Fur growth and replacement in the brushtail possum, *Trichosurus vulpecula*, Kerr.

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ABSTRACT

Seasonal hair replacement in the brushtail possum was described using skin histology and fibre measurement. Possums were held in individual cages under conditions of natural light and temperature for an observation period of 26 months, and skin and fibre samples taken from the mid dorso-lateral position at three week intervals. Counts of hair follicles from transverse sections were used to measure hair follicle activity, and changes in compound follicles. Periods of hair growth were poorly synchronized between individuals, and peak levels of follicle activity did not exceed 40 percent. Derived follicles continued to form in mature possums. The number of follicles present in compound follicles increased by an average of 23 percent per annum, amongst six adult animals of various ages. Much of the fibre growth in possum skin was therefore attributable to follicle neogenesis. On average, only 13 percent of fur fibres were replaced per annum. Primary central follicles producing guard hairs undergo normal shedding and replacement cycles. Levels of growth and seasonal variation amongst members of the original wild population were similar to those of captive animals. Using skin pigmentation as an indicator of hair growth (or "moult"), a survey of commercially collected pelts also verified the high individual variation and tendency for spring-summer growth. Also, variation in the proportions of pigmented pelts over time differed between males and females, and pigmentation was greater in juveniles than in adults. A diffuse topographic growth pattern in possums was demonstrated by fur dyeing and skin pigment patterns. However, much fur growth in possums occured in discrete patches which were attributable to repair of fur lost in intraspecific encounters. Growth of this type was most prevalent about the time of breeding in May, when 89 percent of pelts showed moderate or heavy patchy growth. The median duration of pluck induced follicle activity was 82 days, and growth time of guard hairs was 99 days. These times did not vary with differing ambient temperatures. Peak growth rates were 0.63 mm/day for pluck induced, and 0.58 mm/day for spontaneously growing guard hairs. The force required to extract fibres varied from 0.88 g/fibre under anaesthesia to 0.05 g/fibre shortly after death. Findings were discussed in relation to hair growth in eutherian fur bearers, possible control factors, and commercial management of the species.

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Chapter 1
GENERAL INTRODUCTION

1.1 Possums and fur production in New Zealand

1.1.1 Ecology and history

Brushtail possums, *Trichosurus vulpecula* Kerr, are medium sized marsupials belonging to the family Phalangeridae. They are nocturnal, arboreal, and largely solitary, occupying diverse habitats that offer suitable daytime den sites (Kerle 1984, Green and Coleman 1987). In New Zealand forest habitats, home ranges estimated by various methods averaged 3.5 ha for females and 4.7 ha for males (Green 1984). On farmland, home ranges have been shown to be smaller, although individual animals may disperse over much greater distances (Brockie pers. comm.). This species is regarded as an ecological and behavioural generalist, compared with its congener, *T. caninus*. However, females usually bear only a single young. Births occur mostly in autumn (Pilton and Sharman 1962, Crawley 1973, Bell 1981, Kerle 1984), so that the young start to become independent of the pouch in spring. Time of breeding is highly variable, and in many locations there is a second birth peak in spring. Possums are mostly folivorous, eating a very wide range of plants, but selecting certain preferred species (Fitzgerald 1976,1981).

Possums were introduced from Australia into New Zealand possibly as early as 1837 to give the new colony a fur industry (Pracy 1962). Animals from Tasmania and south eastern Australia were liberated widely around New Zealand and their subsequent spread was greatly assisted by secondary liberations (Wodzicki 1950, Pracy and Kean 1969, Triggs 1987). They are now present over most of New Zealand, except for the northern tip of the North Island and south western South Island (Wodzicki and Wright 1985).

With abundant feed and a lower level of predation possums have become very successful in a variety of New Zealand environments. An estimate of total numbers based on known densities in various habitat types ranges from 34 to 240 million animals, but is probably about 70 million (R.E. Brockie pers. comm.). Their browsing has had widespread effects on indigenous vegetation, changing the species composition of plant communities (Fitzgerald 1976), and

defoliating large forest trees (Meads 1976). Possums are also responsible for some damage to crops and pasture (Spurr and Jolly 1981). More economically important in recent years has been their impact as a vector and reservoir of bovine tuberculosis (Coleman 1981), threatening beef and dairy industries. Control operations are in place to contain possums within 14 infected areas (Livingston 1988).

1.1.2 Commercial exploitation

Possums, or "opossums"[1], were protected during the early years of establishment, although they were illegally taken for their pelts. Even at this time, their economic and ecological impact was the subject of much debate. The report of Kirk (1920), suggesting that possums could be cropped and were unlikely to become a significant pest, had a marked influence on subsequent management policy. Protection was removed in 1921, and limited numbers were trapped from 1922 to 1947 under a system of licensing. But in spite of increasing trapping activity in the war years control became necessary, and from 1951 to 1960 a bounty of two shillings and sixpence was offered. Over this period most skins went to America, either for furrier use or for shuttle cushioning in textile mills (Wodzicki 1950). As the latter application declined, lower quality possum fur came to be used for garment trim and lining.

Possums are hunted for fur using cyanide poison, leg hold traps or by shooting. Trapping and fur handling are described by Pracy and Kean (1969), Marshall (1984), and Moresby (1984). Much trapping is done on a small scale. Relatively few people count full time trapping as their profession, instead working part time or seasonally. However, the trapper population and pelt production fluctuate markedly in response to prices (Keber 1986a). Keber (1986b) described the existing range of marketing routes from producer to exporter. Skins may be collected through "road buyers", sold directly to the exporter, or sold through one of two established auction companies.

The skins are graded prior to sale according to colour, size and condition. In total, about 56 grades are recognised. The two main colour phenotypes are

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1 Debates over the vernacular names "possum" and "opossum" have been conducted in two separate arenas. While the scientific community has settled for "possum" (Bell 1981), those in the fur trade still prefer "opossum".

grey and melanic (brown). These are subdivided by the degree of rufous colouring, which is related to sex and age (Oldham *et al* 1985). The colour classes are: grey, pale grey, red neck (grey), dark brown, red brown, and miscellaneous colours (slate and rusty). Within each colour grade pelts are further divided by size into large, medium-large, medium, and small; and by condition into first, second, third, and fourth (rough). Condition is judged by (i) presence of defects such as holes, "windows" (areas from which the fur has been completely lost), or "ink spots" (pigmentation due to local regrowth of lost fur), (ii) stage of moult cycle indicated by diffuse pigmentation, and (iii) composition and "strength" of the fur. Other defects can be due to poor storage and handling, such as insect damage or oxidation of excessive fat which may result in "fur slip" during the tanning process, through bacterial or fungal degradation.

Most pelts go out of New Zealand in a dried, undressed state. Dressed skins go mostly to Italy and U.S.A. In the June year 1984, these earned $963,851 (Department of Statistics). Most raw (undressed) pelts now go directly to manufacturers, but some are resold through overseas auctions. Currently, the principal markets in order of importance are Korea, U.S.A., Germany (F.D.R.), U.K., Canada, Switzerland, Italy, and Japan (Department of Statistics). The growth of exports to Korea has been the most prominent feature of recent market trends. Korean manufacturers, like most others, use possum mainly for linings and trim in garment manufacture, and have moved up market from their former use of rabbit (Department of Trade and Industry 1984). There is some furrier use, i.e. whole coats made with higher grade possum. According to Kaplan (1974), possum was formerly "let out" (i.e. cut into strips and resewn), but is now mostly worked horizontally. New Zealand furriers presently use a range of styles, but mostly vertical let-out designs. Pearson (1984) reports that these designs are suited to use in garments with reversible linings.

1.1.3 The New Zealand fur industry in a world context

Possum fur production in New Zealand is a small part of the world fur industry, contributing to the lower quality end of the market. The remoteness and change of overseas markets has meant that for many years fur was produced with little appreciation of market needs or potential. However, over the last decade there has been an increase in awareness with investigations of the American and European fur industries by Pethybridge (1982), Giles (1982), and Pearson (1984).

The recent history of the world fur industry has been influenced by factors such as changes in fashion, conflict between factions within the industry, changing patterns of exploitation of wild resources, and new developments in fur production. North America led the world in fur production (both wild and farmed) for many years, but was eclipsed in the mid 1970's by the rise of European fur farming, especially of mink and fox. Some Far Eastern countries are rapidly developing fur manufacturing and may also become important producers. Korea is currently the largest importer of New Zealand possum (Department of Statistics). Others such as Japan have become large fur consumers. The fur farms of Scandinavia and Russia are an important part of the economy of those countries. They are well organized, usually into cooperatives or collectives, sharing facilities such as central feed kitchens, advisory services, research and marketing (Pearson 1984). The industry is coordinated at the international level by the International Fur Trade Federation (I.F.T.F.), of which New Zealand became a member in 1987.

The names "possum" and "opossum" present marketing problems. The fur of Australian brushtail possum (*Trichosurus*) produced in New Zealand is sometimes associated with that of the American opossum (*Didelphis*) (e.g. Prentice 1976, Kaplan 1974). (These two marsupials are distantly related, the former being a member of the diprotodont group which includes the koala, wombats, and kangaroos, while the latter is a polyprotodont marsupial more closely related to bandicoots, marsupial mice, and native cats (Kirsch and Calaby 1977).) The American opossum is regarded as having low quality fur and has a popular image as an scavenger and pest. The words for possum in Japanese mean "rat" and have negative connotations. This factor has presented a barrier to a potentially lucrative market for New Zealand fur producers (Department of Trade and Industry 1979).

In addition to these marketing problems and the handling faults already mentioned, the variability of possum fur is a major obstacle to the production of large uniform lots preferred by international manufacturers (Lockier pers. comm.). Wild possum pelts vary greatly in quality, colour, and fibre composition. Density and length gradients within pelts are also displeasing to manufacturers.

The New Zealand fur industry is presently based on wild possum skins. Small quantities of fitch, farmed rabbit, and "finished" possum are also traded,

although wild rabbit skins were a much larger trade than possum before 1957. Domesticated rabbit (since 1979) and fitch have been farmed in recent years but the quality of fur produced has been inconsistent and initial sales results were in many cases disappointing (Campbell, pers. comm.). However, Kraag (1988) predicted a viable future for commercial scale fitch production modelled upon European mink farming. Chinchillas were imported as an experiment in fur farming, but given the "hobby" nature of production elsewhere, have a doubtful future in New Zealand (Wright and Kettle 1985).

In relation to the international setting then, fur production in New Zealand has suffered from deficiencies in internal organisation, marketing and production standards. However, beneficial changes have been made over the last five to seven years since the creation of producer groups.

1.1.4 Recent developments

The price paid for New Zealand possum fur has fluctuated markedly in recent times (Gibbs 1979), and has been widely attributed to world economic trends, and changes in demand from overseas users coupled to the response of local producers. However, a notable feature of export figures for the past 35 years is the increase in both numbers of pelts and (inflation adjusted) value to a peak in 1980, when export earnings were $23.4 million (Comer 1985, Pearson 1987a).

The boom period was followed by a sharp decline in 1981, but had several significant and lasting effects. First, the need for greater organisation within the industry became apparent. The New Zealand Fur Producers' Association was formed in conjunction with the fledgeling fitch and rabbit breeding interests. This subsequently became the New Zealand Opossum Fur Producers' Association following a split with the latter groups. The New Zealand Fur Council remained as a common forum, but is presently inoperative. Neither of these existing groups have legislative backing, or the power to impose levies (as practiced by Scandinavian fur producers). Some difficulty has been encountered in organizing the possum fur industry, partly because of the individualism which characterizes those involved.

A second result of higher returns was a stimulation of interest in some form of possum farming. Early experiments with pastoral style farming of possums were unsuccessful (Keber 1979, Giles 1982). The few remaining

ventures have developed into intensive "fur finishing". These operations involve live capture and cage holding of wild animals while localized damage to fur is repaired, thus increasing pelt value. A comparatively high degree of organisation and capital outlay is required, including guaranteed ready access to large forested areas, holding facilities, and low cost feed. Holding time and treatment prior to pelting depends on condition, age and reproductive status (Pearson 1987b). A further development which could enhance the viability of cage finishing is the opening of a potential market for possum meat in Hong Kong (Keber 1987). Possum carcasses were sought by Chinese game importers as a substitute for a delicacy meat known as "kor-jee-lai" (or "fruit eating fox") and trial shipments were exported. Pearson (1987b) claims that cage finishing has potential for further developments, such as integration with pest control, creation of new fur markets through larger volumes of consistently higher quality pelts, and allowing a transition to full domestication and genetic improvement of the possum.

