large macopodid species. However, this study shows a full 37% of the sample reached complete closure for the forelimb. Three possibilities exist to explain this result. Either all 37% represent the smaller macropodids where growth plateaus in both the males and the females or all 37% represent a combination of those smaller species and females of the medium- to large-sized species where growth is heteromorphic (females plateau and males do not) or the 37% represents a full mix of all species and sexes.

Table 15 reveals that all but one of the larger specimens in the study with a fusion score of five are female. This result is expected given the plateau in growth observed in this sex. The table also reveals the expected result of both males and females with fusion scores of five for the smaller species in which both sexes exhibit

growth plateaus and for the medium-sized species with no sexual dimorphism (*Dendrolagus* and *Onychogalea*). The unexpected result is in the one male *M. agilis* and the one male *Thylogale* showing full fusion. Both these species are reported to have sexual dimorphism and heteromorphic growth patterns where the male shows continuous growth (Jarman, 1989). The results of this study suggest that perhaps males do plateau in growth, but this occurs either variably or at a time that is later than that covered in Jarman's (1989) study. More data is needed to fully distinguish between these explanations.

The few specimens observed in the macropodids that exhibit modified fusion patterns at either of the long bones can be attributed either to maintenance of completely open joints throughout their lifetimes or more likely an artifact of the bone preparation process. This phenomenon is unlikely to occur just in some animals. However, if it had been the rule and not the exception, it would support Jarman's (1989) conclusions that the animals showed continuous growth. More likely, these epiphyses were partly fused when the animal died and during the drying process the cartilage failed to hold the epiphysis and diaphysis together, in which case the epiphysis was completely removed from the diaphysis and scored as "not fused."

## Conclusion

Although analyses of proximal and distal epiphyses can yield results concerning patterns of fusion, it cannot in and of itself yield quantitative aging

information. Without some external comparison of absolute ages or approximations thereof, it is not possible to evaluate whether all individuals in a particular stage of epiphyseal closure share a similar age or whether several ages are represented within one epiphysis fusion score indicating a prolonged period of growth at that point. For these reasons, the next chapter will examine the epiphysis in relation to eruption of the molars.

The modified fusion patterns in each of the long bones resulted in the inability to assign an epiphyseal rubric score to these specimens. It is predicted that a total fusion score will solve this problem and will also provide a more continuous age estimation method that will correlate more closely with molar eruption scores and therefore provide a better criteria for determining age classes. These questions will be addressed in Chapter Three.

One limit to this study is that in only covering the forelimb, it is not possible to address the discrepancies in the conclusions of Washburn (1946) and Tyndale-Biscoe (1955). In an analysis of the entire skeleton, Washburn (1946) discovered that opossums do not have complete fusion in their entire skeleton and equated this with a retained primitive characteristic shared with reptiles. The mere 36% of the macropodids in this study that had fused epiphyseal regions of the wrist give some support to this open articulation pattern being primitive in the marsupials. A full test of this, however, necessitates a study that examines fusion throughout the Macropodidae skeleton. Future data collection from the metacarpals and phalanges will also allow testing of Jarman's (1989) prediction that forelimb growth in species

without exaggerated musculature occurs by lengthening the manus. If his prediction is true, then the epiphyses in the metacarpals and/or phalanges should remain partly unfused for a longer period of time than do those epiphyses in species that have exaggerated musculature.

# CHAPTER III

# AGE ESTIMATIONS AND MOLAR ERUPTION SEQUENCES IN THE MACROPODIDAE

## Introduction

The proper identification of mammalian cheek teeth is necessary for an assignment to juvenile, subadult or adult classifications. Several factors complicate this identification process in the Macropodidae. The first complicating factor is that there is variation in cheek tooth morphology between and within the family, but little of this has been described in the literature. Only the cheek teeth have been described for *Dendrolagus* (Tate, 1948; Groves, 1982), *Macropus parma* (Maynes, 1972), *Macropus rufus* (Sharman *et al.*, 1964), *Macropus giganteus* (Kirkpatrick, 1969) and *Dorcopsis* (Van Deusen, 1857). The second complicating factor arises from the forward movement of the tooth row in the grazing forms of the Macropodidae. In the larger species like *Macropus*, the last premolar (P4) and the first molar (M1) are lost during this process, so that what is M1, M2 and M3 in one specimen of a species may be M2, M3 and M4 in another specimen of the same species. Finally, the similarities between the two sectorial teeth P3 and P4 and between the molariform teeth dP4 (d designates a deciduous or "milk" tooth) and M1 make it difficult to be

as certain if one is observing a P3/dP4 complex or a P4/M1 complex. These differences can lead to very different age estimations.

## Dental Formulas

Marsupials differ from placental mammals in the number of teeth in their dentition. The total number of teeth can vary from 40-50 depending on the species. One aspect of their dentition that immediately stands out is that marsupials can have as many as five incisors (e.g., *Didelphis*) compared to the typical three in placental mammals. However, they also differ in their cheek teeth. Whereas placentals usually have four premolars and three molars, most marsupials have only three premolars and four molars (with occasional supernumerary molars). The typical dental formula in the Macropodidae is then I 3/1, C 1/0, P 2/2, M 4/4. There is one exception to this; *Macropus* has no canines in either the upper or lower jaw.

## Nomenclature of Cheek Teeth

As described by Thomas (1887), the reduction in overall premolar numbers for the marsupials has led to some differences in premolar nomenclature in the literature. Basing tooth names solely on the three premolars that are present in the marsupials as a group, several authors (mainly American) identify the premolars as P1, P2, and dP3, with a P3 replacement (Groves, 1982; Wroe, 1996; Luckett and Hong, 2000). Other authors (mainly Australian) use the more historical designation of P1, P3 (loss of P2 is described in the section on "Deciduous Dentition"), and dP4, with a replacement P4 (Van Deusen, 1857; Flower, 1867; Thomas, 1887;

Kirkpatrick, 1964; Sharman *et al.*, 1964; Maynes, 1972). The following study will use P3, dP4, and P4 both because it is the older terminology and because it is the terminology used by those individuals using molar eruption scores to identify the ages of macropodids (Kirkpatrick, 1964; Sharman *et al.*, 1964; Maynes, 1972).

## Deciduous Versus Permanent Teeth

Flower (1867) stated that there was only one deciduous tooth present in marsupials. This tooth corresponded to the last premolar in placental mammals and was homologous throughout the Marsupialia. His paper overturned the common belief that all teeth with the exception of the molars were replaced. This opened the door for a discussion on whether the marsupial dentition was to be seen as a permanent series with one deciduous tooth, or as deciduous teeth with only one permanent tooth. This question has evolutionary implications, for either having one set of permanent dentition is the primitive condition, in which case deciduous teeth in placentals is a secondary acquisition, or as some argue, having a deciduous set of teeth is a primitive condition from lower vertebrates and a condition secondarily lost in the marsupials.

Oldfield Thomas (1887) was the first to attempt to determine the homologies of these cheek teeth. He concluded that there was a primitive marsupial condition for both four premolars and molars but that in some cases reduction set in. How reduction is accomplished varied. He postulated that the three premolars present in marsupials are homologous to the first, third and fourth premolars of placental and

extinct early mammals and in most marsupial species the second premolar does not erupt. Part of his evidence stemmed from fossils and a specimen of *Phascogale* that had a second upper premolar, but no corresponding lower second premolar.

A definitive answer to the question of tooth homologies cannot be achieved without a histological examination of embryonic tissue. Kukenthal (1892) provided the first such examination. He discovered the embryological rudiments of successional teeth for all but the second premolar. This rudimentary enamel organ actually develops into an emergent successional tooth in the third premolar (fourth *sensu* Thomas, 1887). From this observation, he concludes that the permanent set of teeth found in the marsupial jaw originates from milk dentition, and thus these are embryonic rudiments of permanent dentition, but in only one case does it fully develop. Although Kukenthal did not himself derive any evolutionary conclusions from his research, it would be logical to conclude that deciduous teeth were not a secondary acquisition in placental mammals but were indeed a primitive vertebrate characteristic. Later researchers have also concluded that the developmental successional pathways of placental dentition and marsupial third premolar dentition are homologous (Luckett, 1993).

Luckett and Woolley (1996) performed an extensive examination of developing embryos from 5 days to 97 days of age. Both Archer (1978) and Luckett (1993) agree that the primitive dasyurid condition is most likely that of a small molariform dP4. They further agree that a molariform dP4 is the primitive condition in didelphids, microbiotheriids, and some other marsupials, making it most likely the

primitive condition for the superorder. Luckett and Woolley (1996) then chose *Sminithopsis* (a marsupial mouse) as their research subject to clarify the homologies in dental eruption for dP4 and P4.

They discovered that there are homologous epithelial connections between the dP4 in marsupials and the premolars in placental mammals. They further discovered that there is a true successional P4 connection to the dP4 from a lingual successional lamina. They conclude that the successional dentition patterns characterize all therian mammals whether those successional teeth erupt or are resorbed.

Confusing the picture is that whereas the anterior two premolars develop from deciduous tooth enamel buds and possess the rudiments of successional tooth development (hence making their correct designation dP1 and dP3 as analogous to the dP4), they are historically (and in the recent literature) referred to as P1 and P3. Further complications arise from the heterochronus development of the cheek teeth. The appearance of the buds of the P1 and P3 is retarded. Luckett and Woolley (1996) postulate that this is due to the lack of successional teeth for all these cheek teeth, unlike the early appearance of a tooth bud in the dP4 which is later replaced by the P4. These researchers conclude that this heterochronus development is a derived characteristic of marsupials with three rather than four premolars. A CT study of a Late Cretaceous juvenile *Alphadon* revealed a similar pattern of succession with the presence of an unerupted P4 deep to dP4 (Cifelli *et al.*, 1996).

## Descriptions of Dentition

The Macropodidae as a group have a reduced premolar number of from three to two through loss of the first premolar. This leaves only the second and third premolars, the last of which is deciduous and replaced by a permanent premolar. As indicated in the previous section, the Australian designation for these two premolars will be used in this study. Therefore, these premolars are indicated with the designations P3 and dP4. The replacement process of the dP4 also displaces the P3 from the jaw as both its roots and those of the dP4 are absorbed by the P4. However, the timing and order of this absorption and loss of P3/dP4 is variable. As Maynes (1972) notes, either tooth could have its roots absorbed first and fall out. Furthermore, the process can vary between the right and left side of the jaw.

In the Macropodidae, the P3 shares many common characteristics, although it can vary in its size and the timing of its loss (cheek teeth morphology summarized in Table 16). Along with the dP4, the P3 erupts as the animal is leaving the pouch and prior to the eruption of M1 (Van Deussen, 1857; Tate, 1948; Maynes, 1972). However, its eruption is delayed such that it is actually the second tooth to erupt after dP4 (Maynes, 1972). It is a sectorial tooth, longer than it is broad, and smaller than the next cheek tooth, dP4 (Fig. 1). Its size, relative to the permanent sectorial tooth, P4, varies. The size of P3 is reported as smaller than P4 in *Dorcopsis* (Van Deusen, 1857), similar in size in *M. parma* (Maynes, 1972), and larger than P4 in *M. rufus* and *M. giganteus* (Sharman *et al.*, 1964; Kirkpatrick, 1969). Its main ridge is close

Table 16

Literature Review of Cheek Tooth Morphology

| | P3 | dP4 | P4 | M1 |
|---|---|---|---|---|
| *M. rufus*<br><br>(Sharman *et al.*, 1964) | Sectorial tooth broader than long<br>Sectorial ridge is on labial side and inline with outer dP4 cusps<br>Inner cusps inline with inner cusps of dP4 | Smaller than M1<br>Anterior cingulum rises to paracone | Narrow sectorial ridge<br>Much smaller M1<br>Ridge aligns with longitudinal ridge of M1<br>Prominent post cusp not connected to sectorial ridge | Anterior cingulum separated from anterior loph |
| *M. parma*<br><br>(Maynes, 1972) | Sectorial similar size P4<br>Developed lingual cingulum with small ant and large post cusps that connect to sectorial ridge<br>Sectorial ridge inline with outer dP4 cusps<br>Inner cusps inline inner dP4 cusps | Molariform similar in morphology M1<br>Smaller M1<br>Anterior cingulum rise to meet paracone<br>Paracone functionally continuous with P3 ridge | Longer than M1<br>Sectorial ridge inline with M1 longit. ridge<br>Inner posterior cusp<br>Blunt and curved anterior border | Anterior cingulum separated from anterior loph |
| *M. giganteus*<br><br>(Kirkpatrick, 1964) | No information | No information | Smaller than M1<br>Replace P3/dP4 at 18 months | Strong longitudinal ridge |
| *Dorcopsis* | No information | No information | Sectorial ridge in midline<br>Longer than M1<br>Inner post cusp is large | No information |
| *Dendrolagus* | Sectorial broader than long<br>Longer than dP4<br>Outer cusps with anterior division and continuous w/ outer dP4/M1 cusps<br>Medial cingulum with ant. and post. cusps<br>Post. cusp larger | Separation of ant. cingulum not distinct<br>Ant. cingulum rises to meet paracone | Longer than M1<br>Sectorial ridge inline with M1 longit. ridge<br>Post. sectorial ridge rises<br>Inner posterior cusp higher but can see ant. | Ant. cingulum not meet paracone<br>Distinct separation paracone and cingulum<br>Has longitudinal ridge |

to the labial surface and in line with the outer cusps of the remaining cheek teeth so that it forms a continuous functional unit with the paracone of dP4. Its lingual cusps, formed by a cingulum that is raised anteriorly and medially, are in line with the lingual cusps of the remaining cheek teeth, also forming a continuous functional unit. The posterior lingual cusp is much more pronounced than the anterior, but the anterior is distinguishable.

The next cheek tooth, dP4 (Fig. 1), is the first molariform tooth to erupt and is significantly smaller than the M1. Its anterior cingulum extends up towards the labial surface so that it forms an extension of the paracone, making it functionally continuous with both the P3 and M1 (Sharman *et al.*, 1964; Maynes, 1976). There is also less definitive separation between the anterior cingulum and the main cusps as compared to the anterior cingulum in M1.

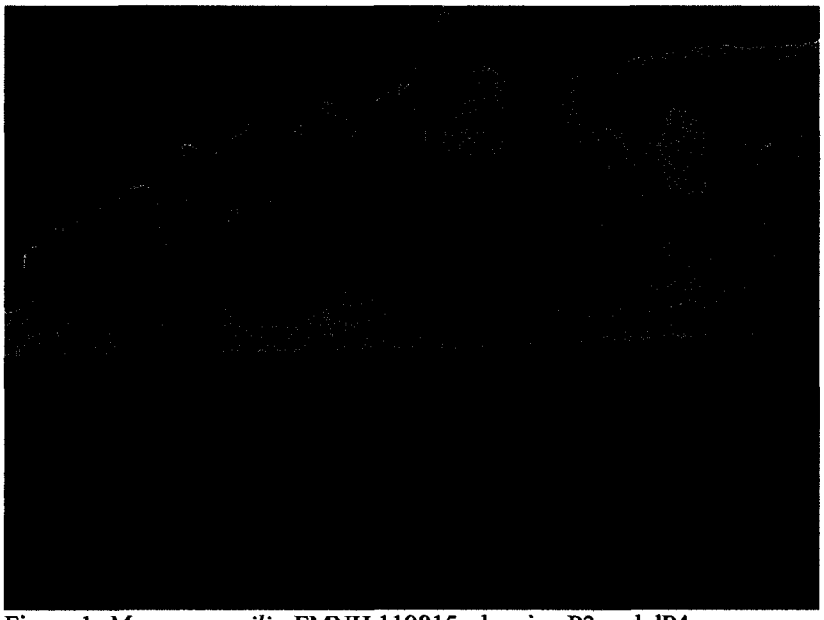

Figure 1. *Macropus agilis*, FMNH 119815, showing P3 and dP4.

The successional premolar, P4, is also a sectorial tooth. It is the most variable in size of all the premolars. In *Dendrolagus* and *Dorcopsis* it is much larger than the M1 (Van Deusen, 1857; Groves, 1982). However, in the larger *Macropus* species it is significantly smaller (Fig. 2) (Kirkpatrick, 1964; Sharman *et al.*, 1964), and in the smaller *M. parma* it is similar in size to the slightly larger M1 (Maynes, 1972). It is narrower than the sectorial P3 and has a central ridge rather than an outer ridge. This ridge is in line with the longitudinal ridge connecting the anterior and posterior lophs of the molar teeth. The shearing blade of the central ridge is more pronounced than in the P3. Whereas the P3 has several distinct lingual cusps formed from its raised cingulum, the P4 has only an enlarged posterior inner cusp that aligns with the inner cusps of the M1. Finally, as mentioned above, the eruption timing of this tooth varies. In most species, it is reported as erupting with the M3 before the M4 has begun to erupt (Kirkpatrick, 1964; Sharman *et al.*, 1964; Maynes, 1972; Groves, 1982). However, in *Dendrolagus lumholtzi* and *Dorcopsis* it is reported as erupting late when the M4 is partly erupted or completely erupted (Van Deusen, 1857; Groves, 1982).

Given the attention spent on describing the premolars in the Macropodidae, relatively little has been focused on the molar teeth. In a discussion of adaptations to diet in the Macropodidae, Sanson (1982) describes the differences between molars in the more derived grazers (*Macropus* and *Onychogalea*) and the remainder of the more primitive browsing macropodids (with the exception of *Petrogale*, which he places in an intermediary classification). In grazers, there is a strong longitudinal

ridge between the anterior and posterior lophs, a broader anterior cingulum and the evolution of molar progression. In browsers the longitudinal ridge and anterior cingulum are less pronounced and no molar progression is evident.

Figure 2. *Macropus antilopinus*, FMNH 120569, showing P4 and M1.

## Age Estimation Studies in the Macropodidae

Previously, age estimation in the Macropodidae has been conducted by head-body length measurements of pouch young or by tooth eruption and forward progression of the tooth row. Measurements of head-body length are consistent between field and captive animals while the young are still in the pouch (Shield and Woolley, 1961; Sharman *et al.*, 1964). This is true for a wide variety of marsupials including *Didelphis* (Petrides, 1949; McManus, 1974), *Dasyurus* (Schmitt *et al.*, 1989), *Setonix* (Shield and Woolley 1961), *Macropus* (Sadlier, 1963), and *Potorous* (Hughes, 1962). It has been hypothesized from these widespread results that the nutritional environment of the young inside the pouch is relatively stable barring extreme conditions, in which case death of the pouch young occurs. However, once the young exit the pouch, the correlation between age and body size drops significantly.

Molar eruption sequences in field studies have been successfully used and checked against known ages in captive animals (Shield, 1958; Sadlier, 1963; Ealey, 1967; Maynes, 1972; Lentle *et al.*, 2003). Several authors have also used the forward movement of the molars in the maxilla as an estimation of age (Sadlier, 1963; Ealey, 1967; Lentle *et al.*, 2003). Forward progression of the molars has only been used in conjunction with molar eruption. Shield (1958) created the first scoring system for molar eruption based on protrusion of both the anterior and posterior lophs above the gum line. Later researchers based their scoring systems on Shield's (1958) work. In all of these systems, a fully erupted molar gets a roman numeral.

The partially erupted molar behind it receives a decimal score (the ranges varying between researchers), e.g., X.0 for both lophs below the maxilla and X.1 for an anterior loph through the maxilla but below the gum, with the posterior loph below the maxilla. Sharman *et al.* (1964) note how in actuality it is impossible to differentiate a score of X.0 from X.1 since in neither case is there a visible loph.

Several methods have been used to measure the forward movement of the molars in the jaw. Sharman *et al.* (1964) used the position of the molar relative to the descending process of the zygomatic. Different scores were given when a loph was opposite the process, when the troph between the lophs was opposite the process, and when the process was between two molars. They did note that it was difficult to achieve accuracy with their method in living animals. They also concluded that the amount of variation in the position of the process relative to the molar between animals of known same age was great enough as to render this methodology of little use. However, they also concluded that this method was better than the highly subjective criteria of tooth wear. In a similar study, Kirkpatrick (1964, 1969) observed three macropodid species that ranged in age from one to three years and measured both molar eruption and molar progression. From these data he derived a molar index which he then regressed on known age. Rather than using the zygomatic process, Kirkpatrick (1969) used a reference line that extended across the anterior edge of the two orbits. As the eruption and progression in the right and left sides of the jaw can be different, he averaged the score for the two sides when

necessary. The advantage to Kirkpatrick's (1969) molar index is that it is less subjective than the assessment of how far along a specimen is in molar eruption.

In a study of the yellow-footed rock-wallaby (*Petrogale xanthapus*), Poole *et al.* (1985) used three different methods of scoring molar eruption and progression. They then evaluated the efficacy of each method. They concluded that when molar progression based on the descending process of the zygomatic was measured by x-ray, rather than by hand (Sharman *et al.*, 1964), it was as equally correlated with age as either molar eruption score or molar progression using the anterior orbit reference line. They reported similar percentages of variance above 95%.

In a study of the agile wallaby (*Wallabia agilis*), Newsome *et al.* (1977) concluded that molar eruption stages are not only dependent on age but also affected by sex, with molars in males erupting slightly earlier than molars in females. They postulated that in other sexually dimorphic macropodids, there will be similar differences between male and female eruption times. Using the methodology of Kirkpatrick (1964), Newsome *et al.* (1977) scored 10 stages of molar progression and five stages of molar eruption. Age was expressed in days. They then regressed both measures on age in days and found a high correlation between molar eruption and molar progression, though the molar eruption data provided a smoother curve.

In *Macropus parma*, Maynes (1972) was able to graph his molar eruption stage by using captive animals of known ages (weeks) and tracing the best fit curve to the data. This plot then showed the mean age for each molar eruption stage. He also used 11 animals to calculate a regression formula based on age and molar index

(derived from molar progression as done in Kirkpatrick, 1964). In the earlier study of Sharman *et al.* (1964) on *M. rufus*, a regression formula was created by regressing molar scores on age in months. Though the two regression formulas are not directly comparable, the ages calculated from each regression can be compared. The results show great variability (e.g., in *M. parma* a molar score of II.4 represents an age of 130 weeks; in *M. rufus* the same molar score represents 168 weeks after conversion from months). This suggests that whereas molar scores are good at estimating age in a species, they are not directly useful when applied to another species, especially when those two species vary widely in size and growth patterns as do the two species of *Macropus* above. This study seeks to create a regression formula based on specimens across the family, rather than within one species, so that a single formula is applicable to all family members.

## Materials and Methods

### Specimens

As age estimations using molar scores was not initially part of the study protocol, it was not possible to return to all four museums to collect tooth identification data and photographs. Therefore, data for this portion of the analysis came only from the specimens housed at the Field Museum of Natural History (FMNH), Chicago, and the National Museum of Natural History (NMNH),

Washington, D.C. (Table 17). Specimens were photographed and scores were calculated using the left maxilla.

Table 17

Specimens Used for Molar Eruption Scoring and Tooth Descriptions

| Genera | Total Numbers |
|---|---|
| *Dendrolagus* | 15 |
| *Macropus* | 21 |
| *Onychogalea* | 3 |
| *Petrogale* | 4 |
| *Setonix* | 3 |
| *Thylogale* | 12 |
| TOTAL | 52 |

## Tooth Identification and Descriptions

Extremely young individuals (determined by lack of fusion at the acetabulum of the three bones of the os coxa) were examined and photographed to use as types for the P3, dP4 and M1 (Fig. 3). The type specimen photographed for the P4 was chosen by examining and photographing an individual with obliteration of the skull suture lines and complete fusion in all epiphyses (Fig. 4). Using these type photographs and the descriptions in the literature, all study specimens were

photographed using a digital Konica Minolta camera (4.0 megapixels) with a macro setting. After photography, the dental formula for each specimen was recorded, noting the specific identification of the cheek teeth present. Finally, each specimen was given a molar eruption score.

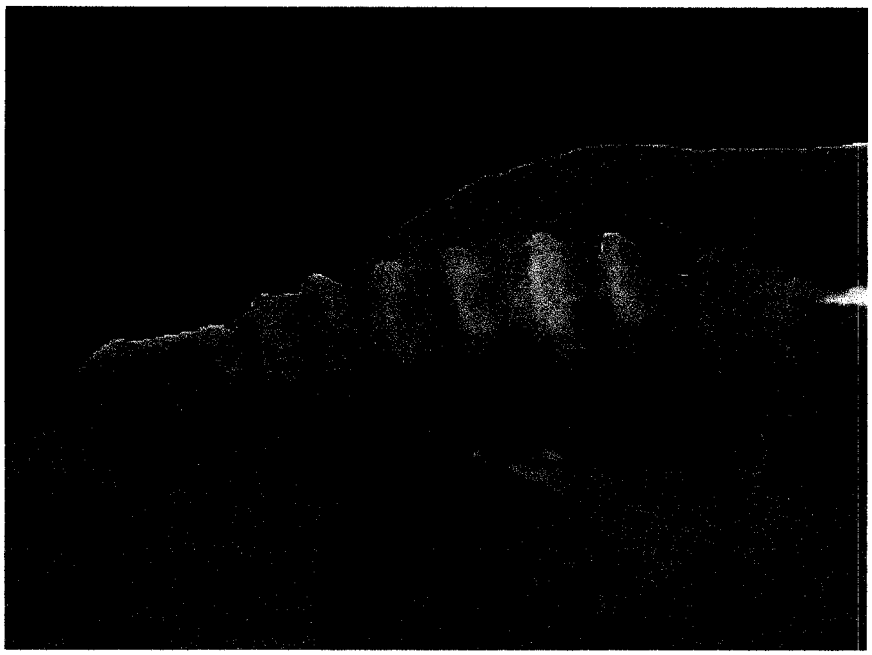

Figure 3. Type for macropodid P3, dP4 and M1 using FMNH 60411.

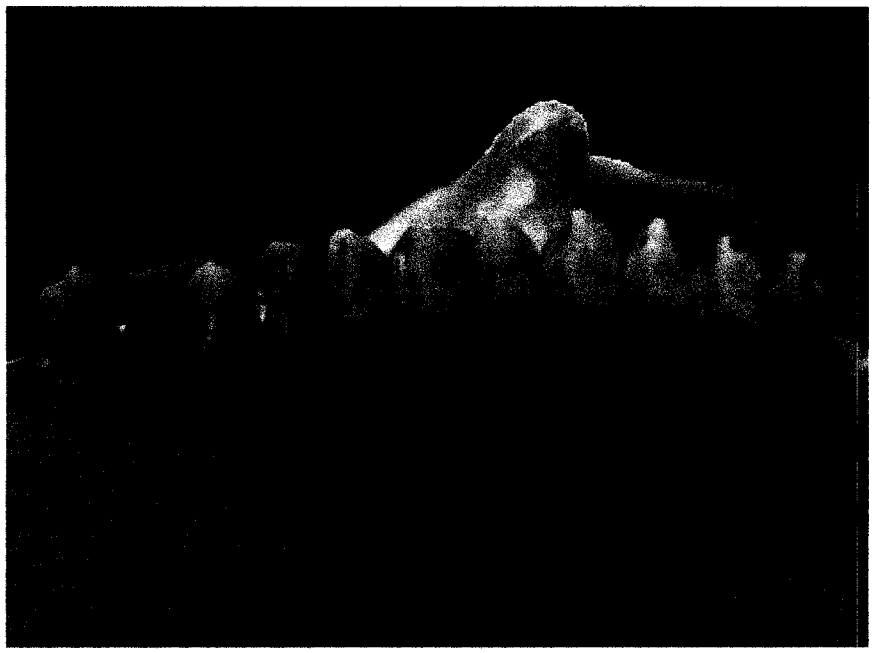

Figure 4. Type for macropodid P4/M1 using FMNH 150720.

## Molar Eruption Score

The molar eruption scoring system of Sharman *et al.* (1964) was used as the basis for the scoring in this study. As his scoring system was designed for field animals and based on eruption through the gums, this system had to be modified slightly. Both Sharman and colleagues' (1964) original system and the modifications for this study are given in Table 18. As an illustration of how the system works, the roman numeral I is used below to indicate complete eruption of the first molar. The table illustrates how the partial eruption of the second molar would be scored in relation to the first molar.