True farming of possums independent of wild resources could be conducted along similar lines to cage finishing, but is not economically feasible at present (Chai 1984). This is principally due to the low reproductive rate of the species (maximum of two per year) in relation to return. According to Chai's 1984 figures, farming is possible at a reproductive rate of 2.0 young/annum, given an improvement in pelt prices to $36, or an increase in reproductive rate to 4.0 with a pelt price of $25.

Finally, the possum boom has meant that research requirements of the possum fur industry gained some recognition (Brockie *et al* 1984, Wright and Kettle 1985), and there has been greater cooperation between commercial interests and scientific/pest control authorities (e.g. Morgan and Warburton 1987). Research aimed at facilitating a system of intensive farming, with special attention to management of reproduction, has been undertaken by the Ministry of Agriculture and Fisheries (Pearson and Ashby 1987).

Brockie *et al* (1984) identified the biology of possum fur as a further topic requiring study. It is perhaps surprising that possums have been commercially exploited for their fur over many years, with continuing problems which have combined to give the raw product a poor reputation, yet so little is known of the basic biology of fur growth upon which the industry is based. The need for such knowledge is even greater as control operations become more organized, and

sustained yield harvesting (Barlow and Clout 1983) and farming of possums are considered.

1.2 Aims and organisation of the present study

As a marsupial, the brushtail possum belongs to a group of mammals in which hair growth and moulting is poorly documented. In New Zealand, pest control is necessary, justifying hunting for fur, and the species is the basis of an established but changing industry with a need for biological information. An investigation with the principal aim of describing seasonal hair growth in this species is therefore warranted.

More specifically, the objectives of this study are:

(1) to provide basic information on the fibre type composition of the coat, and characteristics of fibres, such as length, diameter and growth rate; and to outline the later stages of development of hair follicles relating to acquisition of the adult coat,

(2) to describe follicle population dynamics, including seasonal variation in follicle activity and changes in the compound follicles of adult possums,

(3) to describe topographic moult patterns that result from the integration of follicle activity cycles,

(4) to investigate variation in moult within and between wild possum populations from different latitudes, habitats and genetic stock,

(5) to relate findings to hair growth in other species, other aspects of the biology of possums, and requirements of the fur industry for an understanding of fibre growth in possums.

Methods common to more than one section of the study are described in chapter three. Objective (1) is dealt with largely in chapter four, providing

background to the central topic - objective (2) - described in chapter five. This takes the form of fibre measurements and follicle counts from histological sections, obtained by repeated sampling of a small number of captive animals. A survey of possum pelts, addressing objectives (3) and (4), is detailed in chapter six and provides a means of testing the general applicability of the intensive study. Objective (5), the discussion of the wider implications of the findings is addressed in chapter seven.

Chapter 2
ASPECTS OF HAIR GROWTH

Scope of the review

Much of the research effort in the field of hair biology has been directed towards understanding skin and fibre growth in sheep and humans, with mice and rats widely used as convenient models. By comparison, marsupials have received scant attention. When reviewing literature relevant to seasonal hair growth in brushtail possums regard must be given to peculiarities of other species. The fur-type coats and periodicity of growth in laboratory rodents permit some generalizations about the physiological mechanisms of hair growth, however it has been noted that domestication has tended to disengage hair cycles from environmental control (Ebling and Johnson 1964a, Raushebakh 1972). Although fibre growth is perhaps best understood in sheep, genetic selection for high production and exceptionally long duration of wool growth has led to a de-emphasis of the hair cycle. Notes on pelage in a variety of wild mammals are available, but these are generally lacking in detail.

Hair growth and its various facets have been the subject of numerous reviews (e.g. Noback 1951, Chase 1954, Ebling and Johnson 1964a, Johnson 1965c, 1977b, Ebling and Hale 1970, 1983, Ling 1970, Kollar 1972, Montagna and Parakkal 1974, Spearman 1977, Ryder 1964, 1978, Oliver and Jahoda 1989, Moore 1989) and lengthy reappraisal is unnecessary. It is however appropriate to draw on this diverse material so as to first, describe the general features of hair follicle development and structure making special reference to the brushtail possum and other marsupials, and second, to focus on special aspects of hair replacement: the hair cycle, moult patterns, and control mechanisms.

2.1 Hair follicle development and possums

2.1.1 Evolution of hair, and the marsupials

Hair is unique to mammals, and is one of the key factors in their success. Hair-like sensory structures, or prototrichs, are present in amphibians and

reptiles; but only mammals possess the complex hair follicles which grow and maintain an insulating, protective coat. In the absence of intermediate structures or fossil evidence the theories on the evolution of hair follicles and associated integumentary structures remain speculative (Noback 1951, Meyer and Rohrs 1986). According to one widely accepted model, mammalian hair arose from mechanoreceptor papillae in the hinge region between the scales of synapsid reptiles, and had the initial function of monitoring interscale position. These organs were later adapted for sensing environmental contacts, and eventually acquired an insulatory function in the therapsid ancestor of modern mammals (Maderson 1972).

All extant mammals share some highly developed features of follicle and pelage structure. Group arrangements with compound follicles have been observed in monotremes and marsupials (Poulton 1894, Spencer and Sweet 1899, Lyne 1957a, 1966), as have guard hairs (Toldt 1935, Lyne and McMahon 1951) and tylotrichs (Straile 1961, Mann 1968). Therefore, these specializations of skin and hair structure would appear to predate at least the division between marsupials and eutherians which, on palaeontological evidence, occurred around the middle Cretaceous (about 100 million years B.P.) (Clemens 1977). It is therefore reasonable on phylogenetic grounds to apply knowledge of follicle and fibre structure from eutherian species such as sheep, rats, mice and humans in a study of brushtail possums. The study of possum fur may be seen in an evolutionary context not, as Gibbs (1938) believed, because marsupials have some "primitive" hair features which are intermediate between monotremes and eutheria; but as Tyndale-Biscoe and Renfree (1987) concluded regarding mammal reproduction, marsupials have much in common with eutherians. In addition they possess some uniquely derived and perhaps equally elaborate solutions to their survival problems.

2.1.2 Development and structure of skin

Hair producing skin is a complex organ owing many of its properties to the interrelations of the two main layers: the epidermis and the dermis. The mammalian epidermis is composed of keratinocytes forming the germinal epithelium, together with Langerhans cells and melanocytes from the neural

crest. The germinal layer develops from an unstratified ectoderm, which is overlayed with periderm and differentiates into spinous, granular, clear and horny layers (Sengel 1976). Loss of the periderm is followed by desquamation. In brushtail possums the periderm is retained until after birth at 17 days gestation (Lyne *et al* 1970). A rapid increase in thickness of the possum epidermis coincides with the initiation of the first follicles. Epidermal cornification and hair formation is similar to other species, except that glycogen is not detectable during follicle development. Lyne (1970b) also found no Langerhans granules in the possum. Henrikson (1969) reported that possum epidermis comprised distinct malpighian and horny cell layers. The basal cells contain filaments, while upper cells of the malpighian layer also contain membrane coating granules and keratohyalin granules. Henrikson also observed the filament and matrix structure of keratin in the horny layer.

Bolliger and Hardy (1944) noted that the epidermis of pouched young is pigmented, whereas that of adults is not. Lyne (1970b) accounted for this phenomenon when he described the distribution of melanocytes during development. Epidermal melanocytes are most numerous during follicle initiation, and most are probably incorporated into hair follicles. Epidermal melanocytes discharge melanin granules into neighbouring keratinocytes. Dermal melanocytes, on the other hand are less numerous, remain present later in development, and do not discharge granules.

Dorsal and dorso-lateral dermis derives from somatic dermatome; lateral and ventral regions from outer somatic lateral-plate mesoderm (Sengel 1976). Fibroblasts give rise to intercellular collagen, elastic and reticular fibres. The upper papillary layer of the dermis is composed of loose connective tissue and a range of cell types including fibroblasts, mast cells and macrophages. Below this the thicker reticular layer contains denser collagen and fewer cells. No detailed studies on the structure or development of the dermis in possums have been published. However, Bolliger and Hardy (1944) remarked that the dermis of the dorsal region is one millimetre or less thick, and that there are no papillae projecting into the dermis of possums.

The dermis becomes invaded by vascular and nerve networks. Up to six horizontal blood vessel plexuses have been identified in the mammalian skin (Durward and Rudall 1958). Three are described in sheep (Ryder 1955). Lyne (1970a) observed that in brushtail possums nerves first appear in the dermis at

21 days after birth, and by 54 days a dermal network is well established with branches to central primary follicles. There have been no detailed investigations of skin vascularization in marsupials.

2.1.3 Development of the pilary complex

The hair follicle and associated glands and muscle - the pilosebaceous unit - undergo integrated development largely from ectoderm, within the dermal connective tissue (Pinkus 1958). Greater understanding of hair follicle morphogenesis has recently been gained using monoclonal antibody labeling, transplant, and tissue culture techniques (Holbrook *et al* 1989).

Hardy and Lyne (1956a) formalized the description of development of Merino sheep hair follicles into eight stages which have been generally applied. Follicle formation begins with localized thickening of the epidermis ("primordia", "epidermal plugs", or "anlagen") in association with an aggregation of dermal cells. As the epidermis grows down, it invaginates to enclose the dermal papilla and form the follicle bulb. At the same time, this cell proliferation leads to the formation of a hair cone, the outer cells of which become the Henle layer of the internal root sheath. As the hair cone progresses up the follicle, the hair tip keratinizes and a hair canal forms in the upper follicle (Lyne 1957b). The hair tip enters the preformed hair canal and finally emerges from the epidermis. The mature structure consists of a hyalin sheath and fibroblasts enclosing the external root sheath and keratinizing epithelial layers which are continuous with the surface epidermis. The dermal and epidermal components are separated by a basal lamina. During periods of growth the germinal epithelium surrounding the papilla divides and differentiates into internal root sheath and fibre cell lines. The internal root sheath comprises Henle and Huxley layers and internal root sheath cuticle. Hair follicles are invested with nerves and capillary networks, larger follicles having vessels within the dermal papilla (Durward and Rudall 1958).

Lyne (1957a) applied the same eight stages when describing the development of hair follicles in the bandicoot (*Perameles nasuta*). Sweat gland formation is later than in sheep; the ental swelling regresses without associating with an erector muscle, and the hair canal opens at the skin surface when first

formed. In Gibbs' (1938) earlier study of the brushtail possum, the hair canal was described as developing from sebaceous gland cells into a blind sac which is later pierced by the emerging fibre, as in sheep. However, Lyne (1970a) pointed out that sebaceous gland tissue is not involved in hair canal formation in the first generation of hair follicles in possums.

Sebaceous glands generally develop as outgrowths on the ental side of the follicle (i.e. side forming an obtuse angle with the skin surface) (Lyne 1966). In primary follicles, sweat gland and ental swelling for attachment of the arrector pili (hair erector muscle) also grow from the ental side. Sebaceous glands usually have an acinar structure and produce sebum. This complex holocrine secretion is composed mostly of lipid, and is credited with a hair waterproofing function (Strauss and Ebling 1970). Bolliger and Hardy (1944) reported that in brushtail possums sebaceous glands form a collar around each follicle or follicle bundle, with an opening to the ectal (acute) side. In addition, central primary follicles may have a second sebaceous gland deeper in the skin opening on the ental (obtuse) side. Apocrine sweat (sudoriferous) glands are usually simple coiled glands with a duct opening to the ental side of the hair canal of primary follicles, above the sebaceous gland. In possums, as in most marsupials (Hardy 1947), apocrine sweat glands are associated with central primary follicles only (Bolliger and Hardy 1944). A third type of cutaneous gland is the eccrine sweat gland. These are merocrine glands opening directly to the skin surface. Possums possess eccrine sweat glands in the glabrous skin of the paws and naked prehensile region of the tail (Green 1963).

Skin glands may be modified for specialised functions (Mykytowycz and Goodrich 1974). Green (1963) described a wide range of skin gland structures in possums. Possums possess paracloacal glands (Bolliger and Whitten 1948), chin glands (Kean *et al* 1964) and sternal patches with well developed sebaceous and apocrine glands (Bolliger and Hardy 1944, Bolliger and Tow 1945, Oldham 1986). Fur over the sternal patches is stained dark red-brown in adults, especially males, and the secretions undoubtedly serve an important social function.