Table 18

Molar Eruption Scoring System

| *Scoring* | *Description* | | | |
|---|---|---|---|---|
| | ***Sharman et al.(1964) Study*** | | ***Current Study*** | |
| | **Anterior Loph** | **Posterior Loph** | **Anterior Loph** | **Posterior Loph** |
| I.0 | Not visible | Not visible | Not Visible | Not Visible |
| I.1 | Score not Used | Score not Used | Open but below bone | Open or closed below bone |
| I.2 | Through gum | Below Gum | Through bone | Open but below bone |
| I.3 | Partly erupted | Just breaking through gum | Not as high as next tooth | Not as high as next tooth |
| I.4 | Fully erupted | Completing eruption | Even with next tooth | Not as high as next tooth |

## Timing of P4 Eruption

Molar scores were compared to the presence of the P3/dP4 premolar complex or the P4/M1 premolar complex by qualitatively recording the numbers of specimens falling into each scoring group. The genus *Macropus* was broken down into three body size categories: 1) small (those species less than 5 kg); 2) medium (those species between 5 and 20 kg); and 3) large (those species greater than 20 kg) (adapted from Jarman, 1989) in order to investigate whether the discrepancies in the literature between *M. parma* and *M. rufus* are linked to body size. As the data

revealed that no specimens had an erupted P4 prior to the presence of the M3, a contingency table was created to qualitatively assess at what stage in molar eruption the P3/dP4 complex was replaced by the dP4/M1 complex.

## Analysis

All analyses and plots were generated using SPSS for Windows, release 11.5. Total fusion score and the individual epiphyseal fusion scores for each long bone (independent variable, X) were regressed against molar eruption scores (dependent variable, Y) using a least squares regression ($p < 0.05$). This method of regression calculates the best fitting line for the observed data by minimizing the sum of squares of the deviations of each data point from the regression line. The assumptions of least squares regression are that the variables are continuous, the error is uncorrelated, and the distribution is normal. Pearson correlations ($p < .05$) were also computed for the molar score with each of the long bone scores. Bivariate plots showing regression lines and 95% confidence intervals, as visual representations of X and Y, were generated using the scatterplot command.

# Results

## Tooth Descriptions

Due to limitations in specimen availability, a complete description of P3 – M1 in all genera of the Macropodidae was not possible in this study (notably no

specimens of *Wallabia* were available). However, some important gaps in the literature were filled in (italicized portions of Table 19). With a few exceptions the photographs of the macropodid genera fell within the general characteristics described in the literature (Figs. 5 – 11). One such exception is that in *Dendrolagus* the P4 is a very long sectorial tooth (Fig. 5). It has a characteristic dip in the middle of the ridge and, though small, it has two anterior inner cusps that can be distinguished (Fig. 5). Another distinguishing characteristic of the dentition in *Dendrolagus* is that the anterior cingulum of the molar teeth is not clearly separated from the paracone as it is in other macropodids (compare Fig. 5 and Fig. 6).

## Timing of P4 Eruption

Tables 20 and 21 combine all the molar scores into groups based on the last completely erupted molar and then look at how many specimens for each taxa possess that score within the P3/dP4 complex and the P4/M1 complex. As Table 19 shows, the molar eruption score of III.___ is the boundary between the two tooth complexes. Table 20 examines the presence or absence of the P4/M1 complex while the M4 is erupting (i.e., a score of III.1-III.4). The presence of many cells with a zero value prohibited quantitative analysis. However, qualitative trends are visible and discussed in the following discussion section.

Table 19

Descriptions of Cheek Teeth in the Macropodidae

| | P3 | dP4 | P4 | M1 |
|---|---|---|---|---|
| *Dendrolagus* | Sectorial tooth broader posteriorly;<br>Broader than long;<br>Longer than dP4;<br>Outer cusps that wear as a dip in ridge and continuous w/ outer dP4/M1 cusps;<br>Medial cingulum with ant. and post. cusps;<br>Post. cusp larger | Separation of ant. cingulum not distinct;<br>Ant. cingulum rises to meet paracone;<br>Anterior cingulum narrow | Longer than M1;<br>Sectorial ridge inline with M1;<br>Longit. ridge;<br>Post. sectorial ridge rises;<br>Inner posterior cusp higher but can see ant.;<br>Heavier wear in middle of ridge;<br>Ridge inline with outer M1 cusps | Ant. cingulum not meet paracone;<br>Distinct separation paracone and cingulum;<br>Has slight longitudinal ridge |
| *Macropus small* | Sectorial broader than long;<br>Ridge inline with outer dP4 cusps;<br>Similar size dP4;<br>No clear cingulum<br>Wear pattern produces angle on inner surface | Ant. cingulum not clearly separated;<br>Ant. cingulum rise to meet paracone;<br>Longitudinal ridge;<br>Smaller than M1 | Larger M1;<br>Sectorial with only an inner posterior cusp;<br>Remaining cingulum is thin;<br>Low ridge;<br>Sectorial ridge inline with longitudinal ridge M1 | Ant. cingulum not meet paracone;<br>Distinct separation cingulum and lophs;<br>Has longitudinal ridge |
| *Macropus medium* | Sectorial;<br>Slight lingual cingulum with posterior cusp large;<br>Sectorial ridge inline with outer dP4 cusps;<br>Similar size dP4;<br>Smaller M1 | Ant. cingulum not clearly separated;<br>Ant. cingulum rise partway to meet paracone;<br>Longitudinal ridge;<br>Smaller than M1 | Larger M1;<br>Sectorial with only an inner posterior cusp;<br>Remaining cingulum is thin low ridge;<br>Sectorial ridge inline with longitudinal ridge M1 | Anterior cingulum forms shelf with no rise to paracone;<br>Strong longitudinal ridge |

(Continued on following page)

Table 19 (Continued)

| | P3 | dP4 | P4 | M1 |
|---|---|---|---|---|
| *Macropus large* | XXX | XXX | Smaller M1;<br>Sectorial ridge inline with longitudinal ridge;<br>Lingual cingulum absent except for posterior cusp | Anterior cingulum forms shelf with no rise to paracone;<br>Strong longitudinal ridge |
| *Onychogalea* | *Smaller than dP4;*<br>*Outer cusps with anterior division when worn and continuous w/ outer dP4/M1 cusps* | *Ant. cingulum not clearly separated;*<br>*Ant. cingulum rise to meet paracone;*<br>*Longitudinal ridge;*<br>*Smaller than M1* | *Pathology in tooth and cannot determine morphology* | *Anterior cingulum forms shelf with no rise to paracone;*<br>*Strong longitudinal ridge* |
| *Petrogale* | XXX | XXX | *Similar size w M1;*<br>*Strong post inner cusp w/ no ant.;*<br>*Sectorial ridge inline with longitudinal ridge* | *Ant. Cingulum not meet paracone;*<br>*Less distinct separation paracone and cingulum;*<br>*Has longitudinal ridge* |
| *Setonix* | XXX | XXX | *Sectorial ridge inline with longitudinal ridge;*<br>*Larger M1;*<br>*Lingual cingulum; Large posterior cusp and slight anterior* | *Ant. cingulum not as pronounced;*<br>*Longitudinal ridge less evident* |
| *Thylogale* | *Similar size w/ dP4;*<br>*Smaller M1;*<br>*Both post and ant inner cusps w/ post larger;*<br>*Sectorial ridge inline with outer dP4 cusps* | *Ant cingulum slopes to paracone;*<br>*Has longitudinal ridge* | *Longer than M1;*<br>*Post inner cusp high but rest is distinguishable;*<br>*Sectorial inline with longitudinal ridge* | *Ant. cingulum shows notched separation from paracone;*<br>*Has longitudinal ridge* |

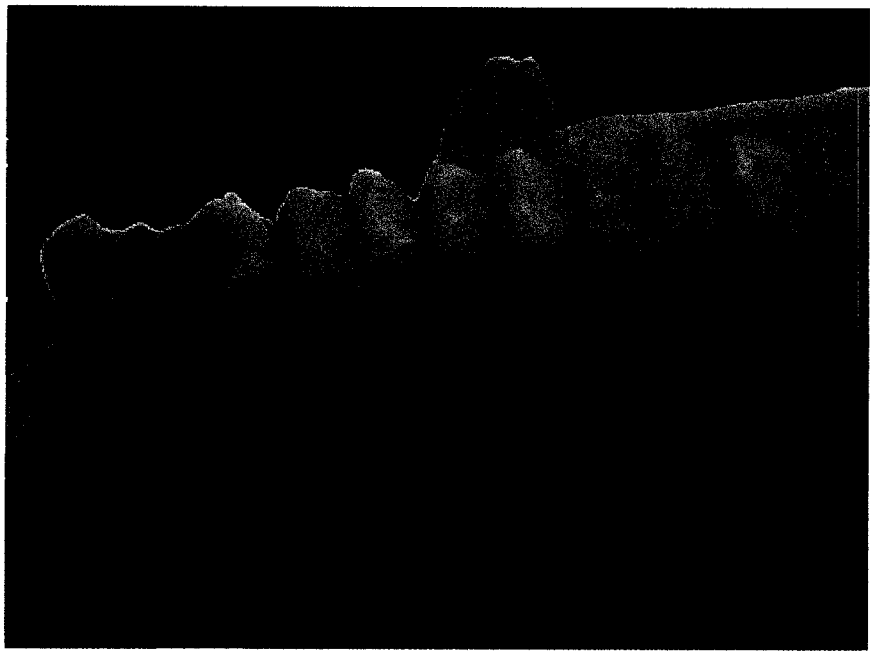

Figure 5. NMNH 399284, *Dendrolagus*, showing the P4/M1 complex.

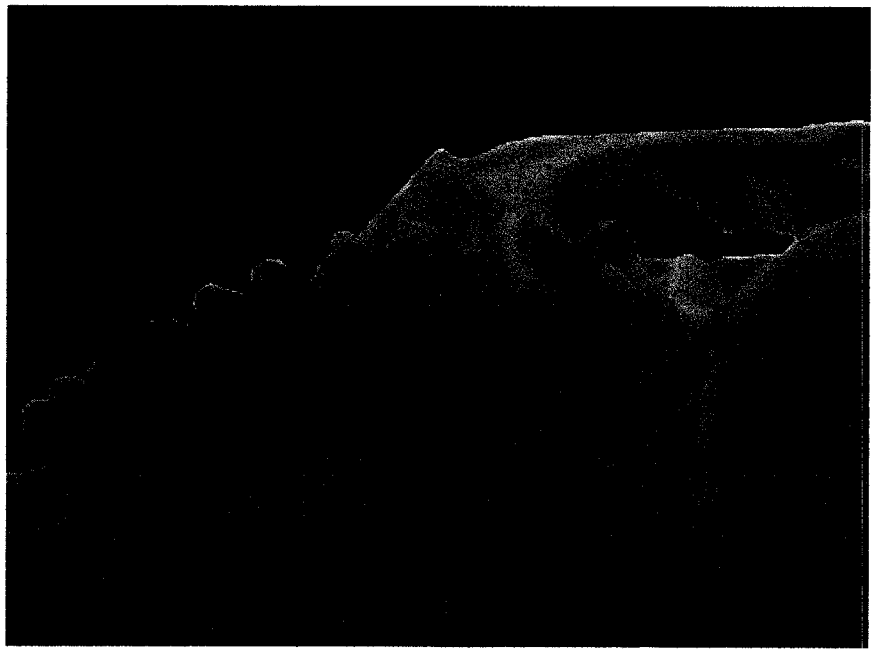

Figure 6. FMNH 119821, *Onychogalea*, showing the P3/dP4 complex.

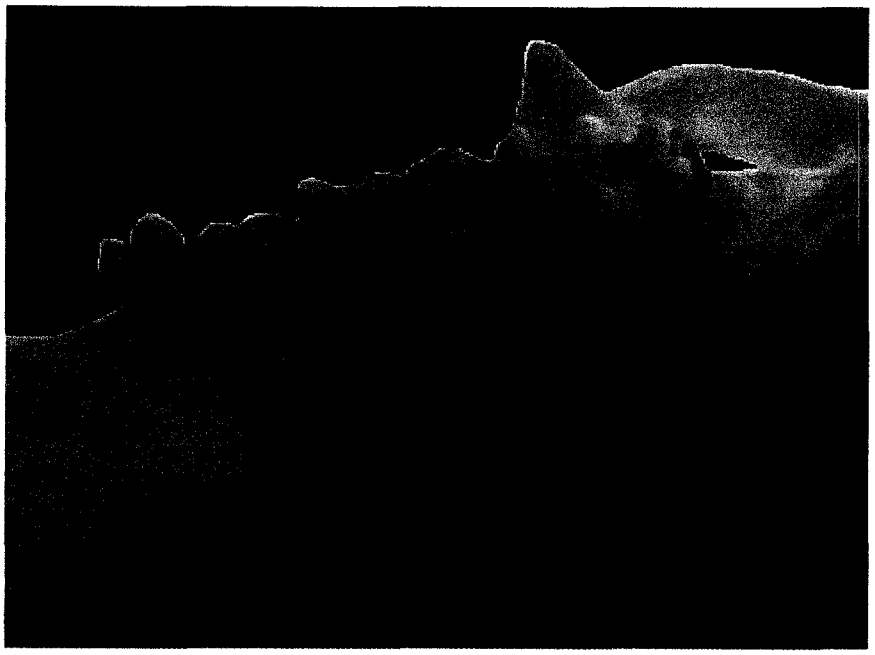

Figure 7. NMNH 237646, *Onychogalea*, showing the P4/M1 complex.

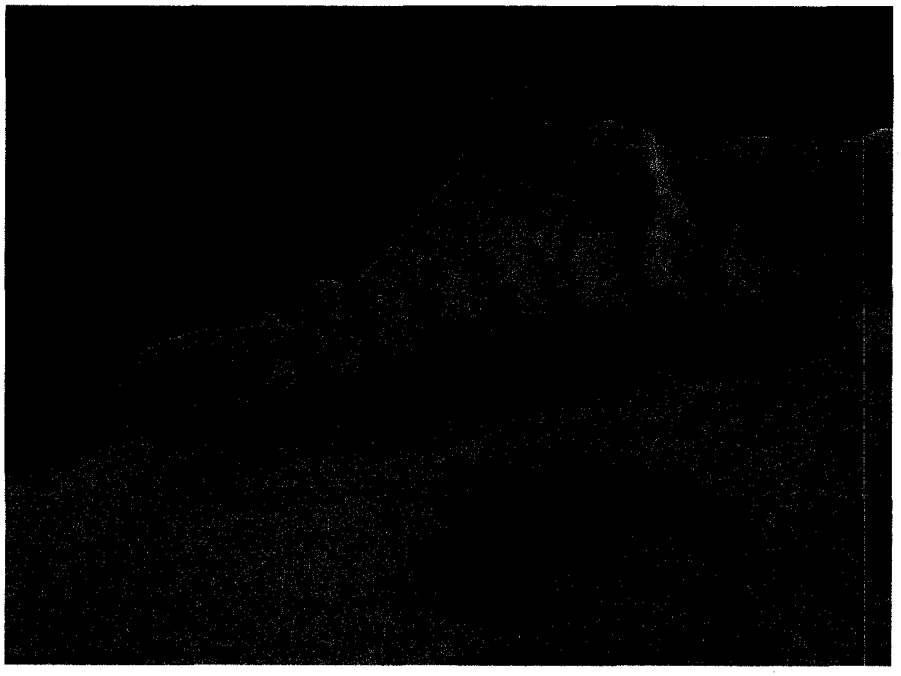

Figure 8. FMNH 67712, *Setonix*, showing the P4/M1 compelx.

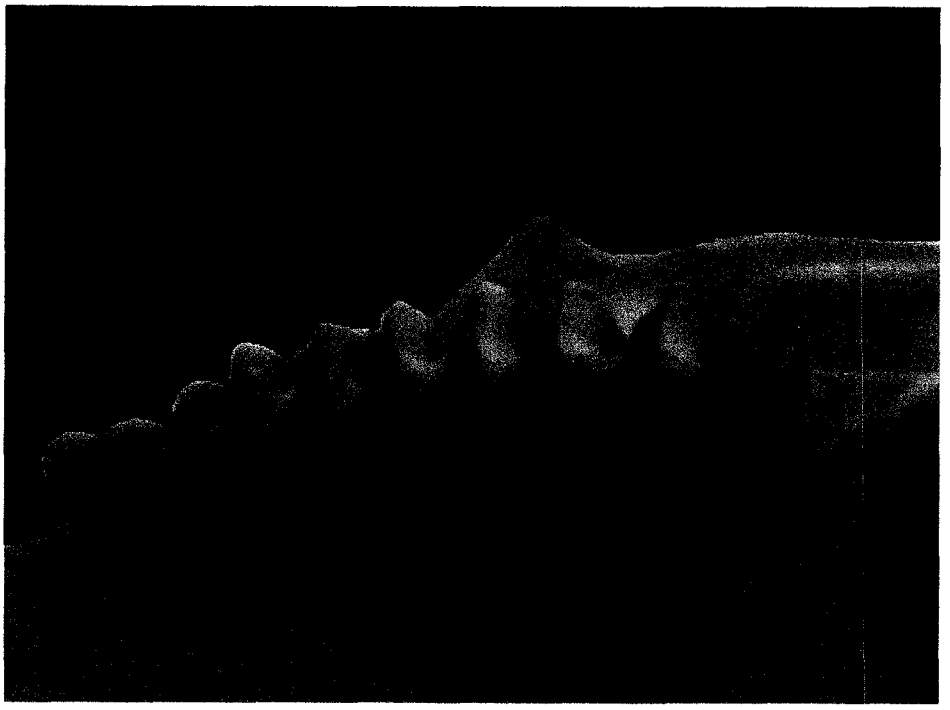

Figure 9. NMNH 238325, *Thylogale*, showing P3/dP4 complex.

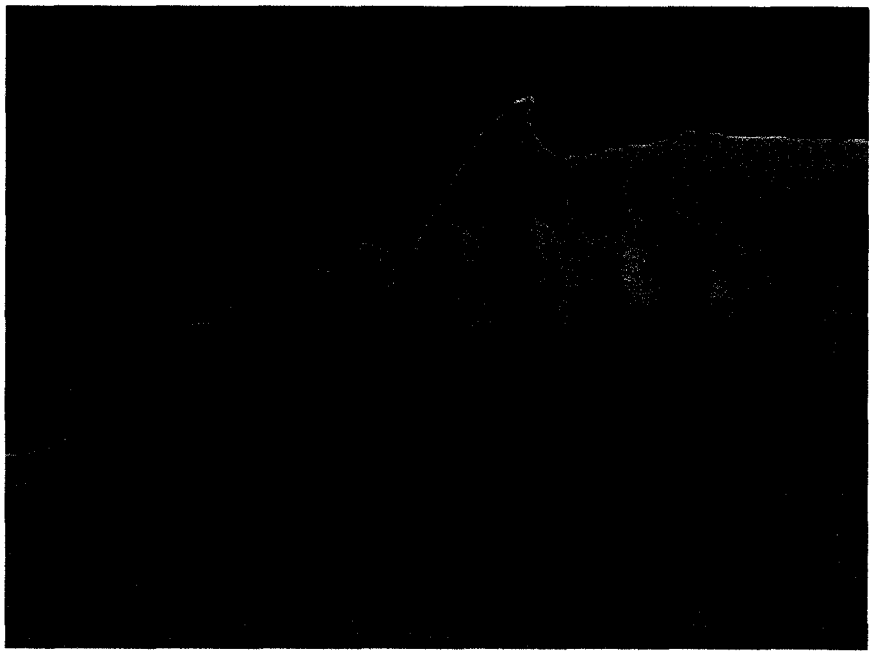

Figure 10. NMNH 60627, *Thylogale*, showing P4/M1 complex.

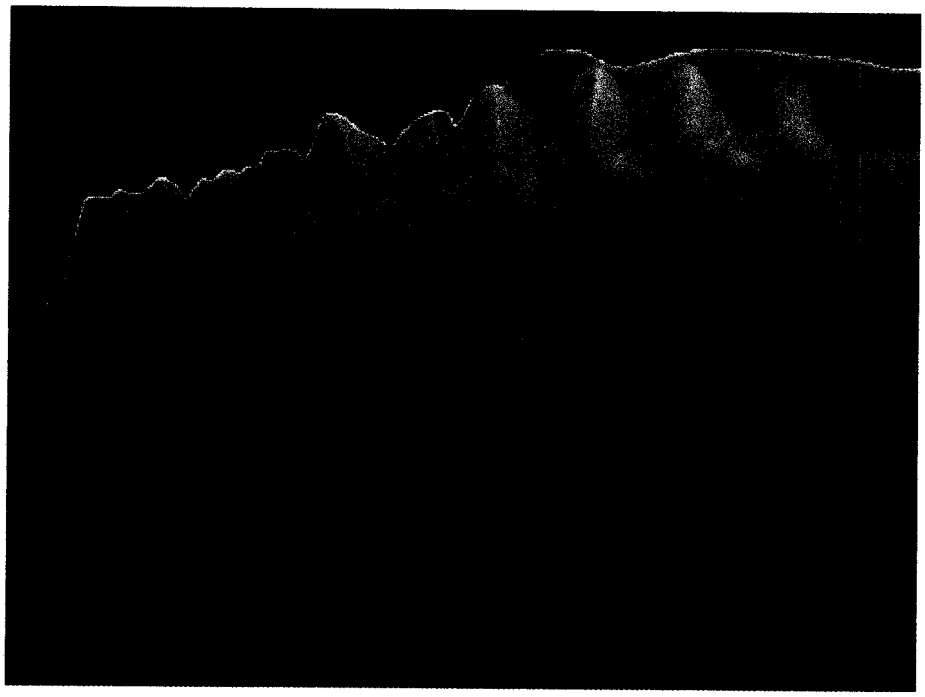

Figure 11. NMNH 155604, *Petrogale*, showing P4/M1 complex.

Table 20

Molar Eruption Scores with P4 Present

| Taxa | I.__ | II.__ | III.__ | IV.__ |
|---|---|---|---|---|
| *Dendrolagus* | 0 | 0 | 10 | 3 |
| *Macropus* small | 0 | 0 | 3 | 0 |
| *Macropus* medium | 0 | 0 | 3 | 2 |
| *Macropus* large | 0 | 0 | 1 | 3 |
| *Onychogalea* | 0 | 0 | 1 | 0 |
| *Petrogale* | 0 | 0 | 3 | 1 |
| *Setonix* | 0 | 0 | 3 | 0 |
| *Thylogale* | 0 | 0 | 8 | 1 |

Table 21

Comparing Stage of 4th Molar Eruption with Presence of P3 or P4

| Tooth Complex | III.0 | III.1 | III.2 | III.3 | III.4 |
|---|---|---|---|---|---|
| P3/dP4 | 2 | 1 | 5 | 0 | 2 |
| P4/M1 | 0 | 0 | 2 | 12 | 18 |

## Epiphyseal Fusion Score Relationships with Molar Eruption Scores

Because the majority of cells would be empty in a contingency table of individual molar scores and epiphyseal fusion scores, no statistical test would be possible. However, grouping the molar scores does yield testable frequency tables (Tables 22 – 24) for the three long bones. Bowker's test for symmetry (with a continuity correction factor to account for cells with fewer than five observations) reveals a significant lack of symmetry in all three of the frequency tables ($p<.05$).

## Relationship Between Epiphyseal Fusion and Molar Scores in the Macropodidae

Both a Model I regression and a Pearson correlation coefficient were calculated for total fusion score and epiphyseal fusion scores for the long bones on molar eruption scores. The regression information is summarized in Table 25. The Pearson correlation results are given in Table 26. Visual comparisons of the regression lines are given in Figures 12 through 14.

Table 22

Frequencies of Molar Score Range and Humerus Fusion Score

| *Observed/expected* | *HFS 1* | *HFS 2* | *HFS 3* | *HFS 4* | *HFS 5* | *Total* |
|---|---|---|---|---|---|---|
| Molar score I.0-I.4 | 1 /0.025 | 1 /0.101 | 0 /0.228 | 0 /0.633 | 0 /1.013 | 2 /2.530 |
| Molar score II.0-II.4 | 0 /0.063 | 2 /0.253 | 2 /0.570 | 1 /1.582 | 0 /2.532 | 5 /6.330 |
| Molar score III.0-III.4 | 0 /0.633 | 1 /2.532 | 7 /5.696 | 17 /15.823 | 25 /25.316 | 50 /63.290 |
| Molar score IV.0 | 0 /0.279 | 0 /1.114 | 0 /2.506 | 7 /6.962 | 15 /11.139 | 22 /27.850 |
| **TOTAL** | 1 /1.270 | 4 /5.060 | 9 /11.390 | 25 /31.650 | 40 /50.630 | 79 /100.000 |

BTS: based on chi-square distribution value 73.0158, df = 12, $p < .0001$

Table 23

Frequencies of Molar Score Range and Ulna Fusion Score

| *Observed/expected* | *UFS 1* | *UFS 3* | *UFS 4* | *UFS 5* | *Total* |
|---|---|---|---|---|---|
| Molar score I.0-I.4 | 1 /0.049 | 1 /0.346 | 0 /0.568 | 0 /1.037 | 2 /2.470 |
| Molar score II.0-II.4 | 0 /0.099 | 3 /0.691 | 1 /1.136 | 0 /2.074 | 4 /4.940 |
| Molar score III.0-III.4 | 1 /1.333 | 9 /9.333 | 16 /15.33 | 28 /28-000 | 54 /66.670 |
| Molar score IV.0 | 0 /0.519 | 1 /3.629 | 6 /5.960 | 14 /10.889 | 21 /25.930 |
| **TOTAL** | 2 /2.470 | 14 /17.280 | 23 /28.400 | 42 /51.850 | 81 /100.000 |

BTS: based on chi-square distribution 34.4781, df = 9, $p < .0001$

Table 24

Frequencies of Molar Score Range and Radius Fusion Score

| *Observed/expected* | *RFS 1* | *RFS 2* | *RFS 3* | *RFS 4* | *RFS 5* | *Total* |
|---|---|---|---|---|---|---|
| Molar score I.0-I.4 | 1 /0.050 | 0 /0.025 | 1 /0.400 | 0 /0.550 | 0 /0.975 | 2 /2.500 |
| Molar score II.0-II.4 | 0 /0.100 | 0 /0.050 | 3 /0.800 | 1 /1.100 | 0 /1.950 | 4 /5.000 |
| Molar score III.0-III.4 | 1 /1.350 | 1 /0.675 | 9 /10.800 | 17 /14.850 | 26 /26.325 | 54 /67.500 |
| Molar score IV.0 | 0 /0.500 | 0 /0.250 | 3 /4.000 | 4 /5.500 | 13 /9.750 | 20 /25.000 |
| **TOTAL** | 2 /2.500 | 1 /1.250 | 16 /20.000 | 22 /27.500 | 39 /48.750 | 80 /100.000 |

BTS: based on chi-square distribution 34.4781, df = 9, $p < .0001$

Table 25

Model 1 Regressions with Molar Score as the Independent Variable

| Regression | Variable (Y) | R2 | Pr > F |
|---|---|---|---|
| Y = -.430 + 1.362X | HFS | 0.587 | <.001 |
| Y = 1.005 + .947X | UFS | 0.355 | <.001 |
| Y = 1.140 + .887X | RFS | 0.314 | <.001 |
| Y = 5.515 + 2.938X | TFS | 0.416 | <.001 |

HFS = humerus fusion score, UFS = ulna fusion score, RFS = radius fusion score, TFS = total fusion score

Table 26

Pearson Correlation Coefficients for Molar Scores and Epiphyseal Fusion Scores

| Variable | Correlation Coefficient | P value |
|---|---|---|
| HFS | 0.751 | <.001 |
| UFS | 0.596 | <.001 |
| RFS | 0.525 | <.001 |
| TFS | 0.622 | <.001 |

HFS = humerus fusion score, UFS = ulna fusion score, RFS = radius fusion score, TFS = total fusion score

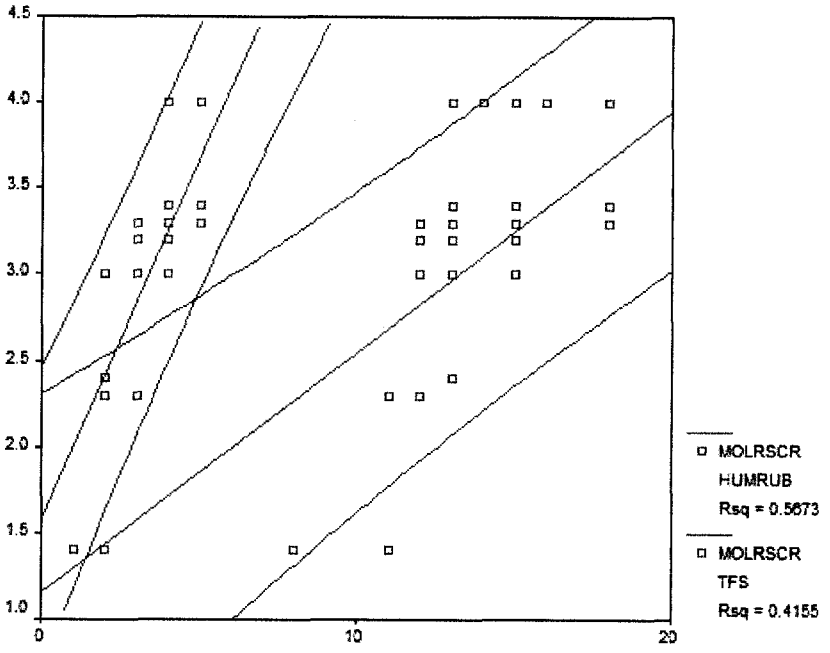


Figure 12. Regression plot 1. Comparison of regression lines of total fusion scores and humerus epiphyseal fusion scores with molar eruption scores, 95% confidence limits shown, Y-axis = molar eruption score and X-axis = epiphyseal fusion score.