Possums, like many other mammals, are well equipped with vibrissae. Hollis and Lyne (1975) examined the vibrissa follicles of possums and found them to be structurally similar to those of eutherian mammals (Davidson and Hardy 1952).

2.1.4 Hair follicle generations and arrangement

In addition to defining stages in the development of individual follicles, Hardy and Lyne (1956a) described successive generations of follicles, which develop into different follicle types producing different fibre types, and together make up a distinct group arrangement. Hardy and Lyne (1956b) also elaborated and standardized the terminology for hair follicle types first proposed by Wildman and Carter (1939). In general, two populations of primary central follicles (PCX and PCY) are followed by primary laterals (PLX and PLY) which form on either side of the PCX and PCY, to give a "trio" formation. Secondary follicle development begins with secondary original (SO) follicles arising from epidermal primordia. Derived secondaries (SD) form by lateral branching of SO follicles, so that many follicles may come to share a common opening to the skin surface (Hardy and Lyne 1956a, Lyne 1966, Rougeot *et al* 1984b).

In the Merino sheep, PCX follicles first appear at about 64 days gestation and initiation of most subsequent follicles is completed by birth. The large number of secondary follicles result in an S/P ratio of approximately 22 (Carter 1965). The primitive condition of sheep is represented in unimproved sheep such as the Soay (Ryder 1966a), or feral and dairy goats (Ryder 1966b, Lambert *et al* 1984). In feral type goats, primaries produce coarse medullated guard hairs and there are usually about 15 to 20 secondaries per group (S/P ratio of 5-7), which grow fine down or cashmere. In mice (Dry 1926, Mann 1962, Claxton 1966) the first primaries are initiated after 14 days gestation and most follicles are established by 21 days (2 days after birth). Adult mouse follicles are arranged in roughly parallel rows so that group identity is lost (Slee 1962). A few unbranched secondaries are formed to give an S/P ratio of 0.3 (Claxton 1966). Guard-hairs (tylotrichs), awls and auchenes are grown by central primaries, and zigzag (underfur) fibres by later formed primaries and secondaries. In some species, for example humans and elephant seals (*Mirounga leonina*), secondaries are absent. In the latter case primary follicles are solitary (Ling 1965).

These few examples illustrate the variation in a basic pattern of follicle development which produces a wide diversity of group arrangements and pelage structures amongst eutherian mammals. Development of follicle groups in

marsupials follows a similar basic pattern. Detailed ontogenetic studies of hair follicles have been conducted in only two marsupial species - the bandicoot and the brushtail possum.

In the bandicoot (Lyne 1957a), primary follicle primordia first form on the mid side at 7-11 days after birth. Original lateral primaries (PLO) are added to form a trio stage, and SO follicles form on the ental side of the group (unlike eutherian mammals). Branching in lateral follicles occurs below the sebaceous gland to form bundles of between two and five hairs. Original central primaries and some PLO follicles grow a coarser, grooved hair type, whereas the hair of derived and secondary follicles is of smaller diameter and round section.

Gibbs (1938) first investigated hair follicle formation by histological and macroscopic observations of pouched young of possums. She found faster development in anterior regions and described follicle formation from epidermis. In a more detailed study Lyne (1970a) applied the stages used in his earlier work on sheep and bandicoots to define the timing of development in possums. The first generation of original primary central (PCOX) primordia are first evident two days after birth, and grow rapidly to produce emergent hair at 14 days. The second population of central primaries appears at 40-50 days, and a trio stage is achieved when PLO follicles arise in association with each central follicle at 60-70 days. At about 85 days after birth the SO follicles are added, at which time some PCOX follicles are entering their second hair cycle. By 100 days all original follicles have been initiated and at around 110 days derived follicles appear. All PLO and SO follicles branch. PCOY follicles branch infrequently, and PCOX do not branch. The resulting group arrangement in adult possums is a central primary with approximately four lateral bundles of smaller follicles sharing a common opening to the skin surface, as observed by Hardy (1947). The development of hair follicle groups in possums is diagrammatically represented in figure 2.1.

Lyne (1970a) noted some special features of PCOX follicles. PCOX follicles develop more rapidly than later follicles, initially forming smaller follicles and fibres. (However, follicle formation is generally slower in the possum than in the bandicoot.) The largest groups are those with a PCOX at their centre. Unlike ovine PCX follicles, sebaceous gland cells have no role in the formation of the hair canal of possum PCOX follicles. Sweat glands associated with this follicle type have an interrupted development. PCOX follicles receive large nerve networks and at 41 days after birth develop epidermal pads,

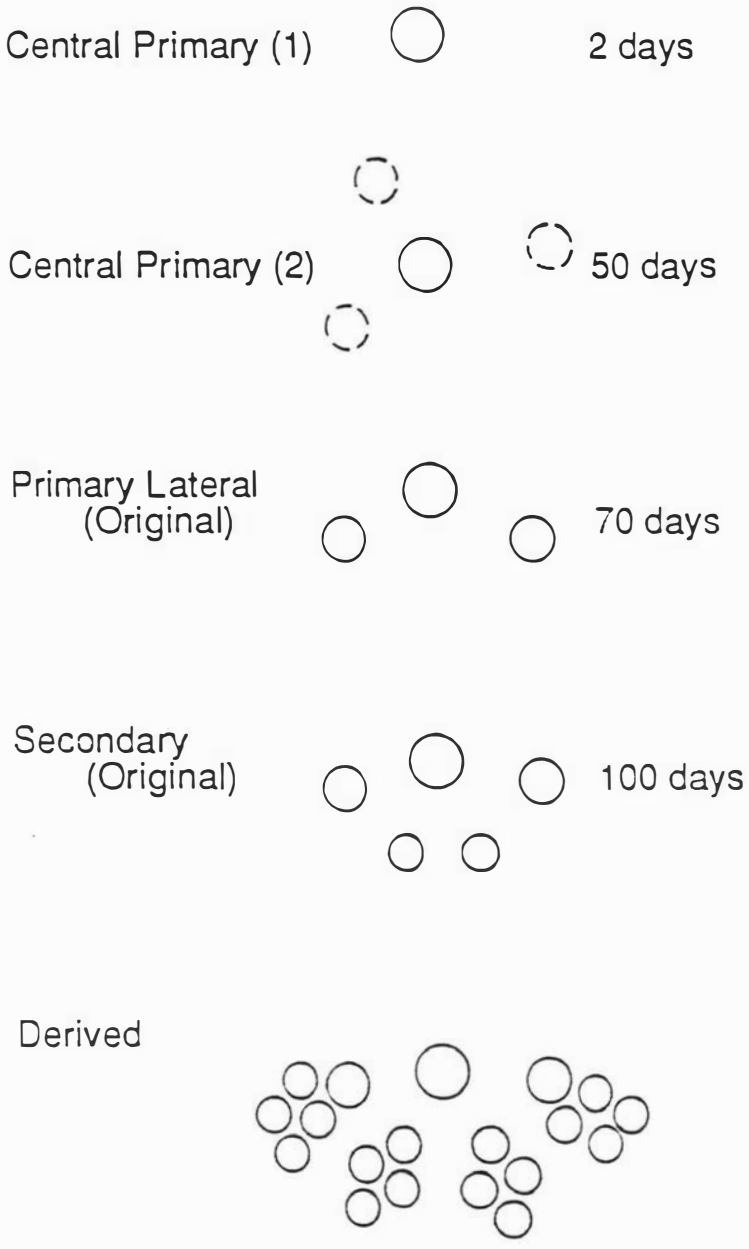


Figure 2.1 Hair follicle group development in possums. Circles represent follicles in transverse section. Dashed circles represent follicle primordia. Successive follicle generations are indicated by decreasing circle size. See text for explanation of follicle groups.

which is typical of tylotrich formation in other species (Straile 1961, Mann 1969).

On the basis of these two species and Hardy's (1947) descriptions of group arrangement in an additional 11 species, it is possible to make some general statements about follicle development and structure in marsupials. Hardy suggested that a group consisting of a central follicle with one to four lateral bundles is a characteristic feature. Although 12 of the 13 marsupial species that she examined showed this pattern, it is not unique to the suborder. Follicle groups with one central primary plus lateral bundles occur in eutherian mammals such as the cat (Schwarz *et al* 1976) and the rabbit (Whiteley and Ghadially 1954, Weddell and Pallie 1955). Some other marsupial features are sweat glands and erector muscles associated with central primary follicles only. Erector muscles are attached to connective tissue sheaths rather than directly to the primary follicles. The development of SO and derived follicles on the same side as the sweat gland (ental side of primaries) was noted in the brushtail possum by Duerden (1939), and is unique to marsupials (Lyne 1966).

2.1.5 The mechanism of follicle pattern formation

The mechanism by which spatial arrangements of hair follicles form within the skin has been approached from different viewpoints, and several theories have been proposed. A commonly adopted starting point is the role of dermal cells in the induction of epidermal growth and development of hair follicles, which has been repeatedly demonstrated in papilla transplant experiments (Cohen 1965, 1969, Oliver 1969, Kollar 1972, Jahoda *et al* 1984). This has led to the suggestion that positioning of follicles is determined by distribution of dermal papillae (Oliver 1980). Moore and Jackson (1984) have gone on to speculate that the allocation of a fixed population of dermal founder cells determines wool follicle group composition. This idea is similar to the competition hypothesis put forward by Fraser (1951). Observations such as the correlation between fineness and follicle density, and differences between follicle generations in growth rate and crimp led Fraser to suggest that adjacent follicles compete with each other for a limited amount of fibre substrate, or space within the dermis (Fraser and Short 1952).

On the basis of his work showing dermal/epidermal interactions, Cohen (1969) postulated that the blood vascular pattern of the dermis organizes papillae condensation. Induced primary follicles then cause local modifications to the vascular system which affect the development of subsequent generations of follicles. Alternatively, a mechanical stress hypothesis has been proposed by Oster *et al* (1983). Motile mesenchymal cells set up forces which deform the extracellular matrix. The stress patterns determine centres of aggregation, and thereby the spatial arrangement of skin primordia. Claxton (1964) and Claxton and Scholl (1973) applied a model which requires the diffusion of an inhibitor to create an inhibitory field around developing follicles. That is, all epidermal cells have the potential for folliculogenesis, but differentiation at one site inhibits neighbouring cells. This model becomes increasingly complex, requiring further signals as each follicle generation is added.

Many aspects of pattern formation in skin can be explained by a reaction-diffusion (RD) system model (Nagorcka and Mooney 1989, Nagorcka 1986). The RD system proposed by Nagorcka comprises two morphogens residing in the epidermis. They are postulated to form a feedback mechanism with other substances which can diffuse between epidermis and dermis. Pattern formation is a spontaneous result of the RD system properties, dependent on the size and shape of the epidermis. Predicted patterns are consistent with successive waves of primary and secondary follicle initiation (Nagorcka and Mooney 1985), follicle orientation and development of accessory structures (Mooney and Nagorcka 1985), and fibre characteristics (Nagorcka and Mooney 1982). Hence, the RD theory has considerable explanatory power, but as Mooney and Nagorcka (1985) acknowledge, further evidence for the existence of morphogens is required to support their mathematical model.

All of the above theories are speculative. This area of skin biology therefore shows potential for experimental testing of the diverse mechanisms that have been proposed, particularly with the increasing availability of biochemical techniques.

2.1.6 Fibre types and coat structure

The coat of most mammals contains fibres of differing size and form according to their follicular development (Danforth 1925). The combination of fibre types gives a characteristic coat structure, which often varies topographically and seasonally. The double coat of ungulates typically consists of coarse, long, medullated guard hairs with fine woolly down (Ryder 1978). Fur pelage found in many other mammals, including carnivores, rodents, lagomorphs and marsupials, may show a greater range of fibre types which tend to intergrade.

De Meijere (1894) and Toldt (1910, 1912, cited in Toldt 1935) surveyed the coats of many mammals and classified their hair types. Dry (1926) defined fibre types in mice. Current fibre terminology and classification is founded in this early work.

The longest pelage hairs are usually straight and relatively coarse. Hairs of this structure were called leithaare ("leading hair") by Toldt and monotrichs by Dry. Some have been described as having tactile sensory function and in this context are termed tylotrichs (Straile 1960, Mann and Straile 1965, Mann 1969). Characteristics of their follicular structure include the epidermal pad and annular complex, similar to that seen in vibrissae follicles (Mann 1968). First described in rabbits (Straile 1960, 1961), they are present in many mammals, including the Opossum, *Didelphis virginiana* (Mann 1968). Brushtail possums possess such long straight fibres (Bolliger and Hardy 1944), and as noted above, Lyne (1970a) described the formation and enervation of the first generation of primary central follicles, which produce these fibres.