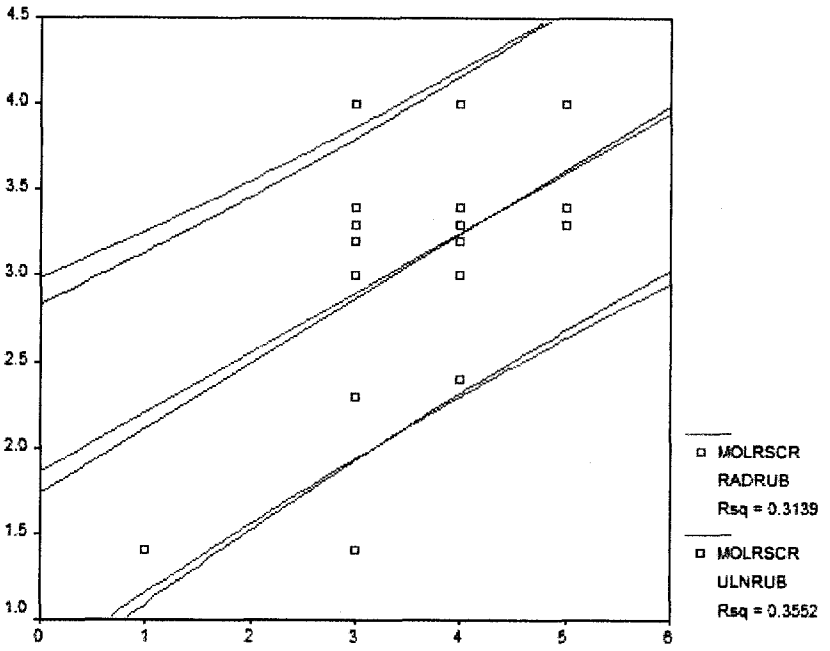


Figure 13. Regression plot 2. Comparison of regression lines of ulnar epiphyseal fusion scores and radial epiphyseal fusion scores with molar eruption scores, 95% confidence limits shown, Y-axis = molar eruption score and X-axis = epiphyseal fusion score.

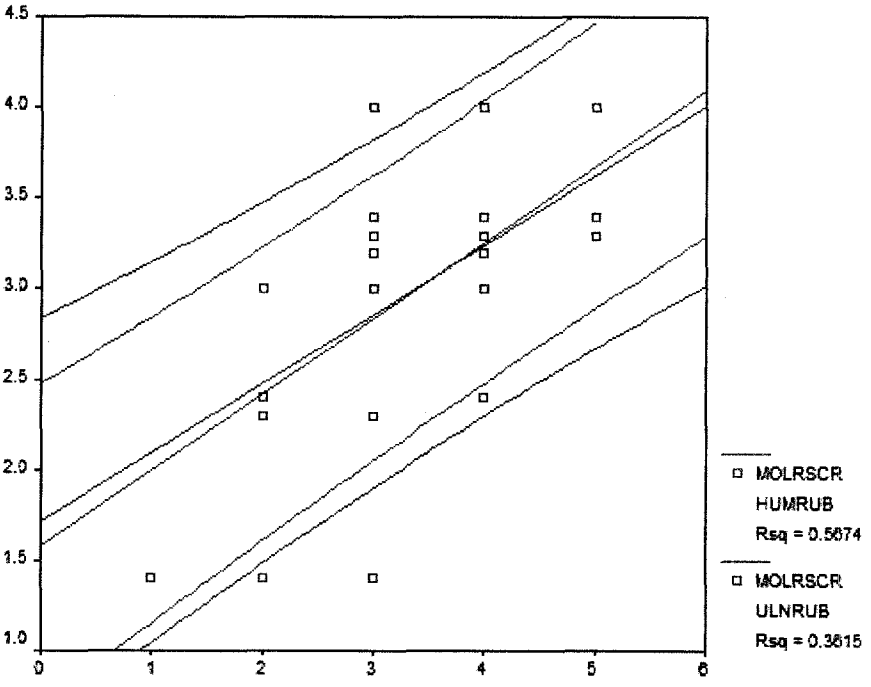


Figure 14. Regression plot 3. Comparison of regression lines of humerus epiphyseal fusion scores and ulnar epiphyseal fusion scores with molar eruption scores, 95% confidence limits shown, Y-axis = molar eruption score and X-axis = epiphyseal fusion score.

# Discussion

The data for P4/M1 complex and molar eruption scores show that P4 is erupting concomitantly with the M4. No specimen showed the presence of P4 with the erupting M3. Thirty-eight of the 54 specimens showed the presence of the P4 when the M4 was approaching the half-erupted stage (scores of III.3-III.4). This contrasts with most reported results in the literature in which the P4 is reported as erupting with the erupting M3 (Kirkpatrick, 1964; Sharman *et al.*, 1964; Maynes, 1972; Groves, 1982). If previously reported results were true here, it would mean the P4/M1 complex would be present with a molar eruption score of II.___. Van Deusen (1857) mentions that in *Dendrolagus lumholtzi* M4 is fully erupted prior to the presence of P4. This could not be evaluated here as no samples of this species were available for photography.

Based on the differentiation into browsing and grazing forms, the M1 descriptions of Sanson (1982) need to be modified after a closer analysis. Of the species covered in this study, Sanson places *Dendrolagus*, *Thylogale* and *Setonix* in the browsing group. He characterizes *Petrogale* as an intermediate herbivore and *Macropus* and *Onychogalea* as true grazers. This study reveals an enlarged anterior cingulum and a strong longitudinal ridge connecting the anterior and posterior lophs in *Macropus*, *Onychogalea*, *Petrogale,* and *Thylogale*. The anterior cingulum is narrower and the longitudinal ridge is less distinct in the other macropods. Although the first three species make sense in light of Sanson's divisions, *Thylogale* is

included in his grouping as a true browser and should not have a distinct longitudinal ridge.

Sprent and McArthur (2002) show that both *M. rufogriseus* and *Thylogale billardieri* have a similar diet of 91% grasses and broad-leaf forbs. Diet selection revealed *M. rufogriseus* choosing grasses 74% of the time and *T. billardieri* choosing broad-leaf forbs 38% of the time. From this, Sprent and McArthur (2002) conclude that diet selection in the two species matches predicted grazer and browser categories. In contrast, I would argue that a 38% choice of forbs by *Thylogale* means approximately a 60% selection for grasses; that is, if 91% of the diet is grasses and forbs, and an animal is characterized as a grass-eater, then 60% and 74% are not that different. The choice of grasses in preferred diet selection and of the M1 morphology may point to *Thylogale* being an intermediary form in the continuum similar to *Petrogale*.

Regression analysis of the epiphyseal fusion scores on the molar eruption scores showed that the regression lines of the ulna and radius are indistinguishable. This was to be expected given the results of Chapter Two, in which the patterns of epiphyseal fusion and the fusion scores for these two long bones were not significantly different. It was expected at the beginning of the study that the total fusion score would have a more significant regression relationship with molar eruption scores than would any of the three long bones because of the greater number of categories (a range from 6-18, versus 1-5 in the long bones). However, the humerus was a better predictor of age than was the total score. Perhaps since

continuous growth is postulated to be occurring at the wrist and manus and not at the shoulder, there is less variability in the humerus data. Since the regression equations of the ulna and radius are the least significant, this interpretation has validity but needs further data for drawing a firm conclusion.

## Conclusion

With a broader descriptive base of the morphological differences in cheek teeth within the Macropodidae, it is possible to identify the P3/dP4 complex and the P4/M1 complex. After this, calculating molar eruption scores can be done, making them available for age estimations and comparisons to the humeral and ulnar fusion scores of the previous chapter. There is still work to be done, though, as no data exists for the majority of the species of *Macropus*, either from this study or in the literature. Such data could be used to confirm the division of the genus into a small- to medium-sized group and a large-bodied group. Data is also lacking for *Wallabia* and *Dorcopsis* (outside of some description of the P4). As there is debate over the phylogenetic position of *Wallabia* relative to *Macropus* (Flannery, 1989; Kirsch *et al.*, 1997), a description of the dentition in this genus could be useful.

Of all four postcranial age estimation methods, the humerus fusion score rather than total fusion score was the most significant. This was unexpected but can be of value as it is a simpler method and does not require the presence of all three forelimb bones.

Sharman *et al.* (1964) suggested that the variability in epiphyseal fusion patterns in marsupials would prove problematic in generating accurate age estimation methods. However, this study shows that using epiphyseal fusion scores and total fusion scores yields regressions and correlations that are significant at the familial level. These results also suggest that the varied age results of Sharman *et al.* (1964) in *M. rufus* and of Maynes (1972) in *M. parma* for similar molar eruption scores can be addressed by generating regression formulas by combining the species in the family rather than looking at just one species.

# CHAPTER IV

# USE OF EPIPHYSEAL AND TOTAL FUSION SCORES TO EVALUATE MORPHOLOGICAL INDICES IN THE MACROPODIDAE

## Introduction

As Chapter Three shows, previous studies regressing molar eruption scores on Macropodidae of known age produces significant regression lines. Molar scores then were shown to be good estimates of macropodid age after pouch exit. An alternate method of age estimation by determining the degree of epiphyseal fusion has been suggested in the literature and was attempted in *Trichosurus*, an opossum in the family Phalangeridae. In the Macropodidae such a postcranial method of estimating age is specially of interest due to the problematic nature of cheek tooth identification. As reported in Chapter Three, the results of this current study indicate strong correlations between long bone epiphyseal and total fusion scores (TFS) with molar eruption scores and also show a significant result when regressing either of the two on molar scores. However, this only indicates that such epiphyseal markers correlate with age. It does not reveal if those scores can indicate age categories. Nor does it address the question of whether individuals with partly unfused epiphyses differ significantly in morphological measures from individuals with completely closed epiphyses. This is an important question to consider since most members of

this family maintain partly unfused epiphyses to varying degrees, at least late into life. This chapter considers how the epiphyseal measures of age separate out in multivariate space functionally and morphologically.

## Materials and Methods

### Specimens

Macropodidae skeletal specimens were examined at the following museums: Field Museum of Natural History (FMNH), Chicago; American Museum of Natural History (AMNH), New York; National Museum of Natural History (NMNH), Washington, D.C.; Museum of Vertebrate Zoology (MVZ), Berkeley. Specimens were included for data collection if they met two criteria: 1) complete fusion of the three bones of the os coxa and 2) humeral and femoral epiphyses that were whole (although not necessarily fused to the diaphysis). A total of 157 Macropodidae were measured. Table 27 gives numbers of specimens for each genus in the study. As museum sample sizes are often less than that needed for statistical analyses, all specimens meeting the above criteria were measured, whether those specimens were wild-caught or zoo-raised (Table 27). The number of individuals measured in each genus ranged from two (*Dorcopsis*) to 50 (*Macropus*), with an average of 18.

Table 27

Specimens Included in Epiphyseal Fusion Study

| Genus | Total | Male | Female | Unknown | Wild | Zoo |
|---|---|---|---|---|---|---|
| *Dendrolagus* | 38 | 8 | 22 | 8 | 17 | 21 |
| *Dorcopsis* | 2 | 1 | 1 | 0 | 2 | 0 |
| *Dorcopsulus* | 4 | 2 | 2 | 0 | 4 | 0 |
| *Macropus* | 50 | 18 | 25 | 7 | 23 | 27 |
| *Onychogalea* | 6 | 3 | 3 | 0 | 4 | 2 |
| *Petrogale* | 18 | 7 | 7 | 4 | 11 | 7 |
| *Setonix* | 13 | 4 | 6 | 3 | 9 | 4 |
| *Thylogale* | 22 | 4 | 13 | 5 | 13 | 9 |
| *Wallabia* | 4 | 2 | 2 | 0 | 3 | 1 |
| TOTALS | 157 | 49 | 81 | 27 | 86 | 71 |

## Measurements

### Linear Measurements

Twenty-four postcranial measurements (Table 28) were recorded for each specimen. Measurements were made either from Mititoyo digital calipers accurate to 0.01mm (all measures except long bone lengths for larger *Macropus* spp.) or from an osteometric board accurate to 1.0 mm (femur, tibial and ulnar lengths in the larger *Macropus* spp.). Measurements were recorded from the left side of the specimen unless this bone was absent or, as in the case of three specimens (NMNH 284462,

Table 28

Postcranial Measurements

| Measure | Description |
|---|---|
| 1) Humeral Length (HUML) | Humeral head to distal trochlea |
| 2) Radius Length (RADL) | Radial head to end styloid process |
| 3) Femur Length (FEML) | Greater trochanter to lateral condyle |
| 4) Tibia Length (TIBL) | Medial condyle to medial malleolus |
| 5) Ulna Length (ULNAL) | Olecranon process to styloid process |
| 6) Humeral Head Length (HUMHDL) | Proximal head to distal extent head |
| 7) Humeral Head Width (HUMHDW) | Medial edge before neck to lateral edge |
| 8) Capitulum Length (CAPL) | Proximal edge to distal extent of capitulum |
| 9) Capitulum Width (CAPW) | Trochlear border to lateral edge |
| 10) Biepicondylar Width (BIEPIW) | Medial epicondyle to lateral condylar ridge |
| 11) Trochlea Length Med (TRCLM) | Proximal to distal extent at medial border |
| 12) Trochlea Length Inter (TRCLI) | Proximal to distal extent at narrowest point |
| 13) Trochlea Length Lat (TRCLL) | Proximal to distal extent at lateral border |
| 14) Scapular Width (SCAPW) | Edge caudal angle to edge cranial border |
| 15) Scapular Length (SCAPL) | Glenoid fossa to edge vertebral border |
| 16) Glenoid Width (GLENW) | Medial to lateral edge |
| 17) Glenoid Length (GLENL) | Cranial to caudal edge |
| 18) Trochlea Width (TRCW) | Posterior mediolateral extent |
| 19) Deltopectoral Length (DELTL) | Greatest proximodistal extent of crest |
| 20) Olecranon Length (OLECL) | Proximal olecranon to inner trochlear notch |
| 21) Radial Head Max (RHDMX) | Width of head at farthest two points |
| 22) Radial Head Min (RHDMN) | Width of head at closest two points |
| 23) Radius Articular Length (RDSTL) | Medial edge distal articular surface to styloid |
| 24) Radius Articular Width (RDSTW) | Anteroposterior extent distal articular surface |

AMNH 65427, FMNH 98158), showed gross pathologies from a previous injury. Available specimens with gross pathologies on both the right and left sides were omitted from the study.