Guard hairs, also called contour-hairs (Salaman 1922), grannenhaare (Toldt 1935) and awls and auchenes (Dry 1926), possess a paddle shaped portion of the fibre shaft towards the tip. These hairs arise from central primary follicles (Salaman 1922, Robinson 1958, Claxton 1966, Pastirnac and Gruia 1987). Bolliger and Hardy (1944) described the presence of numerous wavy hairs in possums grading in length from long broad-tipped awns, down to shorter down fibres.

The finest, shortest fibres are generally described as down, underfur or underwool. Toldt's (1935) names were wollhaare ("wool hair") and flaumhaare ("down hair"). In fur bearers, these fibres are often crimped or wavy with a uniserial ladder medulla (Brunner and Coman 1974). For mice, Dry (1926) used

"zig-zags" in reference to their shape. They are grown by the later formed primaries, secondaries and derived follicles (Claxton 1966, Keiji *et al* 1988). Priestley (1967) described zig-zags and other fibre types in rats, similar to those of mice.

Toldt (1935) classified brushtail possum with those marsupials having coats of fox-like colour and fibre composition. His illustration of possum fur indicates a maximum (staple) length of about five centimetres. Bolliger and Hardy (1944) give an "average" fibre length of two to three centimetres. Their fibre density estimate was 75-300 hairs per square millimetre. This compares with approximately 120 hairs per square millimetre in rabbits quoted from several sources by Robinson (1958), 50-150 for mice, and 100-150 for the monkey *Nycticebus* (Carter 1965). Lyne and McMahon (1951) and Brunner and Coman (1974) further described fibre characteristics of brushtail possums as well as other phalangers. Maximum diameter is 70 μm, and at their widest point guard hairs are oval to eye-shaped in cross section. Medullae are either of uniserial ladder or narrow aeriform lattice type. Cuticle scales over much of the fibre shaft form a diamond petal pattern.

Possums then, have a typical "fur bearer" coat. Hair morphology, medulla and cuticle scale patterns, fibre type composition, and density are comparable with other species of their approximate size, both marsupial and eutherian. It should be noted however that the fur of possums and diprotodont marsupials generally has a "wiry" or "fluffy" feel in comparison to many fur bearers. This is perhaps due to more irregular orientation of crimp and awn tips.

2.2 The hair cycle

Hair growth is not continuous. Follicles undergo cycles of growth and quiescence marked by morphological and biochemical changes. Figure 2.2 summarizes some of the changes in follicle structure that occur throughout the hair cycle. This occurs in all mammals, including humans and sheep. Most of what is known about hair cycles is based on work with rodents, and little is known from marsupials.

A classification of hair cycle stages was first proposed by Dry (1926) following his work with mice. The three main phases are: **anagen**, the fibre growth phase; **catagen**, the short period of follicle regression; and **telogen**, the "mature" resting phase when the fully grown fibre is anchored in the follicle.

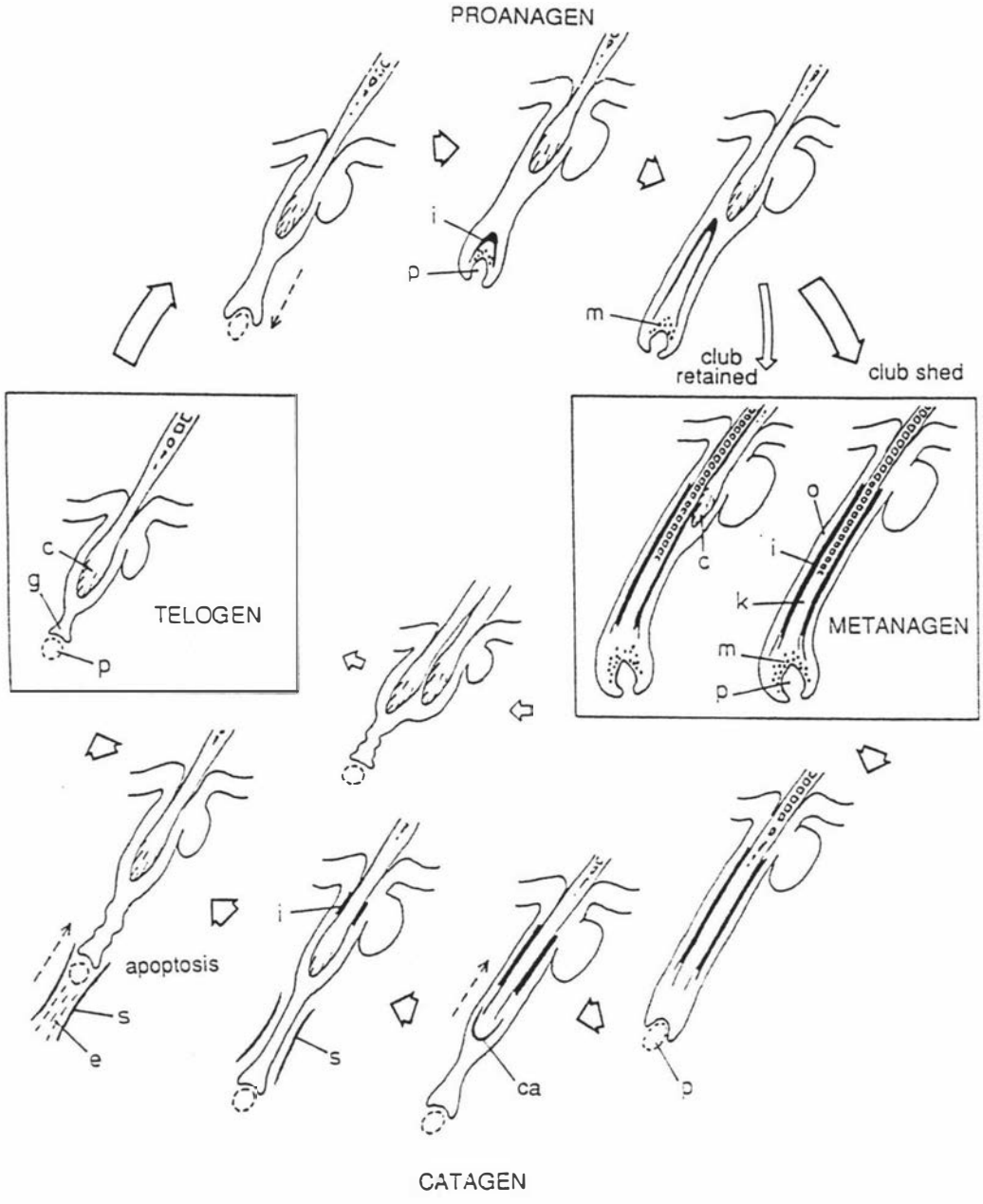


Figure 2.2 The hair cycle. Some follicular structures which change during the hair cycle are indicated; c - club end, ca - capsule, e - epithelial strand, g - hair germ, i - internal root sheath, k - keratogenous zone, m - matrix of the bulb containing dentritic melanocytes, o - external root sheath, p - dermal papilla, s - hyalin and connective tissue sheath.

2.2.1 Anagen

The period of follicle activity has been further divided into six sub-stages (Chase *et al* 1951, Chase 1954). This activation process can be viewed as a recapitulation of development. The beginning of anagen (anagen I) is marked by the initiation of mitotic activity in the hair germ, which then grows down around the papilla (anagen II). At this stage the first keratinization of the internal root sheath occurs. Maximum follicle length is achieved at anagen III. The bulb is completely formed, and includes melanotic and dendritic melanocytes. By anagen IV the fibre has formed, but is still enclosed within the internal root sheath, in a similar fashion to a hair cone during ontogeny. The tip of the hair projects through the internal root sheath and by the end of anagen V is level with the epidermis. Finally, the emergent hair continues to grow throughout anagen VI, the longest and most commonly seen sub-phase.

A shortened terminology (Chase 1965) is also used. The initial stages, anagen I - IV above, are referred to as proanagen. Anagen V is called mesanagen. And the main period of growth, anagen VI is called metanagen.

The structure of the anagen follicle is very different from the mature quiescent state. The bulb consists of a dermal papilla separated by a basement membrane from the matrix of mitotic epithelial cells interspersed with dendritic melanocytes. Cells arising from this proliferative zone divide into six keratinocyte lines disposed in concentric layers: Huxley's and Henle's layers and cuticle of the internal root sheath, and the cuticle, cortex and medulla of the fibre. A cellular shunt system has been shown to operate whereby changes in cross sectional area and shape of the fibre are largely complemented by changes in internal root sheath, particularly the Huxley's layer, so as to maintain the overall size of the follicle (Straile 1965, Priestley and Rudall 1965, Priestley 1967).

Cortical cells elongate as they move up into the keratogenous zone, where by cell differentiation and protein synthesis they become packed with hard keratin (Chapman and Ward 1979). The internal root sheath accompanies the fibre moving up the follicle, and undergoes a similar process of differentiation. Trichohyalin granules present during early stages give rise to a fibrous structure in terminal cells. The internal root sheath then degrades at about the level of the sebaceous gland, probably through the action of keratolytic enzymes (Montagna

and Parakkal 1974, Pinkus *et al* 1981). This region of the anagen follicle is known as the zone of sloughing (Straile 1965).

Curves of growth rate during anagen of different hair types, hair generations, and body regions have been shown in rats (Hale and Ebling 1975) and guinea-pigs (Jackson and Ebling 1972). Larger fibre types grew at a faster rate. Ventral hairs grew slower than mid-side or dorsal hairs. Fibre growth rates varied sigmoidally during anagen, with a maximum growth rate of approximately 0.9 mm/day in rats and 0.7 mm/day in guinea-pigs.

2.2.2 Catagen

As the follicle completes the productive phase, melanogenesis and then mitoses cease, and a fibre anchoring structure is built. This final stage of fibre differentiation and transition from activity to quiescence has been divided into eight substages by Straile *et al* (1961), again working with mice. Some stages of this process are illustrated in figure 2.2. The first stage of catagen is indistinguishable from anagen VI (metanagen), except for a decline in mitosis. This is followed by a decrease in melanotic melanocytes so that pigmentation, along with medullation ceases. The dermal papilla shortens and becomes more spherical by a change in cell shape. The bulb narrows, and retracts from around the papilla. Epithelial cells between the dermal papilla and keratogenous zone constrict and later form an epithelial strand.

A capsule, continuous with the internal root sheath, forms around the lower keratogenous zone. As these structures move up the follicle, continuing to keratinize, a brush-like club forms on the base of the fibre. Following an examination of the ultrastructure of the murine telogen follicle, Roth (1965) indicates that the club is derived from cells of the germinal matrix, and describes the numerous desmosomal connections which form between the club cuticle and external root sheath. However, from their work with dogs and humans, Pinkus *et al* (1981) claim that the club is formed not from bulb matrix cells, but instead from the stratified squamous epithelium of the external root sheath. They therefore describe club formation as "tricholemmal keratinization". The internal root sheath is viewed as playing an inhibitory role, since keratinization occurs where external root sheath is not opposed by internal root sheath.

As catagen progresses the club moves up the follicle leaving the dermal papilla and external root sheath cells behind, resulting in a narrow column of

epithelial cells enclosed in a vitreous (glassy) membrane and connective tissue sheath (Straile *et al*, 1961). Corrugation of this epithelial strand is associated with thickening of the vitreous membrane (Kligman 1961). Finally, the epithelial strand shortens so that the dermal papilla and hair germ move upward, (leaving a few epithelial and connective tissue cells which degenerate or disperse). The mechanism by which the follicle shortens has been identified in mice as apoptosis by Weedon and Strutton (1981). This is an orderly process of cell deletion by phagocytosis, in contrast to degeneration by autophagic vacuoles (Montagna and Parakkal 1974). A study of cell ultrastructure has revealed the same apoptotic mechanism in mEGF (mouse epidermal growth factor) induced catagen in sheep (Hollis and Chapman 1987).

Overall then, catagen is a process of differentiation and re-organisation, rather than degeneration (Kligman 1961, Straile *et al* 1961).