Estimates of Body Mass

Any study seeking to investigate ontogentic patterns must find a way to minimize or eliminate the allometric component of body size variability from all remaining variability in the analysis. Because variation in specimens due to body size can be extensive, it has the potential to swamp out other sources of variation. One method of correcting for body size is to use log-transformations of the raw data (Oxnard, 1973). Another method is to divide each original measure by another measure known to statistically correlate allometrically with size. Two such variables are femur length and femur midshaft diameter (Alexander et al., 1979). Femur length in this study would not be appropriate. Whereas femur length has been shown to have a strong correlation with body size in carnivores and ungulates (Janis, 1990; Van Valkenburgh, 1987), in the Macropodidae, the femur (along with the tibia) has a strong correlation to the unique locomotory behavior of bipedal hopping (Badoux, 1965; Bennett, 1987). Femur diameter is also not an appropriate correcting factor for this study because it was calculated as the ratio of anteroposterior width / mediolateral width. As such, in this study it is a ratio itself. To then divide it into the other measures of the study as a correcting factor puts a ratio in the denominator of a ratio and potentially renders the results of multivariate analyses less meaningful.

Yet a third method for body size correction is to divide each raw measure by the body mass of the specimen. However, when dealing with museum samples, these data are often not available. A common alternative is to use mean sex-specific body mass values taken from the literature. However, such a solution introduces problematic variability in studies with small sample sizes because the few individuals sampled may deviate from the literature mean to a significant degree and therefore skew the results. In this study three species are represented by fewer than five specimens (Table 27), and so this method of correction is not ideal. A traditional alternative to the above size correction factors is to form meaningful ratios from the raw data (Mosimann, 1970; Ashton *et al.*, 1975; Manaster, 1979). Although these ratios do not eliminate size as a variable (Corrucini, 1973; Atchley *et al.*, 1976), they do reduce the degree to which it influences the generation of canonical functions from multivariate analyses (Dodson, 1978). Ratios are calculated using both the numerator and denominator values specific to each individual specimen and therefore also avoid the problems mentioned above with the other body-size correction options. Finally, ratios are also good measures for capturing shape information and information contained in growth series (Dodson, 1978; Hill, 1978). When ratios are used in multivariate techniques such as PCA and discriminant function analysis, methods which traditionally have a first axis incorporating size that accounts for a very large part of the variability, ratios render the first axis less of a size component (Dodson, 1978). In so doing, the first axis no

longer accounts for very large percentages of the variability, and also the signs of the coefficients are no longer the same.

## Morphometric Indices

Seventeen standard indices (Table 29, indices 1-17) were chosen from the literature (Gebo and Sargis, 1994; Sargis, 2002). Four other indices (Table 29, indices 18-21) were created because they were considered to hold unique functional or discriminating significance to this study. Unlike the humeral head or the capitulum, the trochlea of the humerus is a more complicated surface. Both proximodistal breadth and anteroposterior depth were measured in three locations to capture some of this complexity: 1) at the most medial edge of the articular surface, 2) at the narrowest portion, and 3) at the most lateral edge where the trochlea meets the capitulum (Figure 15). Mediolateral extent of the trochlea was measured on the posterior aspect of the articular surface at its widest point. The four unique ratios were created from these linear measures.

Table 29

Indices

| Index | Formula * 100 |
|---|---|
| 1) Intermembral (INTRNX) | HUML+RADL/FEML+TIBL |
| 2) Humerofemoral (HMFMNX) | HUML/FEML |
| 3) Brachial (BRCHNX) | RADL/HUML |
| 4) Troch-Cap Width (TRCAPNX) | TRCW/CAPW |
| 5) Troch-Cap Length (TRCPLNX) | TRCLM/CAPL |
| 6) Capitulum Width (CAPWNX) | CAPW/HUML |
| 7) Capitulum Length (CAPLNX) | CAPL/HUML |
| 8) Biepicondylar Width (BIEPINX) | BIEPIW/HUML |
| 9) Deltopectoral (DLTNX) | DELTL/HUML |
| 10) Olecranal (OLCNX) | OLECL/HUML |
| 11) Trochlea Shape (TRCNX) | TRCW/TRCLM |
| 12) Capitulum Shape (CAPNX) | CAPW/CAPL |
| 13) Scapular Shape (SCAPNX) | SCAPW/SCAPL |
| 14) Glenoid Shape (GLENNX) | GLENW/GLENL |
| 15) Humeral Head Shape (HUMHDNX) | HMHDW/HMHDL |
| 16) Radial Head Shape (RDHDNX) | RHDMN/RHDMX |
| 17) Radial Articular Shape (RDDSTNX) | RDSTW/RDSTL |
| 18) Trochlea Length Max (TRLMXNX) | TRCLM/HUML |
| 19) Trochlea Length Min (TRLMNNX) | TRCLI/HUML |
| 20) Trochlear Length Med (TRLMDNX) | TRCLI/TRCLM |
| 21) Trochlear Length Lat (TRLLTNX) | TRCLI/TRCLL |

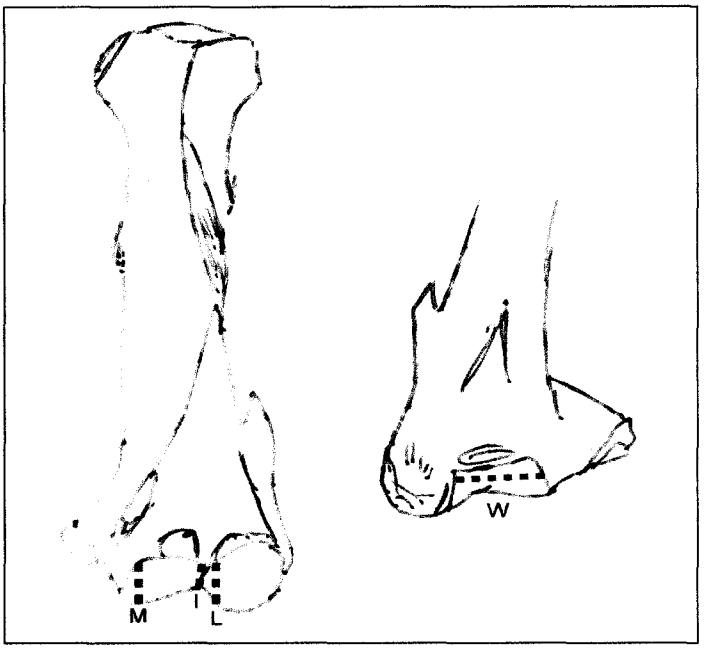


Figure 15. Measures of the trochlea. M= medial, I = narrowest point, L = lateral, W = width

## Analysis

### Assessing Measurement Error

A preliminary study was conducted on the Phalangeridae prior to data collection for the Macropodidae. For the preliminary section of the study, 44 specimens of *Trichosurus vulpecula* from the MVZ were measured. These specimens were part of the larger data collection across the order but are not included in this study of the Macropodidae. During the preliminary study, all postcranial measurements were taken three times. Each time a complete set of measurements was taken, the researcher returned to the first measure and began again. This process helped eliminate placement of the calipers in the same location

based on memory rather than by knowledge and discernment of correct caliper placement. Analysis of measurement error was based on Bailey and Byrnes (1990).

To assess measurement error, Model II ANOVAs were used to partition the total variability of the study into variability among specimens and variability within the three measures taken on one specimen. Measurement error was then expressed as a percentage using the following calculation:

$$\% \text{ ME} = 100 * (S^2_{within} / S^2_{within} + S^2_{among})$$

When % ME was greater than 5 %, the measure was eliminated from the analyses and was not included in this study of Macropodidae. This criterion affected only three preliminary study measures (extension of acromion past glenoid, scapular notch depth and ulnar styloid length).

Assessing Sex Differences

All statistical analyses were performed using SPSS for Windows, release 11.5. Analysis by generalized linear model with a Type III sum of squares was used to test the indices for sex differences across both the family and the subfamilies (Table 30).

Table 30

GLM Sum of Squares Results for Sex Differences in Indices

| Index | Macropodidae |
|---|---|
| | Significance of p |
| INTRNX | .158 |
| HMFMNX | .116 |
| BRCHNX | .464 |
| SCAPNX | .817 |
| GLENNX | .145 |
| HMHDNX | .974 |
| TRCAPNX | .234 |
| TRCNX | .140 |
| CAPNX | **.007** |
| TRCPLNX | .135 |
| CAPWNX | **.013** |
| TRLMXNX | **.018** |
| TRLMNNX | .157 |
| CAPLNX | .069 |
| BIEPNX | .685 |
| DLTNX | .888 |
| OLCNX | .872 |
| RDHDNX | .668 |
| RDDSTNX | .346 |
| TRLMDNX | .597 |
| TRLLTNX | .185 |

Indices with $p < 0.05$ in bold

Discriminant Analyses

Discriminant analysis was chosen as a multivariate technique to assess whether individuals possessing partly unfused epiphyses (TFS scores of 15-17 and long bone rubrics of 4) grouped with those individuals possessing complete fusion (TFS = 18 and rubrics = 5). The canonical axes generated in a discriminant function maximize the variation between groups and minimize variation within. Therefore, if fusion scores grouped together despite the maximization of group centroid differences, it would be a strong indication that those scores formed an age category. As given in the canonical function coefficients, the weights assigned to the variables give some information about the importance of that particular variable in separating the groups (Zar, 2007). In all discriminant analyses run, four of the indices consistently failed the preliminary tolerance test at a significance level of $p < 0.001$. Those four indices (TRCCPLNX, CAPLNX, BIEPINX, TRLMDNX; Table 29) do not appear in any of the following analyses.

## Results

### Analysis of Sex Differences

Three indices (CAPNX, CAPWNX, TRLMXNX) revealed a significant difference between the sexes when comparing specimens in the family (Table 30). Although there were significant differences in these indices between the sexes, they were retained in all future statistical analyses because of their strong potential

information about shape and because the greatest degree of sexual dimorphism (as shown in Chapter Two) occurs in the genus *Macropus*. In this study, specimens of this genus account for only 50 of the total 157 specimens. However, a caveat is inserted here that the effect of these significant differences may play a role in driving subsequent significant results.

## Discriminant Function Analysis of Indices by Total Fusion Scores

Discriminant analysis of the 21 indices based on total fusion score did not result in strong separation of group centroids (Table 31). Whereas the overall dispersion was not large for this analysis (Figure 16), there was a slight degree of clumping of TFS values 16-18 along the first functional axis. Only 27.4% of the variation in the data is accounted for by the first axis (Table 32). Indices of the trochlea and capitulum (TRCNX, TRCAPNX, CAPNX) were the most influential in separating the fusion scores.

Table 31

Functions at Group Centroids Based on Total Fusion Scores

| TFS | Function 1 | Function 2 |
|---|---|---|
| 6 | 1.172 | -1.756 |
| 7 | 1.738 | -1.760 |
| 8 | 1.149 | .350 |
| 9 | OMIT | OMIT |
| 10 | .737 | -2.007 |
| 11 | -.106 | -.957 |
| 12 | .797 | .044 |
| 13 | .755 | 1.140 |
| 14 | .186 | 1.167 |
| 15 | .337 | .250 |
| 16 | -.256 | .245 |
| 17 | .071 | -.337 |
| 18 | -1.063 | -.143 |

Note: no specimens were observed with a TFS of 9.

Table 32

Discriminant Function Coefficients Based on Total Fusion Scores

| Index | **Function 1** | **Function 2** |
|---|---|---|
| INTRNX | .450 | .362 |
| HMFMNX | -.815 | .638 |
| BRCHNX | .019 | .343 |
| SCAPNX | .258 | .359 |
| GLENNX | .123 | -.576 |
| HMHDNX | .197 | -.275 |
| TRCAPNX | -.894 | **1.629** |
| TRCNX | **1.095** | -.723 |
| CAPNX | -.220 | **1.098** |
| CAPWNX | -.155 | .125 |
| TRLMXNX | .838 | -.552 |
| TRLMNNX | -.550 | .522 |
| DLTNX | .481 | .315 |
| OLCNX | .061 | -.115 |
| RDHDNX | .381 | -.045 |
| RDDSTNX | .498 | -.186 |
| TRLLTNX | .202 | -.379 |
| % Var. | 27.4 | 19.4 |

Indices with the greatest weight are indicated in bold-faced type.