2.2.3 Telogen

The final disappearance of the internal root sheath, marks the end of catagen and the start of the resting phase, telogen. The follicle is now half to a third of its length during anagen, and consists of an external root sheath housing the club end of a fully grown fibre, as described above. The ball of dermal papilla cells is capped by the secondary hair germ (the remaining epithelial bulb cells), which may be separated from the rest of the external root sheath by an epithelial strand. Lyne (1970b) reports that in the possum, as in other species (Montagna and Parakkal 1974), melanocytes are retained in an amelanotic state in the resting hair germ. Glycogen and alkaline phosphatase are also absent (Chase 1954) reflecting the dormant state. Ling (1965, 1972) states that the resting follicles of some seals fail to undergo structural regression to the extent seen in other species.

2.2.4 Cycle stages and shedding

Figure 2.2 illustrates the main structural changes to follicles during the hair cycle, and draws attention to two additional points.

First, there are generally two stable states separated by two phases of rapid transition. The hair cycle has been viewed as a continuous process which can be

divided somewhat arbitrarily into Dry's three phases (Chase 1954). However follicles generally remain in telogen and metanagen for a comparatively long time. While proanagen and catagen involve the greatest change in form of the follicle, they are also the most rapid. Proanagen and catagen can therefore be seen as transitional phases between the period of fibre growth and the resting state when the fibre has reached maturity. Such a view was taken by Davis (1962b) who suggested the term "metagen" be used for the functional growth phase of the cycle in mature follicles. However the term has not become widely used.

Second, shedding and growth are separate, but not completely independent events in the hair cycle. In figure 2.2 the retention of the club end throughout anagen is depicted as an alternative route through the cycle. This results in an accumulation of clubs in a single follicle, as reported in rodents (Chase 1954, Ebling 1964, Khateeb and Johnson 1971a), and should not be confused with the compound follicle formed from derived follicles (Lyne 1966, Rougeot *et al* 1984b). Another relation between shedding and growth (not illustrated in the figure) is the loss of club fibres during telogen, with a delay in the initiation of anagen. This occurs in some ungulates such as the goat (Ryder 1966a) and some carnivores (Maurel *et al* 1986) as a means of achieving seasonal changes in pelage density. Thus, despite the generalizations that have been made in reviews of hair growth cycles (e.g. Ebling and Hale 1970, Schwarz *et al* 1986) shedding can occur before, during or after growth of the new fibre.

The mechanism of shedding has received scant attention. When examining the fine structure of murine telogen follicles, Roth (1965) noted numerous desmosomes anchoring the external root sheath to the cuticle of the cortex (surface of the club end). Vandevelde and Allaerts (1984) approached the problem of shedding by histochemical examination of the distribution of protein-bound thiol and disulphide groups in telogen follicles, also from mice. They supported the suggestion of Pinkus *et al* (1981) that cells of the club end are derived from external root sheath, and found a thiol-disulphide conversion corresponding to a succeeding hair generation and shedding of the club hair. Vandevelde and Allaerts therefore suggested that this conversion (part of the process of keratinization in the external root sheath) causes loosening of the old club end.

2.2.5 Effects of plucking on the hair cycle

It has been well documented that resting follicles can be induced to enter anagen by plucking the club end fibre. Hair growth can also be initiated by chemical irritants, X-radiation wounding of the skin, or simply scraping the skin (Chase 1954, Ghadially 1958, Argyris 1969), but light shaving, or clipping generally does not stimulate follicle activity.

Silver *et al* (1969) found differences between early anagen induced by plucking, and early spontaneous anagen in mice, although middle and late anagen were the same. Mitotic activity commenced throughout the entire follicle when anagen was induced by plucking. This contrasts to spontaneous anagen in which only the lower follicle, especially the hair germ, is the site of initial mitoses. Silver *et al* also described impairment of growth in the plucked follicle. Plucking has therefore been viewed as "wounding". Repeated plucking results in decreased growth response, indicating that damage to the follicle is cumulative (Horton and Whiteley 1969, Hamilton and Potten 1974).

Nevertheless, plucking has been experimentally useful. Follicles can be brought into phase at known intervals, or put out of phase with unplucked skin. Lyne (1965) used plucking to synchronize hair cycles in the skin of chinchillas (*Chinchilla laniger*), and described the induced growth cycle for each follicle type present in chinchilla skin. Plucking experiments have been used especially in the debate over the nature of factors controlling hair growth, as discussed below.

When a growing fibre is plucked from the skin of rats, the hair shaft breaks at the level of the keratogenous zone leaving the germinal matrix in the follicle (Ebling and Johnson 1964b). Fibres thus induced have smoothly tapering tips (Hale and Ebling 1979) and grow at the same rate and to the same dimensions as spontaneously initiated hair (Hale and Ebling 1975). By comparing the times of eruption between plucked and unplucked skin of rats, Johnson and Ebling (1964) showed the effects of plucking on different stages of the hair cycle. When plucking an anagen follicle, new hair eruption was advanced and the period of subsequent activity reduced. During telogen, the interval between plucking and eruption remained constant (at 12 days), except at the end of telogen when eruption was delayed. Hale and Ebling (1979) interpreted similar results as indicating that plucked telogen follicles are induced

into anagen immediately, and the constant interval until eruption represents the time taken for anagen I to anagen V. Johnson and Ebling (1964) found that once mitotic activity was initiated, plucking out the club end fibre had no effect. However, Hale (1981) comparing growth in plucked and unplucked flanks of rats found that premature removal of an old club from an anagen follicle shortened the duration of subsequent telogen. She concluded that the hair cycle can not be regarded as a discrete event, as old clubs from the previous cycle can influence behaviour of the follicle.

2.3 Patterns of hair follicle activity

The hair cycles of individual follicles are commonly coordinated with those of adjacent follicles, so that fibre growth and shedding are synchronized over a wide area of skin. This gives a temporal pattern of hair replacement. In addition, slight variations in cycle phase across the skin surface create characteristic topographic patterns of follicle activity, or moult patterns.

2.3.1 Temporal patterns

Temporal patterns often have seasonal periodicity which is adapted to climatic changes. Biannual follicle activity is an especially common pattern (Ling 1970, Maurel *et al* 1986). Summer coats usually grow during spring, and winter coats, often possessing greater insulative properties, grow in autumn. Replacement of the entire coat is typical of boreal mammals, and may even be marked by a colour change. Some examples are the stoat (*Mustela erminea*) (Rothchild 1942), the mountain hare (*Lepus timidus*) (Flux 1970), and the Djungarian hamster (*Phodopus sungorus*) (Logan and Weatherhead 1978) . Further species in which spring and autumn pelage changes have been observed in detail include: cattle (Dowling and Nay 1960), moufflon (*Ovis musimon*) (Ryder 1960, 1973), blacktailed deer, (*Odocoileus hemionus*) (Cowan and Raddi 1972), red deer (*Cervus elaphus*) (Ryder and Kay 1973, Ryder 1977), raccoon dog (*Nyctereutes procyonoides*) (Korhonen *et al* 1984), and mink (*Mustela vison*) (Bassett and Llewellyn 1949, Maurel *et al* 1986). In cases such as mink and deer, all follicles become active as the coat grows in spring and autumn,

while in winter and summer all follicles are resting. By contrast, Al-Bagdadi *et al* (1977) found biannual peaks of follicle activity in male beagle dogs, but mean activity was never below 50 percent or above 90 percent. Observing shedding in an Alsatian bitch, Hale (1982) concluded that there were about three moults per year related primarily to the oestrus cycle.

The insulation of summer and winter coats of a range of northern and arctic species have been compared. Both Scholander *et al* (1950) and Hart (1956) found greater seasonal change in thickness of fur and thus insulation in larger mammals. In small mammals, the insulative value of arctic and tropical forms were found to overlap. In those smaller than a fox there appear to be physical limits to the thickness and length of the fur, requiring these species to have behavioural adaptations for added insulation, or metabolic heat generation in cold climates.

Some mammals undergo only one moult per year. Examples are the silver fox (*Vulpes fulva*) (Bassett and Llewellyn 1949) and the European badger (*Meles meles*) (Maurel *et al* 1986). In the domestic cat, there is a single peak of follicle activity over summer-autumn (Ryder 1976), but hair replacement is relatively gradual and diffuse (Baker 1974). In species with annual or poorly defined moults, seasonal change tends to be less marked. Cases of three moults per year have been noted, for example the common shrew (*Sorex araneus*) in which there are two spring moults (Borowski 1968).

Marked seasonal pelage change often involves not only renewal of fibres, but also variation in coat structure brought about by a divergence in the timing of growth between follicle types, i.e. a difference in the behaviour of primary and secondary follicles, or subdivisions of these. Two examples serve to illustrate this point. In the red fox (*Vulpes vulpes*) Maurel *et al* (1986) described three hair types: guard hairs, fine hairs, and intermediate hairs. This species of fox has two moulting periods, but only the spring moult can be clearly observed. The spring moult begins with shedding of all fibre types. Subsequent growth of the summer coat involves all guard hairs, but not the fine hairs. Only in the autumnal moult are the fine hairs replaced to provide a thickened coat during winter. In autumn however, there is no growth of guard hairs or intermediate fibres (from primary follicles). The winter coat is maintained from December to March, when all follicles are in telogen. Anagen in individual follicles lasts for about four months, but different follicle types replace their fibres at different times.

The second example is that of the blacktailed deer, in which Cowan and Raddi (1972) reported four hair types ranging from kemp-like guard hair to woolly down. There are two moults per year in this species giving a dense winter coat containing all four hair types, and a flatter summer coat in which down and longer, finer guard hairs are absent. This is achieved by marked differences between follicle types in shedding and growing times and also duration of growth. Some grow fibres twice per year, while others produce only one fibre per year. Some primary follicles are even capable of growing fibres differing between seasons in both colour and morphology.

Compound follicles have been implicated in seasonal adaptive pelage changes (Rougeot *et al* 1984b). For example, in angora rabbits Rougeot and Thebault (1983) found that a decrease in down during summer can be attributed to regression of derived secondary follicles. Coat density is restored in autumn by the renewal of these derived follicles. Rougeot *et al* (1984b) claimed that this process is distinct from follicle neogenesis, which is believed to occur only in special cases, such as wound repair and antler growth (Lyne and Brook 1964, Billingham *et al* 1959).

2.3.2 Topographic patterns

The most marked topographic patterns of hair replacement arise when follicle activity initiated in one region of skin spreads in wavelike fashion over the body surface, as shown in mice by Dry (1926) and Borum (1954). Also using mice, Ebling and Johnson (1964b) demonstrated with a series of grafting experiments that the "waves" are not propagated, but are the consequence of intrinsic follicle cycles which can be slowly modulated by systemic factors.

Well defined, characteristic patterns have been described for a variety of mammal species. Ling (1970) collated many examples and classified moult patterns according to their predominant direction. His five basic categories were: dorsad, ventrad, cephalad, caudad, and diffuse. Ling's tables indicate that patterns of moult are not closely associated with phylogeny. Within species, moult patterns can vary seasonally. In mink, for example, the spring moult is caudad, whereas the autumn moult is ventrad - proceeding in almost the opposite direction (Bassett and Llewellyn 1949, Maurel *et al* 1986). Moult patterns also vary with age. Maturational or "juvenile" and "post-juvenile"

moults have been noted in most detailed studies (e.g. Kryltzov 1964, Linzey and Linzey 1969, Olsen 1980). Once adult pelage is attained moulting is usually described as "seasonal". However, patterns tend to become less regular with increasing age, as shown in wild rabbits (*Oryctolagus cuniculus*) (Stodart 1965) and the old-field mouse (*Peromyscus polionotus*) (Golley *et al* 1966). Kryltzov (1964) reports that amongst rodents and lagomorphs, diffuse moults are common in older animals. Age related moult patterns have proved useful in field studies, for example in determining the age of muskrats (*Ondatra zibethica*) (Shanks 1948, Schofield 1955) and cottontail rabbits (*Sylvilagus floridanus*) (Negus 1958). Physiological condition reflecting reproductive status can also affect moult pattern as well as timing (e.g. Negus 1958, Borowski 1963, Olsen 1980). Meyer *et al* (1980) report that some domesticated mammals have more irregular moulting than their wild counterparts.