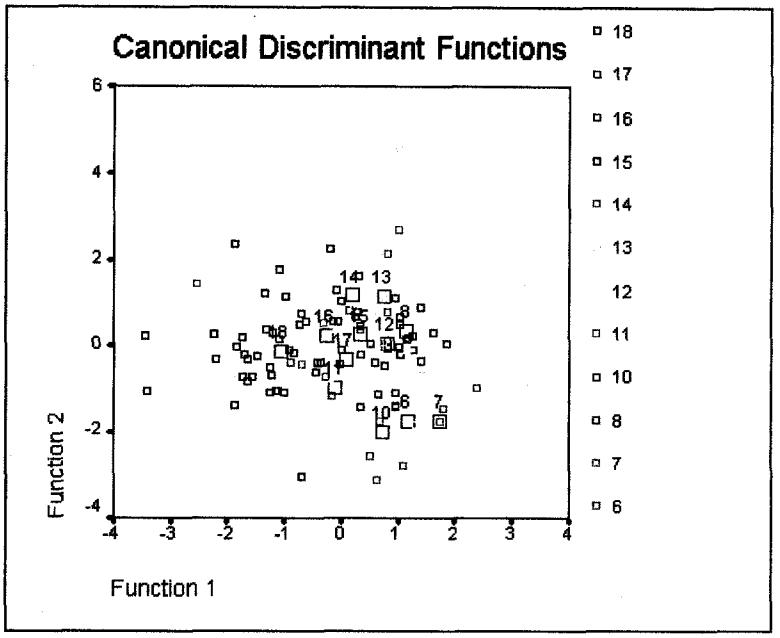


Figure 16. Canonical discriminant plot of TFS on indices for the Macropodidae. Numbers 6-18 correspond to possible total fusion scores, with 6 being unfused and 18 totally fused.

## Discriminant Function Analyses of Indices by Humerus Epiphyseal Fusion Score

Discriminant analysis of the 21 indices based on the humerus epiphyseal fusion score (HFS) did result in good separation of group centroids (Figure 17 and Table 33). There were three groupings: HFS 5, HFS 3 and 4, and HFS 1 and 2. As in the discriminant analysis of TFS, there was variation explained by the first function (Table 34). There were no indices that strongly influenced the first discriminant function, whereas the intermembral index and brachial index strongly influenced the second axis (Table 34).

Table 33

Functions at Group Centroids Based on HFS

| HFS | Function 1 | Function 2 |
|---|---|---|
| 1 | 1.122 | -.422 |
| 2 | 1.058 | 02.117 |
| 3 | .597 | -.062 |
| 4 | .498 | .571 |
| 5 | -.968 | -.127 |

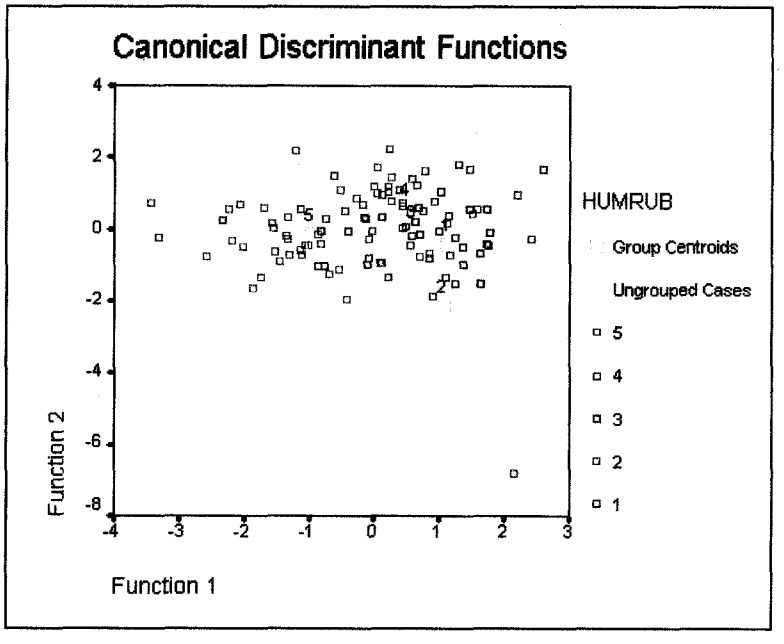


Figure 17. Canonical discriminant plot of HFS on indices for the Macropodidae. Numbers 1-5 represent humeral epiphyseal fusion scores, with 1 being unfused and the youngest age category.

Table 34

Discriminant Function Coefficients Based on HFS

| Index | **Function 1** | **Function 2** |
|---|---|---|
| INTRNX | .627 | **-1.602** |
| HMFMNX | -.907 | **1.649** |
| BRCHNX | -.073 | -.133 |
| SCAPNX | .095 | .261 |
| GLENNX | .257 | -.307 |
| HMHDNX | .071 | -.358 |
| TRCAPNX | -.543 | -.116 |
| TRCNX | .773 | .240 |
| CAPNX | -.125 | .497 |
| CAPWNX | -.073 | -.079 |
| TRLMXNX | .553 | .197 |
| TRLMNNX | -.613 | .208 |
| DLTNX | .460 | .388 |
| OLCNX | .056 | .044 |
| RDHDNX | .423 | .064 |
| RDDSTNX | .485 | -.083 |
| TRLLTNX | .231 | -.400 |
| % Var. | 48.6 | 25.8 |

Indices with the greatest weight are indicated in bold-faced type.

Discriminant Function Analyses of Indices by Ulna Epiphyseal Fusion Score

Discriminant analysis of the 21 indices based on the ulna epiphyseal fusion score (UFS) resulted in a strong separation of group centroids but lacked any particular groupings (Table 35 and Figure 18). Unlike the previous two discriminant analyses, the percent variation explained by the first function was relatively high at nearly 60%. However, unlike in multivariate cases where the first axis is a function of size, here the coefficients of the variables did not all carry the same sign. Indices that strongly influenced the discriminant functions included the trochlear shape index on the first discriminant function and the trochcap-length index on the second (Table 36).

Table 35

Functions at Group Centroids Based on UFS

| UFS | Function 1 | Function 2 |
|---|---|---|
| 1 | 1.234 | -1.210 |
| 2 | Omit | Omit |
| 3 | 1.016 | -.016 |
| 4 | .243 | .631 |
| 5 | -.819 | -.252 |

Table 36

Discriminant Function Coefficients Based on UFS

| Index | **Function 1** | **Function 2** |
|---|---|---|
| INTRNX | .174 | .104 |
| HMFMNX | -.808 | .774 |
| BRCHNX | -.009 | -.016 |
| SCAPNX | .115 | .451 |
| GLENNX | .146 | -.436 |
| HMHDNX | .209 | -.512 |
| TRCAPNX | -.817 | **1.353** |
| TRCNX | **1.128** | -.731 |
| CAPNX | -.020 | .245 |
| CAPWNX | -.330 | .599 |
| TRLMXNX | .714 | -.647 |
| TRLMNNX | -.497 | .413 |
| DLTNX | .326 | .216 |
| OLCNX | -.199 | -.011 |
| RDHDNX | .308 | .165 |
| RDDSTNX | .337 | -.129 |
| TRLLTNX | .333 | -.594 |
| % Var. | 59.6 | 30.3 |

Indices with the greatest weight are indicated in bold-faced type.

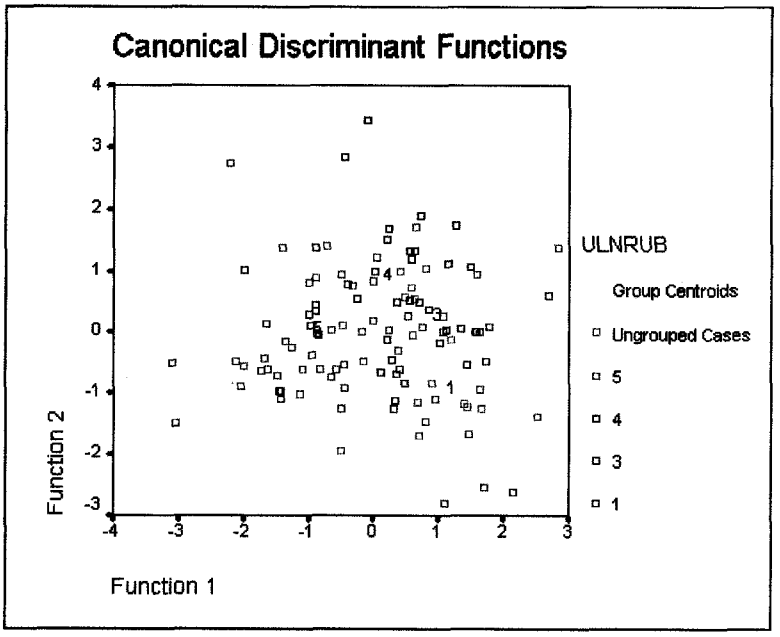


Figure 18. Canonical discriminant plot of UFS on indices for the Macropodidae. Numbers 1-5 represent ulnar epiphyseal fusion scores, with 1 being unfused and the youngest represented age category. Note that there are no representatives for group two in this data set.

## Discriminant Analyses of Indices by Radius Epiphyseal Fusion Score

Discriminant analysis of the 21 indices based on the radius epiphyseal fusion score (RFS) gave clearly separated centroids in the discriminant analysis (Figure 19 and Table 37). However, the plot did not show any groupings. Similar to the discriminant functions for TFS and HFS, the first function did not explain a very large portion of the variability in the data. For the discriminant coefficients of the functions, three indices of the trochlea strongly influenced both functions (Table 38).

Table 37

Functions at Group Centroids Based on RFS

| RFS | Function 1 | Function 2 |
|---|---|---|
| 1 | 1.486 | -1.175 |
| 2 | -.522 | .624 |
| 3 | .678 | .150 |
| 4 | .253 | .709 |
| 5 | -.871 | -.333 |

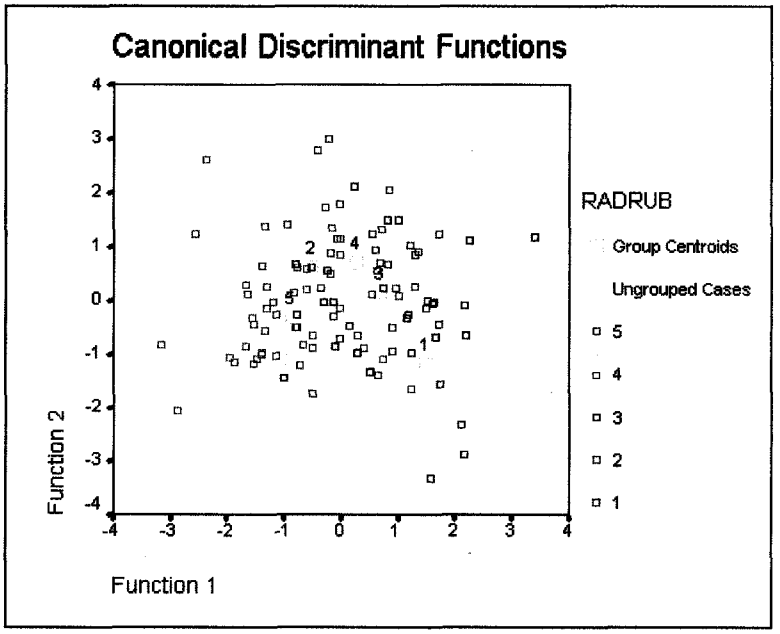


Figure 19. Canonical discriminant plot of RFS on indices for the Macropodidae. Numbers 1-5 represent radial epiphyseal fusion scores, with 1 being unfused and the youngest represented age category.

Table 38

Discriminant Function Coefficients Based on RFS

| Index | **Function 1** | **Function 2** |
|---|---|---|
| INTRNX | .283 | -.099 |
| HMFMNX | -.880 | .845 |
| BRCHNX | -.019 | -.108 |
| SCAPNX | .202 | .430 |
| GLENNX | .110 | -.351 |
| HMHDNX | .290 | -.590 |
| TRCAPNX | **-1.377** | **1.583** |
| TRCNX | **1.440** | -.835 |
| CAPNX | -.434 | .546 |
| CAPWNX | -.137 | .234 |
| TRLMXNX | **1.121** | -.854 |
| TRLMNNX | -.612 | .523 |
| DLTNX | .458 | .242 |
| OLCNX | -.147 | .020 |
| RDHDNX | .379 | .125 |
| RDDSTNX | .351 | .028 |
| TRLLTNX | .277 | -.471 |
| % Var. | 46.5 | 26.3 |

Indices with the greatest weight are indicated in bold-faced type.

## Discriminant Function Analyses Omitting *Macropus* Specimens

Discriminant functions for the Macropodidae without the genus *Macropus* were run for TFS and HFS to investigate whether the strong degree of sexual dimorphism in *Macropus* might have contributed to any of the observed results in degrees of spread or clumping of groups in the discriminant function plots. For TFS, omission of *Macropus* resulted in a first discriminant function that explained 32.6% of the variability rather than 27.4%. The indices that strongly influenced the functions remained similar, except that on the first function the intermembral index was now an order of magnitude higher (Table 39) . The dispersion of points was greater than with *Macropus* included, but retained the same pattern (Figure 20).

For the humerus, the first function explained 53.1% of the variability rather than 48.6%. The indices with the strongest influence on the discriminant functions were not noticeably different from when the analysis was run with *Macropus* included (Table 39). The plot spread, similar to the new TFS plot, was also greater than in the original discriminant analysis, but the same pattern of spread appeared with groups one and two clumping near each other, as did groups three and four (Figure 21).

Table 39

Discriminant Function Coefficients for TFS and HFS with *Macropus* Omitted

| Index | TFS Function 1 | TFS Function 2 | Humerus Function 1 | Humerus Function 2 |
|---|---|---|---|---|
| INTRNX | **-2.836** | .442 | **-2.475** | **3.063** |
| HMFMNX | **2.341** | .292 | **2.192** | **-2.599** |
| BRCHNX | .937 | -.444 | .440 | -.794 |
| SCAPNX | -.011 | .732 | .274 | .833 |
| GLENNX | .092 | .118 | .113 | -.188 |
| HMHDNX | .428 | -.229 | .248 | -.241 |
| TRCAPNX | **-1.275** | **1.816** | .137 | **1.250** |
| TRCNX | .421 | -.690 | -.157 | -.361 |
| CAPNX | -.220 | .350 | .109 | .259 |
| CAPWNX | -.160 | **1.029** | .131 | .645 |
| TRLMXNX | .186 | **-1.004** | -.535 | -.556 |
| TRLMNNX | -.507 | .727 | -.252 | .733 |
| DLTNX | .276 | .452 | .414 | .416 |
| OLCNX | .107 | -.444 | .147 | .140 |
| RDHDNX | .145 | .308 | .209 | .288 |
| RDDSTNX | .436 | -.169 | .284 | -.196 |
| TRLLTNX | .545 | -.208 | .321 | -.915 |
| % Var. | 29.1 | 20.1 | 53.1 | 26.9 |

Indices with the greatest weight are in bold-faced type.