Moult patterns have been broadly classified into three types; mosaic, wave and seasonal (Ebling 1965a, Ryder 1965, Chapman 1986). In the mosaic type, follicle cycles are said to be completely asynchronous. The usual examples given are humans and guinea pigs. Wave replacement refers to progression of a front of follicle activity, as in laboratory rodents. Seasonal moulting occurs in species with marked seasonal coat changes, as in many ungulates. However, this classification is not very useful, mainly because the categories describe different qualities and therefore are not mutually exclusive. "Wave" patterns can be seasonally periodic - for example, as in the rodents described by Kryltzov (1964) - or continuous, as in laboratory rats and mice. In many cases of "seasonal" replacement there is also a topographic pattern, as shown for some ungulates by Meyer *et al* (1980). The examples of "mosaic" replacement have also been shown to have underlying pattern. In humans, waves of hair growth are evident in early life (Pecoraro *et al* 1964), and seasonality in shedding has been recorded (Orentreich 1967). In guinea-pigs, Jackson and Ebling (1971,1972) report synchrony between follicles of the same type, and suggest that a wave pattern is present but obscured by differences between follicle types. This oversimplified classification can thus lead to confusion; for example whether hair replacement in the domestic cat is mosaic (Baker 1974, Muller *et al* 1983) or seasonal (Ryder 1976). It would be more informative to characterize hair growth patterns by the degree of each of the three component features of follicle activity: (i) local synchrony, (ii) wave progression, and (iii) seasonality.

2.4 Control of hair cycles

2.4.1 Theories of control

There have been various attempts to explain cycles of activity in individual follicles, and their coordination which produces patterns of moult. The theory that a mitotic inhibitor substance accumulates in anagen follicles and is lost during telogen (Chase 1965, Chase and Eaton 1959) influenced much of the earlier work on the control of hair growth. The inhibitor theory appeared to explain induction of growth by plucking (i.e. by removal of the inhibitor) but was not consistent with results of plucking experiments obtained by Johnson and Ebling (1964). Also contributing to decline of this concept was a failure to identify a tenable inhibitor, other than by an extension of the chalone hypothesis (Bullough and Laurence 1964, Bullough 1975). It was claimed that in addition to an inhibitory effect, a stimulator might also be evident (Davis 1962a). Indeed, control of mitotic activity in the hair follicle might be better explained by stimulatory action (Argyris 1962, Johnson 1965a).

The proposition that the pattern of hair growth is determined developmentally was put forward by Nay and Fraser (1954) and Slee (1962). From their observations of murine follicle development they suggested that synchronous development of follicles results in synchrony of activity, which remains for subsequent cycles. Slee (1965) noted that the sequence of shedding over the body of Wiltshire Horn sheep follows the chronological order of initiation in the embryo. Ling (1970) labeled this "morphogenetic induction", and pointed out that species in which moulting proceeds in different directions in alternate seasons do not readily fit the theory.

Durward and Rudall (1958) compared the blood vascular system of active and quiescent areas of skin in rats and rabbits. They suggested that vascularization of foliicles may be influential in spreading the wave of hair growth. However, Ebling and Johnson (1964a) claimed that anagen precedes increased blood supply and therefore can not be causally related to the activity state of the follicle.

The importance of other features of the dermal environment has been recognised. It has been suggested that the duration of telogen and rate of progress of the moult wave is influenced by the collagen content of the dermis

(Ebling and Johnson 1964b). Moretti and his co-workers have investigated the role of mast cells and fibroblasts in influencing hair cycles, particularly by mediating the effects of hormones (Moretti *et al* 1963, 1981). They point out that epidermal and dermal events are coordinated so that a "skin cycle" might be a more appropriate concept. Ebling and Hale (1983) have criticized the Italian and Japanese studies in this area on the grounds that epilation used to induce growth could also inflict damage that produces changes in the dermal environment that they are trying to measure.

Any general explanation of control of hair growth must take account of three essential features. First, hair follicles are not simply switched by some remote cycle regulator, but result from intrinsic rhythms of the follicle, modulated by systemic factors. This was clearly demonstrated in grafting experiments (Durward and Rudall 1949, Ebling and Johnson 1964a, Johnson 1965b). The follicles of rotated autografts retained the rhythm and fibre characteristics of their original site (Ebling and Johnson 1959), suggesting that waves of growth are not propagated. Homografts between rats with hair growth out of phase showed that follicular rhythms were eventually brought into phase with the younger recipient (Ebling and Johnson 1961). A similar result was achieved with parabiotic rats (Ebling and Johnson 1964b). These experiments showed that there is both an inherent rhythm and systemic adjustment via a humoral mechanism.

Second, the interaction between the dermal papilla and the germinal matrix is likely to be of key significance. Papilla/epidermal recombinant experiments have established that the papilla induces epidermal proliferation (e.g. Cohen 1965, Jahoda *et al* 1984). It has been proposed that this relationship applies to normal hair cycles, in which the papilla is the cyclic centre and source of factors signaling growth and regression (Oliver 1980, Oliver and Jahoda 1981).

Third, control factors operate hierarchically. Environmental stimuli act upon neuroendocrine mediators, ultimately altering cell biochemistry. These complex sets of factors interact, and although effects of some factors are known in a limited number of species, the exact mechanisms of message transmission are not well understood. According to the generally accepted theory of hormonal control (Johnson 1981), moulting cycles are adjusted to meet environmental conditions through the action of seasonally fluctuating hormone

levels. Numerous experiments have shown the influence of various hormones on the hair cycle, and recent attention has concentrated on the role of the pineal gland in adjusting annual cycles to meet environmental changes.

2.4.2 Environmental factors

A relationship between day length and timing of hair and sexual cycles has been known in various species for many years. Moulting in ferrets, for example, has been affected by manipulating photoperiod (Bissonnette 1935, Hammond 1952, Rust and Shackleford 1969). Harvey and MacFarlane (1958) used reversed photoperiod to show that decreased daylength was followed by shedding and new growth to a winter coat, whereas with increased day length some shedding occurred to give a summer coat. Similarly, repeated experiments with mink have shown that reduced photoperiod accelerates fur maturation by shortening telogen (Bissonnette and Wilson 1939, Bassett and Llewellyn 1949, Weiss *et al* 1980, Prasolova *et al* 1984). By contrast, earlier completion of moult was induced by increasing daylength prior to the summer solstice in the silver fox (Bassett *et al* 1944), a species moulting once per year.

Khateeb and Johnson (1971b) exposed field voles (*Microtus agrestis*) to long days (16L:8D) in autumn resulting in growth of a summer coat, whereas short days (6L:18D) induced a winter coat. Another vole, (*M. montanus*) raised under long days completed maturational and adult moult sooner than those raised under short days (Pinter 1968). But availability of food also had an effect, and Pinter speculated that under natural conditions, light, diet and temperature combine to control pelage changes.

Artificial long days in winter advanced the onset of spring moult in white tail deer bucks (*Odocoileus virginianus*) (French *et al* 1960) and in roe deer does (Lincoln and Guiness 1972). Kay and Ryder (1978) subjected red deer to annual changes of photoperiod compressed into six months. A corresponding doubling in the frequency of shedding and hair growth resulted, although some shedding was incomplete. Shortening of the cycle was achieved by a shortening of the time that the mature coat was retained (telogen), with the duration of growth remaining the same. In cattle exposed to reversed photoperiods seasonal coat changes were reversed (Yeates 1955). Feral doe goats kept in continuous light

continued to exhibit cyclic growth, but showed a reduced cycle period - three cycles in a year compared with two in normal light (McDonald *et al* 1987). Lengthening of the cycle period again under prolonged continuous light led these authors to suggest that photorefractoriness was becoming evident. Such a period of apparent unresponsiveness to photic cues was more clearly shown in the moulting cycle of mink (Duby and Travis 1972).

Although seasonal moulting has largely been abolished in improved sheep breeds, an annual rhythm of wool production remains, and the primitive pattern of shedding and growth persists on the legs of Merino and Southdown sheep (Hutchinson 1965). It has been demonstrated that the circannual rhythm of wool growth is determined primarily by photoperiod (Morris 1961, Hart *et al* 1963, Hutchinson 1965). In some breeds of sheep, seasonal shedding has been retained. Slee (1965) showed that exposure to continuous light retarded shedding in the Wiltshire Horn sheep. Rougeot (1961) treated Limousine ewes with two reverse semestrial light cycles and observed corresponding changes in kemp follicle activity. Changes in follicle activity were even more marked in a similar experiment conducted on moufflon sheep (Rougeot *et al* 1984a).

Do nocturnally active mammals such as the possum use light for setting seasonal cycles? Goldman (1983) reviewed a set of experiments designed to answer this question using Syrian (or golden) hamsters (*Mesocricetus auratus*) in artificial burrows. Not only were activity rhythms and testicular regression entrained by photoperiod, but hamsters were able to respond to a "skeleton" photoperiod with a light pulse as brief as 30 seconds.

Temperature effects on hair growth have been most easily observed in species with marked colour change, such as mountain hares and stoats. In captive stoats, cold environmental temperature hastened autumn change to winter coat, and delayed spring change to summer coat (Rothchild 1942, Rust 1962). Hewson and Watson (1979) studied regional variation in the moult of wild Scottish stoats, and found that the proportion turning white in winter was associated with snowfall, snowlie and monthly minimum temperature, but not mean temperature. Temperature also influences the extent of whiteness of winter coat in stoats, as it probably does in the Djungarian hamster (Logan and Weatherhead 1978). The rate of spring colour change in Scottish mountain hares was retarded by cold and accelerated by warm spring seasons (Watson 1963, Flux 1970). Watson observed that hares at higher altitude turned white

earlier in autumn, turned dark later in spring, and became whiter in winter than hares at a lower altitude. Both Watson and Flux concluded that moult is triggered by daylength, but the subsequent rate of progress is modified by temperature. However, mechanisms for these modulating effects of temperature are not understood. It should also be noted that these examples are boreal species, and the extent to which temperature influences hair growth in southern temperate or tropical mammals is unknown.

Photoperiod then, is the principal environmental agent determining the timing of moult, at least in mammals of the northern hemisphere, but the use of photic cues varies between species. Temperature and nutrition have also been shown to correlate with the rate of pelage replacement in some cases.

2.4.3 Hormonal control

2.4.3.1 The pineal gland and melatonin

If daylength is the main cue for seasonal physiological cycles, then what is the mechanism for converting the light stimulus into a chemical message, and transmitting that message to the hair follicle? It is now established that the retino-hypothalamic tract conveys photic information to the suprachiasmatic nuclei (SCN) of the hypothalamus, which in turn are connected via the superior cervical ganglion to the pineal gland (Moore and Card 1985). The pineal gland produces a number of indolamines and polypeptides, the most well studied of which is the tryptophan derivative, melatonin. Melatonin production is responsive not only to the daily cycle of light and darkness, but also to a circadian clock located in the SCN. By this means, the pineal is thought to play a key role in daylength discrimination (Stetson and Watson-Whitmyre 1984) and entrainment of seasonal activities to annual changes in photoperiod (Kennaway 1984). It has therefore been described as "an adaptive self-tuning neuroendocrine phase modulator" (Quay 1984). The hormonal signal from the pineal has been shown to influence the secretion of releasing hormones from the hypothalamus, and thereby activity of the anterior pituitary gland (Reiter 1980, Lincoln *et al* 1982). The duration of the nightly melatonin pulse rather than the total amount has, in one case, been found to be a critical parameter of secretion (Goldman 1983).

Various models for the precise interactions of melatonin with the hypothalamus have been proposed, but remain speculative (Stetson and Watson-Whitmyre 1984), and these interactions probably vary between species.

Although much recent attention given to this photic control mechanism has focussed on seasonal reproduction and fertility, effects on hair growth cycles have also been noted. The first experiment demonstrating an effect of the pineal gland and melatonin on hair growth was that of Houssay *et al* (1966). The passage of waves of hair growth was hastened in pinealectomized mice, whereas melatonin administered to both intact and pinealectomized mice slowed growth waves. Mink treated with melatonin implants moulted summer pelage to grow a winter coat six weeks earlier than controls (Allain and Rougeot 1980, Rose *et al* 1984). The same effect was achieved with reduced photoperiod (6L:18D). "Weasels" (*Mustela erminea*) normally moulting to a brown coat grew white winter pelage when treated with melatonin (Rust and Meyer 1969). As in mink, red deer hinds subjected to shortened photoperiod or melatonin treatment were induced into earlier moulting from summer to winter pelage (Webster and Barrell 1985). Change from winter to summer pelage in the Djungarian hamster (*Phodopus*) was accelerated by exposure to long days (16L:8D) and this advance was delayed by melatonin treatment, mimicking the effect of short days (Hoffmann 1973). In summer however, melatonin treatment prevented pelage change, mimicking pinealectomy (Hoffmann 1981). Melatonin implants in golden hamsters (*Mesocricetus*) alter the effects of photoperiod (on gonadal regression and recrudescence) in ways different to that reported in *Phodopus* (Hoffmann 1981, Stetson and Watson-Whitmyre 1984). In golden hamsters melatonin counteracts photoperiod in both summer and winter.

These studies indicate that photoperiodic effects on hair growth are mediated by pineal secretion of melatonin, but that the pineal signal may be used in different ways by different species to regulate the timing of growth. Coat characteristics such as fibre type and pigmentation are also affected. In some species there may be photoperiodic effects which bypass the pineal (Hoffmann 1981), and yet in others pineal mediated photoperiodic cues can be overridden by other factors such as nutrition (Kennaway 1984, Hoffman *et al* 1987).

Whether or not melatonin acts directly on the hair follicle to affect fibre growth has not been fully resolved. Some attempts to locate melatonin receptors in skin have been unsuccessful (Kennaway and Seamark pers. comm.), and there

is ample evidence that melatonin influences hair growth through its effects on other hormones. However, direct effects of melatonin on melanogenesis within the follicle have been reported. Logan and Weatherhead (1978) found that in the autumn moult of *Phodopus* growth of unpigmented hair was accompanied by high tyrosinase levels, just as in the spring moult when pigmented hair is produced. After adding melatonin to hamster hair follicles *in vitro* they observed inhibition of melanogenesis but not of tyrosinase activity (Logan and Weatherhead 1980). They therefore suggested that melatonin directly inhibits (MSH induced) melanin biosynthesis at a post-tyrosinase step in the pathway.

2.4.3.2 The pituitary gland and prolactin

The pituitary gland plays a key role in regulating moulting cycles, mediating between the hypothalamus and various endocrine glands, and probably also acting directly on hair follicles. The importance of the pituitary has been demonstrated by hypophysectomy and autograft experiments. Hypophysectomized rats showed advancement of the time of eruption of hair in the subsequent replacement wave on anterior and dorsal regions (Ebling and Johnson 1964b). In short-tailed weasels, hypophysectomy resulted in regrowth and maintenance of white winter pelage only (Rust 1965). However, growth of pigmented hair following plucking was induced by administration of MSH and ACTH. Pituitaries of hypophysectomized weasels transplanted to the renal capsules likewise restored pigmentation (Rust and Meyer 1968). Normal hair replacement cycles were also disrupted by hypophysectomy in mink (Rust *et al* 1965), so that the winter pelage was grown regardless of previous coat type, and the wave pattern became irregular.

Wool growth has been shown to cease in hypophysectomized sheep (Ferguson *et al* 1965). Bovine growth hormone failed to restore wool growth, but a response was obtained with crude pituitary extract.

Recently, attention has been given to the relationship between melatonin, the pituitary hormone prolactin and hair growth. Observations that changes in photoperiod, nightly melatonin secretion, and prolactin levels are related (e.g. Kennaway *et al* 1982) and are associated with change in reproductive state and hair growth led to the suggestion that melatonin regulates moulting primarily via

its effects on prolactin secretion (Allain *et al* 1981, Martinet *et al* 1983, Rougeot *et al* 1984a). This mechanism was suggested by Lincoln and Ebling (1985) to explain the timing of moult from the scrotum of Soay sheep. In mink, the increase in plasma prolactin was found to be closely related to the lengthening of photoperiod and induction of spring moult (Martinet *et al* 1983, Rose *et al* 1987), and 11L:13D was proposed as the threshold daylength at which prolactin secretion is inhibited. Martinet *et al* (1984) pursued this enquiry with experiments to show the dependence of autumn moult on circulating prolactin levels. Under natural conditions the moult coincided with seasonal decrease in prolactin. Earlier suppression of prolactin by bromocriptine induced an early onset of moult. Conversely, injection of bovine prolactin to delay the seasonal decline resulted in a delayed and incomplete moult. Similar experiments with Djungarian hamsters by Duncan and Goldman (1984b) showed that ovine prolactin treatment inhibited pigmentation. They concluded that low circulating prolactin levels induced moult to winter pelage, and that an increase in prolactin is required for the moult to, and maintenance of summer pelage. In sheep, prolactin is reported to have no influence on wool growth (Ferguson *et al* 1965, Wallace 1979) even though the annual rhythms of blood prolactin and wool growth are correlated.

2.4.3.3 Other endocrine glands

The hypothalamo-pituitary axis has also been shown to exert an influence on hair cycles and the passage of moult through other glands. Observations of coincidence of hormonal state and pelage cycle, and removal and replacement experiments have led to the conclusion that hair growth is generally controlled by the changing balance of stimulatory and inhibitory effects of endocrine systems, interacting with inherent follicle rhythms (Johnson 1977a, Ebling & Hale 1970). Reviews of the hormonal control of hair growth are available (e.g. Ebling and Hale 1983), however the main points are reiterated below.

Activity of the thyroid gland has been noted to correspond to periods of seasonal hair growth, as well as wool growth in sheep. In mink, thyroxine was reported to be high during moult (Boissin-Agasse *et al* 1981), however thyroidectomy and treatment with thyroid powder and TSH has been shown to

have no effects on spring pelage change (Rust *et al* 1965). Ashwell-Erickson *et al* (1986) observed that in two species of seal (*Phoca* spp.), thyroid production and resting metabolic rate were at a minimum in the early moult then increased to a maximum during the main period of hair growth. In rats, Mohn (1958) found thyroxine treatment had no effect on growth induced by plucking but spread of spontaneous growth waves was accelerated. Accordingly, rats made thyroid hormone deficient with propylthiouracil had normal induced growth but retarded wave progression. Hale and Ebling (1975) showed that thyroxine increased the growth rate but reduced the duration of both spontaneous and pluck induced growth, so that the final length of fibre remained the same as that of controls. Propylthiouracil prolonged the growth period.

The role of the thyroid in actually controlling wool growth has also been unclear. Early experiments indicated that thyroxine stimulated increased wool production (Ferguson 1958, Ferguson *et al* 1965). However, Ryder (1979) was unable to manipulate follicle activity in Soay sheep with thyroxine. Maddocks *et al* (1985) established that in Merino rams fed a basal restricted diet, thyroxine is facilitatory rather than regulatory with respect to wool growth. In their experiments, thyroidectomy caused a 60 percent reduction in wool growth, but only 30 percent restoration of normal thyroxine levels was required for recovery of normal wool growth. A 300 percent thyroxine replacement increased wool production only slightly.

Hormones of the adrenal cortex, and ACTH acting through this gland, have generally been found to inhibit hair growth, although reports vary. Circulating cortisol in the seals studied by Ashwell-Erickson *et al* (1986) was high during shedding then declined to low levels during the main period of hair growth. In mink, Pilbeam and Travis (1979) report that cortisol levels were elevated during both summer and winter moults. Weiss *et al* (1980) found that artificial lighting resulted in elevated serum glucocorticoids which corresponded to early winter fur growth, and suggested that the relationship was causal. However, adrenalectomy hastened hair replacement in mink (Rust *et al* 1965). Similarly in adrenalectomized rats, all quiescent follicles immediately became active, but the duration of growth remained unchanged (Mohn 1958). Cortisone retarded eruption of follicles initiated by plucking in rats (Mohn 1958), as did hydrocortisone in mink (Rust *et al* 1965). In the latter case the seasonal moult was delayed. Adrenalin likewise inhibited both plucked and spontaneous

growth and slowed the spread of the hair growth wave (Mohn 1958) Wool growth in sheep is also usually suppressed by adrenal hormones. Ferguson *et al* (1965) report that ACTH and corticosteroids can produce "break" or "tenderness" in wool.

Gonadal hormones might be suspected of influencing hair growth, since there is a relationship between seasonal reproduction and moult, and the acquisition of adult pelage generally coincides with puberty. Furthermore, control of sex hormone secretion by gonadotropins which in turn are influenced by melatonin and photoperiod (e.g. Kennaway *et al* 1982) would seem to provide a mechanism for regulation of seasonal moult via the gonads. To what extent does this operate?

In male mink, maximum testosterone levels occur in January-February when follicles are in telogen, and seasonal moults correspond to low levels of testosterone (Boissin-Agasse *et al* 1981). However, gonadectomy and administration of gonadotropins had no effect on winter coat growth in mink and other boreal species (Rust *et al* 1965). Similarly in the Djungarian hamster, moult occurred in gonadectomized animals, although hormone implanted animals moulted more slowly (Duncan and Goldman 1984a).

Androgens generally exhibit only minor effects on hair growth. Mohn (1958) found that testosterone caused hair of male rats to be normally coarser than that of females. The progress of growth waves in rats was usually not affected by testosterone, although it was retarded by very large doses. Mohn (1958) also found that cortisone potentiates testosterone. In the voles studied by Khateeb and Johnson (1971c) castration in autumn advanced initiation of hair replacement, and the winter coat was of high density. Castration in spring did not affect the time of growth, although the result was a dense winter-type coat.

Oestrogens are potent inhibitors of hair growth. Ovariectomy in female mice and rats has been shown to hasten the growth wave. When oestradiol benzoate was administered to rats, pluck induced growth slowed, and spontaneous growth ceased (Johnson 1958, Mohn 1958). Hale and Ebling (1975) found that oestradiol treatment produced decreases in hair length, growth rate, and also duration of anagen. Jackson and Ebling (1972) likewise found reduced growth rate and delay of initiation of anagen by oestradiol in guinea-pigs, but in this species the duration of anagen was not affected. Rampini *et al* (1971) detected changes in oestradiol metabolism which coincided with hair

cycles. Both Pinter (1968) and Khateeb and Johnson (1971c) considered that oestrogens accounted for the finer hairs grown by female voles. Progesterone has been found to have little or no effect on hair growth in rats (Mohn 1958).

Using mice with a "naked" gene, Nay and Fraser (1955) recorded marked inhibition of the hair growth wave during pregnancy and lactation. At the end of lactation, hair growth resumed all over the animal, except where the growth wave was interrupted. These authors concluded that hormonal inhibition brought about fibre retention during early anagen, but had no effect on follicles in later anagen. Pregnancy and lactation also depresses wool production in sheep. Figures summarized by Corbett (1979) show wool growth reduced by about 30 percent in the second half of pregnancy, and approximately the same amount during lactation.

Pinter (1968) found no precise relationship between sexual maturation and subadult moult in voles. And in laboratory rats, control of coat development has been attributed to prolactin, rather than sex hormones (Rennels and Callahan 1959).

Sex hormones then do not control the timing of hair cycles. They can affect the form of fibre grown, and oestrogens in particular can suppress hair growth. But in general, moulting is linked with pubertal changes and breeding through a higher level endocrine control, as shown for example in red deer (Webster and Barrell 1985) and hamsters (Hoffmann 1981).

In conclusion, the established view that hair cycles are modulated by fluctuations in the activity of thyroid, adrenal and sex glands might explain hair growth control in domesticated animals in which seasonal rhythms have to some extent become disengaged, and perhaps for some species such as the voles studied by Khateeb and Johnson (1971a-c). But as we are reminded by Panaretto (1979), this theory is founded largely on experiments with nonphysiological doses of hormones and inference from correlations between gland activity and pelage change. Furthermore, recent work on more strongly seasonal species, such as mink, indicate a more direct response to photoperiod mediated through prolactin. Whatever the channel of control operating in any particular case, the mechanism of hormone action at the hair follicle remains unknown. There is increasing evidence however of paracrine effects involving growth factors.

2.4.3.4 Local hormones

Recently, there has been interest in signal molecules involved in local interactions between and within cells, or paracrine and autocrine effects. Such growth factors have been shown to play a role in stimulation and inhibition of cell proliferation, cell migration and adhesion, and cell differentiation. These are key processes in hair follicle development and cyclic activity, and growth factors - such as epidermal growth factor (EGF) - have been strongly implicated in these communication and control interactions. Moore (1989) has reviewed the role of growth factors in the regulation of growth and development of skin and hair follicles.

Mouse epidermal growth factor is a polypeptide of 53 amino acid residues (Cohen 1986). The receptor is a transmembrane glycoprotein (Carpenter 1987). When EGF binds at the cell surface, phosphorylation of tyrosine residues on the cytosolic side of the receptor activates a cAMP independent tyrosine kinase, and through a reaction cascade has a range of effects on cell metabolism. The biological actions of EGF are hyperplasia, hypertrophy and mitogenesis (King and Carpenter, 1983). The mechanism of action in cell culture conditions has been proposed to involve the creation of "an imbalance of homeostatic signals that favours cell proliferation" (Carpenter 1978).