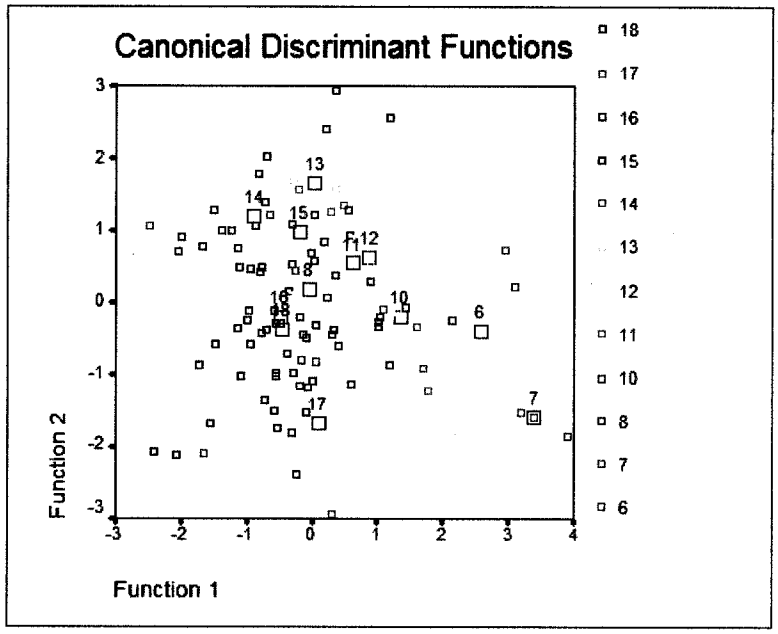


Figure 20. Discriminant function for TFS in Macropodidae with *Macropus* omitted. Numbers 6-18 represent TFS, where 6 is totally unfused and the youngest age group. No specimens were observed for TFS 9

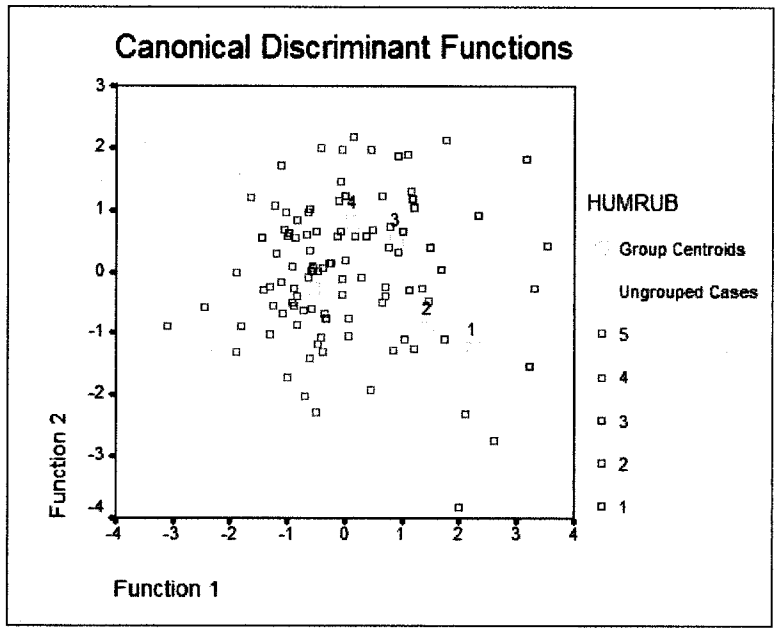


Figure 21. Discriminant function for humerus in Macropodidae with *Macropus* omitted.

# Discussion

As discussed in Chapter Two, there is a high degree of sexual dimorphism in the larger Macropodidae species, though not in the medium-sized or small-sized species. This variation in sexual dimorphism could have had an effect on the outcome of the analyses. However, Dodson (1978) analyzed fossil reptiles that spanned a size range increasing by a factor of 18. He then ran multivariate analyses on his data using both log-transformed raw data as well as log-transformed ratios and found no significant effects from utilizing ratios. The genus *Macropus* contains species with the greatest degress of sexual dimorphism, and it contains the largest species in the family. In this study, *Macropus* represented nearly a third (50 of 157) of all the specimens. Thus the potential existed for the variation of sexual dimorphism within *Macropus* to swamp out any other signal of interest. To check for this, discriminant functions were rerun after removal of the genus *Macropus*.

Whereas the spread of the data plots for the family did increase when *Macropus* was omitted from the analysis (Figures 20, 21 vs. Figures 16, 17), the patterns in the plot did not change significantly under qualitative assessment. For example on the first function for the HFS plot (Figure 21), there is still a progression from lowest to highest age groups going from right to left on the axis. Groups 3 and 4 still clump close to each other as do groups 1 and 2. These results suggest that although including *Macropus* in the analysis does affect the results, it does not significantly change their overall pattern. Since two obvious differences between

*Macropus* and the remainder of the family involve size (i.e., species mean body sizes and the degree of sexual dimorphism), there is evidence that these two size variables are not overriding the multivariate analysis.

The tighter spread for the TFS plot (Figure 16) indicates less ability for the function to discriminate between age groups. Though molar scores and TFS are highly correlated (see Chapter Three), the age groups in this plot still are not widely spread. One interpretation of this result is that there are too many TFS values for effective differentiation. In view of the good spread for each of the three long bone fusion scores (each with only five categories), this interpretation has merit. When viewed along the first discriminant axis, there is a pattern of greater degrees of fusion and therefore older age to the left side of the axis, with less fusion to the right side of the axis. This pattern suggests that there is some similarity in shape between older specimens, even if those specimens do not have complete epiphyseal fusion at all articulations.

Along the first discriminant axis for each of the long bone plots (Figures 17-19), the Macropodidae showed a separation of group 5, then 3 and 4 grouped together, and groups 1 and 2 grouped together. However, along the second axis, groups 3, 4 and 5 were consistently grouped together. In the humerus, group 1 was included in this clump (Figure 17). Thus, on axis one there is most likely something that is consistently influencing the function in a way that is separating the groups along age lines, and on axis two those older groups are clumped. Interpreting this result necessitates reviewing the discriminating variables with the greatest influence

for each function. With the exception of the second function of the HFS discriminant function (in which the intermemebral and humerofemoral indices strongly influence the functions), every function has the trochlear shape index and the trochlea-capitulum ratio as influential indices.

Variables that strongly influence a function in this particular series of discriminant function analyses are those that discriminate the best between the age groups as determined by the particular fusion score. With the exception of the humerus fusion score, the indices of humerofemeral and intermembral relationships are not variables that strongly influence the analyses. This was a surprising result since the growth in the forelimb is reported to be continuous (Jarman, 1989). Jarman (1989) suggested that in species where the male fights close in, the forelimb increases in musculature; in species that fight pushing away from each other, growth continues in the manus. The manus was not covered in this study. Perhaps this is why these two indices do not appear to strongly influence analyses. Yet, the distal ends of the radius and ulna remain only partly fused, as shown in Chapter Two, so it would be expected that across the ages the radius should play a larger role in the denominator of the intermembral index and so change the nature of the index across ages. Perhaps, though only partly fused, the actual growth at this articulation is slight enough that it has no discernable effect on the ratio.

It is very interesting that the measures of the trochlea and capitulum so consistently appear weighted heavily throughout the functions of the analyses. Chapter Two shows that the elbow is the first functional unit to fuse in the forelimb.

If this area fuses first, then the nature of any ratio at the elbow should be fixed and consistent throughout the age groups. In that case there would be no ability of the ratio to discriminate among those age groups. That is not seen here. One possible interpretation is that whereas growth in length has ceased, internal changes in shape are still occurring. Perhaps increasing body size as the animals age is affecting the surface area of the joint. Although this study does not address joint surface areas directly, its results do suggest the possibility of positive allometric change in elbow articular surface area, as opposed to geometric change (Alexander, 1980; Jungers, 1988; Biewener, 1989; Swartz, 1989; Godfrey *et al.*, 1991).

## Conclusion

The initial goal of this portion of the study was twofold: 1) to show that some specimens with partly unfused epiphyses would be morphologically indistinguishable from specimens with complete fusion and 2) to use fusion scores to separate specimens into age categories. The first goal was only partly met in these analyses. There is supporting but not conclusive evidence for the inclusion of more animals in the morphologically adult group than just those with rubric scores of 5 or total fusion scores of 18. The overall trend in the Macropodidae is for the oldest three age groups as determined from long bone fusion scores (epiphyseal fusion scores of 3, 4, 5) to group together on one of the two axes of the discriminant function and the middle two age categories (epiphyseal fusion scores 3 and 4) to group together on the other axis. This pattern is also seen in total fusion scores in

which scores of 16, 17, and 18 group together along the first axis. However, as the overall pattern is for category 5 in the long bone age estimation methods to group separately from the rest when considering all axes of the discriminant function, it is not conclusive that animals with a score of 4 or 5 can be morphologically included with those having a score of 3 or 4. One possible interpretation is that long bone fusion scores are good for placing specimens into age categories; therefore, the major age classes are: adult with scores of 5, subadult with scores of 3 and 4, and juvenile with scores of 1 and 2. On the other hand, total fusion scores are better at showing which partly unfused specimens (those with scores of 16 and 17) can be grouped with those that show complete fusion (scores of 18).

# CHAPTER V

# CONCLUSION

The two overall goals of this study were to provide a quick and easy postcranial method for estimating age of a specimen and also to show that in the Macropodidae some animals lacking total epiphyseal fusion could still validly be used in traditional functional morphology studies. Aside from the overall goals, there were also more specific goals: 1) filling in gaps in the literature of the dentition of the Macropodidae; 2) describing patterns of epiphyseal closure; and 3) assigning specimens to the age categories of juvenile, subadult, and adult.

The first main goal was met more completely than the second. Tyndale-Biscoe (1955) described the tibia as fused, partly fused or not fused. He observed no differences in body weight or tibial length in the first two groups but observed that both differed from the last group. He then created two age groups: sexually mature and sexually immature. For a morphological study, such a basic distinction in the sample is not especially helpful. In this study the scoring of both the proximal and distal epiphyses separately allowed for the observation of fusion patterns. This pattern was then used to create a postcranial age scoring system that correlates significantly with molar scores (a method of age estimation supported in the literature).

As it is difficult to consistently and accurately identify the molar teeth of the Macropodidae, an age estimation based on molar scores is open to error. The postcranial method of age estimation developed in this study avoids the difficulty inherent in correct cheek tooth identification and is therefore a valuable addition to the current available methods for calculating age.

However, it is important to make a cautionary note in regards to age estimation based on epiphyseal closure. Molar scores are themselves indirect measures of age. Whereas the method of age estimation based on molar scores is based in the literature on several independent studies of animals of known age, there are no specimens of known age in this study. Although both the correlation and the regression equation for molar scores and epiphyseal fusion scores are significant, the age estimation for this study is still an indirect method. Future work should include a check of this present age estimation method with animals of known age, either through radiographs or pathology records for captive specimens.

An attempt was made in this study to obtain pathology reports for each of the museum specimens housed at both the NMNH and the FMNH that originated from the Brookfield Zoo, Chicago, and the National Zoological Park, Washington, D.C. It was not possible to make contact with the necessary individuals at the BZ in time to complete this study. For the eleven specimens obtained by the NMNH from the NZP, only three had pathology reports, and two of those specimens were outside the scope of this study (genus *Vombatus* and *Trichosurus*). Work with the zoological

parks could in the future produce more data if radiographs could be taken on known individuals in either a large cross-sectional study or a longitudinal study.

Kingsmill (1962) observed three epiphyseal regions: wrist, knee, and ankle. From this she formulated three age classes: 0-1 yr, with no evident fusion in any epiphysis; 1-4 yrs, with partial fusion in all epiphyses; and 4+ yrs with complete fusion in all epiphyses. However, though she designated those age categories, she also noted that no specimens of her study were observed for the third age class. The problem with such a method is that it provides no method of differentiating fusion patterns in the three regions. But as this dissertation shows, and in agreement with Washburn's (1946) study, epiphyseal regions do not all fuse at the same rate. A method of evaluation of the epiphyses that allows for the capture of the difference in fusion rates is a necessary and important contribution to the literature. Though the method was only applied here to the forelimb, it would work equally well on the hindlimb. In fact, a comparison to patterns in the hindlimb would be of great interest.

The goal of providing information about Macropodidae dentition to broaden the literature base was partially met by this study. As mentioned in the body of the study, one key missing group was information on *Wallabia*. Though much genetic and biochemical work has been done in the last two decades to elucidate Macropodidae phylogeny, there is still room for morphological information. Since teeth are so important to the fossil record both because of their numbers in faunal

deposits and because of the information they generate about diet and body size, it is important to have adequate available information about dentition in extant species.

The goal of separation into age categories was also met by this study. The fusion scores created for the three long bones did clearly separate specimens into adult (FS = 5), subadult (FS = 3, 4), and juvenile (FS = 1, 2). However, these fusion scores did not give support to combining some animals with partly unfused epiphyses with the fully fused specimens, a null hypothesis of the original study. In discriminant analyses, animals with a fusion score of 5 were grouped separate from those with a 4 or 3. If the null hypothesis were true, then animals with a score of 4 should have grouped with those having a score of 5. Closer evaluation of the discriminant plot does reveal, though, that on the second axis animals with scores of 3, 4, and 5 are all grouped together. This result is similar to that of a contingency table which shows that at a molar score of 4 there are animals with both 4 and 5 fusion scores. There is supporting evidence then that there are some similarities in size and shape between animals with a score of 4 and 5, but it is not clear and definitive.

Further study should be conducted on the epiphyseal fusion patterns in males and females in the sexually dimorphic Macropodidae, species with heteromorphic and homomorphic growth patterns, and in males of species that fight by pushing away versus those that fight by holding the opponent close.

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