The distribution of unoccupied and accessible EGF receptors in rat skin and hair follicles was demonstrated by Green and Couchman (1984), by culturing tissue in ^{125}I-labeled EGF and autoradiographing histological sections. They found that EGF receptors were localized in proliferating basal cells of the epidermis and hair follicle external root sheath, but noted a curious loss of EGF receptors from basal epithelial cells directly above the dermal papilla. On the basis of their observations, these authors suggested that EGF was involved in regulation of the hair cycle. Increases in EGF receptor binding coincided with increases in epidermal cell mitosis. Proliferating, undifferentiated components of the hair follicles were labelled, along with the secondary hair germ during telogen. They concluded that this represents a potential mechanism for the initiation of cell proliferation during anagen. Green and Couchman further proposed that a potential source of EGF could be the dermal papilla, which exhibited no labelling throughout the hair cycle.

The effects of EGF in mice and sheep have been examined by Moore and his co-workers, modifying the concept of EGF as a growth promoter. Mice

injected with EGF showed earlier tooth eruption and opening of eyelids, and lower body weight than controls (Moore *et al* 1981). Monotrichs were shorter, finer and curved, with bends corresponding to the time of injection. So, in contrast to the previously described mitogenic effects, EGF acts under these conditions as a specific mitotic inhibitor. In EGF infused sheep, Moore *et al* (1985) found that the response in basal cells of the epidermis and sebaceous glands was a sharp increase in mitotic index on the second day after infusion, returning rapidly to pre-treatment levels. Wool follicles on the other hand, responded in an inverse manner undergoing an equally sharp decline and recovery in mitotic index. The regression of wool follicles resembled a brief catagen phase and resulted in depilation.

Further investigation of growth factors promises to link hormone action and some of the interactions between skin constituents with functioning of the hair follicle at the cellular and molecular levels. However, an integrated approach with contributions from many disciplines will be required to achieve an understanding of these control mechanisms adequate to explain the diversity of hair growth patterns and processes found in nature.

Chapter 3
MATERIALS AND METHODS

The techniques and materials used throughout the study are detailed in this chapter. Additional methods used only within each section are described in chapters four to six. In particular, methods used in a survey of wild possum pelts are contained in chapter six.

3.1 Animal capture and maintenance

Capture and experimental groups

Possums used in the captive study were trapped in box or cage traps from the Keebles Bush reserve, a 14 ha remnant of podocarp-broadleaf forest in the central Manawatu district surrounded by exotic trees and pasture (Esler 1978). Possums were initially captured and selected between December 1984 and January 1985. Only healthy, grey coloured possums were used for captive study. Selected animals were weighed and ear tagged with size 1 bird tags. A two to six week period was allowed for adjustment to captivity before animals were assigned to trials.

Eight animals comprising four females and four males were then assigned to a natural light and temperature group (group A). Two possums, one of each sex, obtained from the Taupo area were included in group A. Subsequent replacements were made to this group until April 1986. Group A was used for the main investigation of seasonal changes in hair follicles. Five animals, two females and three males (group B) were kept under the same conditions as group A, and were used for ancillary experiments. A third group (group C) comprising two animals of each sex, was assigned to a controlled light and temperature group. This group was used for measurement of growth times.

Trapping was continued at Keebles Bush reserve until January 1987 to provide a small monthly sample for comparison with group A. A total of 122 animals caught over the 15th, 16th, and 17th of each month were killed, skin sampled, and their pelts stretched and dried on boards by the method of commercial possum trappers (Marshall 1984).

Housing

The captive animals were individually housed in 35 x 35 x 70 cm wire mesh cages fitted with external wooden nest boxes. The cages of groups A and B were enclosed within a three-walled, naturally-lit building, originally designed for housing poultry. The animals were therefore exposed to natural variations in temperature and light (but not to direct sunlight), such that summer light intensities reached 3.5 x 10^3 uE/m^2/s in the cage, and 22 uE/m^2/s in the nest box. Weekly maximum and minimum temperatures within the shelter were recorded over the 26 month observation period.

Group C animals were kept in similar cages, within a controlled room of the Animal Physiology Unit, Massey University. A 12 hour light, 12 hour dark (L12:D12) photoperiod regimen was maintained over the two year observation period, and temperature controlled.

Diet and animal health

For the first two to three weeks after capture the diet consisted of mixed greenfeed (mostly *Rumex* and *Trifolium* species) and possum pellets prepared by Northern Roller Mills to D.S.I.R. specifications. Thereafter, the animals received only pelletized feed, *ad libitum*. Water was continuously available through drinking nozzles in each cage.

A drench of 10 mg/kg mebendazole ("telmin", Ethnor) mixed with honey was administered shortly after capture to reduce the effects of worm load. Topical applications of isopropanol were used to treat cases of "rumpyness" - alopecia caused by mites (Presidente 1984). Veterinary pathology reports were obtained for all animals which died after the period of adjustment to captivity and before the end of the trial, except those lost through misadventure.

3.2 Skin and fur sampling

Samples of skin and fur were taken from captive possums at three weekly intervals between March 1985 and May 1987. Animals were anaesthetized by i.m. injection of 13-15 mg/kg Alphaxalone/Alphadolone acetate ("Saffan", Glaxovet). The sample site was a mid dorso-lateral position, selected because it corresponds to a position near the middle of the stretched pelt (but not on the

dorsal midline) and has fur characteristics that are generally intermediate in relation to the rest of the pelt (section 4.5). In group A animals, sequential samples were taken from an area approximately 8 x 4 cm (see figure 3.1). Adjacent sites were about 2 cm apart. This distance was judged on the basis of earlier investigation to be sufficient to avoid abnormal hair growth induced around previous biopsy sites.

Fur was clipped with scissors to within 1 mm of the skin surface from an area of 1-2 cm^2. Clipped fur was retained for fibre measurement. Isopropanol was used to sterilize the skin and hold back the fur around the biopsy site. Skin samples were cut with a 4 mm or 6 mm diameter disposable biopsy punch (Stiefel Laboratories), and excised with a scalpel. The shallow wound was closed with a single silk stitch. This was usually groomed out by the possum within three days. Animals were weighed at the time of each sampling as an indicator of growth and condition.

Skin and fur samples were similarly taken from possums caught at Keebles Bush reserve during monthly trap sampling. In addition, fur was sampled from three other sites (figure 3.1).

3.3 Histology

The tissue sample was held in a flat position during fixation and processing by spreading the tissue between two halves of a folded 50 x 12 mm manila card label, in which two 3 mm holes had been drilled to facilitate diffusion of fixative and processing solvents. After fixing in Bouin's fluid for 8-24 hours, the tissue (enclosed in card) was washed and stored in 70 percent ethyl alcohol. Prior to embedding, the circular disk of tissue was divided asymmetrically and the larger segment embedded in wax for transverse sectioning. The smaller segment was retained in alcohol as backup, or for longitudinal sectioning.

The tissue was processed and embedded in paraffin wax according to the schedule shown in table 3.1.

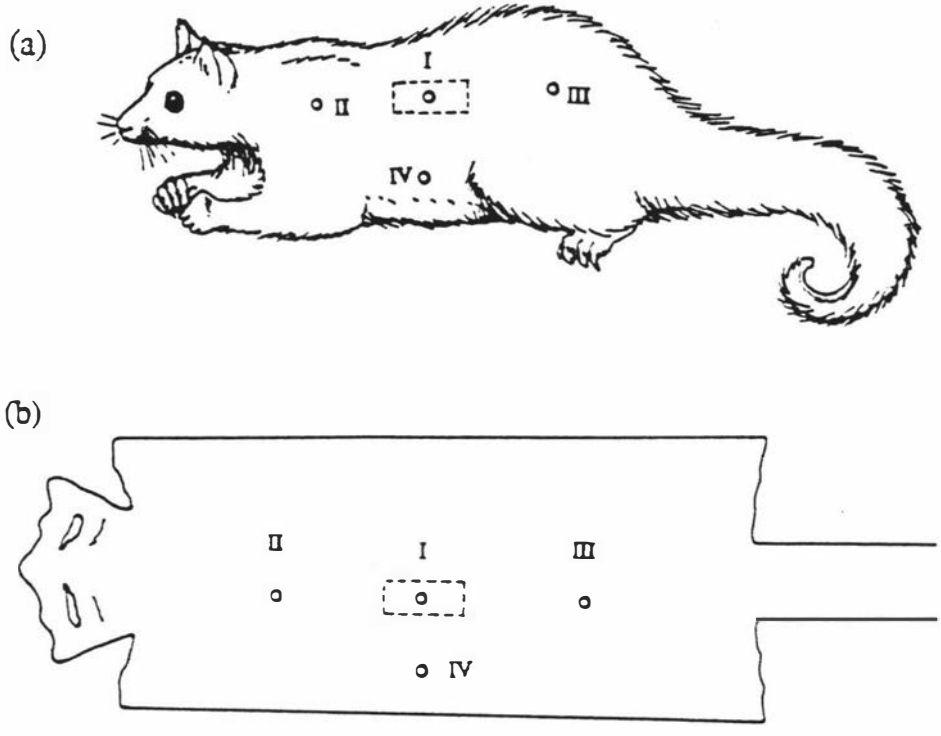


Figure 3.1 Skin and fur sampling sites; (a) on anaethetized possum or whole carcass, (b) on stretched and dried pelt. The dashed box deliniates the area from which successive biopsies were taken during the study of captive possums. Sites I to IV were sampled in animals captured at monthly intervals at Keebles Bush Reserve.

Table 3.1 Histology processing schedule.

Solvent	Time (hours)	Solvent	Time (hours)
70% ethyl alcohol	2	chloroform	1
95% " "	1	xylene	1
100% " "	1	xylene	1
100% " "	1	wax (58°C)	2
100% " "	1	wax (58°C)	2

Serial transverse sections of hair follicles of 7-8 microns thickness were cut on a Reichert sledge microtome. Excessively oblique sections were avoided by cutting at an angle of approximately 10° to the plane of the skin surface.

Most sections were stained in Mayer's haemalum and eosin (Humason 1979). A picric acid bath was added to the staining schedule before the haematoxylin if insufficient staining of fibres was achieved during fixing. Some sections were stained by the sacpic method (modified from Auber 1952, Ryder and Stephenson 1968).

3.4 Follicle scoring

The level of follicle activity in each sample was determined by counting anagen and telogen follicles on a group by group basis. Tissue sections at or slightly above the level of the sebaceous gland, with a large number of follicles in near transverse section were selected for scoring. The follicles of up to 15 contiguous groups were scored; that is, 400-600 follicles for most adult possums. All well sectioned groups within the defined area were scored regardless of their composition. For each group, the information recorded included: number of bundles, number of central primary follicles in anagen, number of central primary follicles in telogen, number of lateral (primary lateral and secondary) follicles in anagen, number of lateral follicles in telogen. The presence of any shed-empty follicles was noted. These data were processed using the computer program listed in appendix I, to give statistics for each sample.

Central primary follicles (identified by their associated sweat gland canal) were sometimes accompanied by derived follicles, i.e. bundles formed from central primary follicles. These follicles were scored as central primaries if there were one or two, and as lateral bundles if there were three or more follicles.

Follicles were scored as anagen if a growing fibre or eosinophilic internal root sheath was evident. Features of growing fibres are: medullation, pigmentation, cuticular scales, and usually weak picric acid staining. Follicles were scored as telogen if they possessed a club-end, which is strongly picrophilic, non pigmented and round in section. These characteristics of anagen and telogen follicles are seen in plates 9, 10 and 11, and further description of follicle scoring criteria is given in chapter four.

3.5 Fibre Measurement

Fibre Length

Fur length was measured on a device which held the clipped sample in place and facilitated the removal of guard hair to measure underfur length. The device (figure 3.2) consisted of a board with a graduated scale. At the zero mark of the scale was mounted a metal block, against which the cut proximal ends of the fibres were placed. The fibres were held in place by a clamp with two springs. When the pressure of both springs was applied, the sample could be groomed flat and free of loose fibre. Guard-hair length could then be read. When the heavier spring was released, the fur sample was held in place by light pressure while guard hairs were removed with forceps. The length of remaining underfur could then be read off the scale. Monotrichs were removed before measuring guard-hair length, since these are relatively rare and could not be reliably measured. A glass slide was placed on top of the fibre when making readings, and length was read at the tip of third to fifth longest fibre.

Fibre diameter

Measurement of fibre diameter using a F.F.D.A. and projection microscope is described in section 4.6.

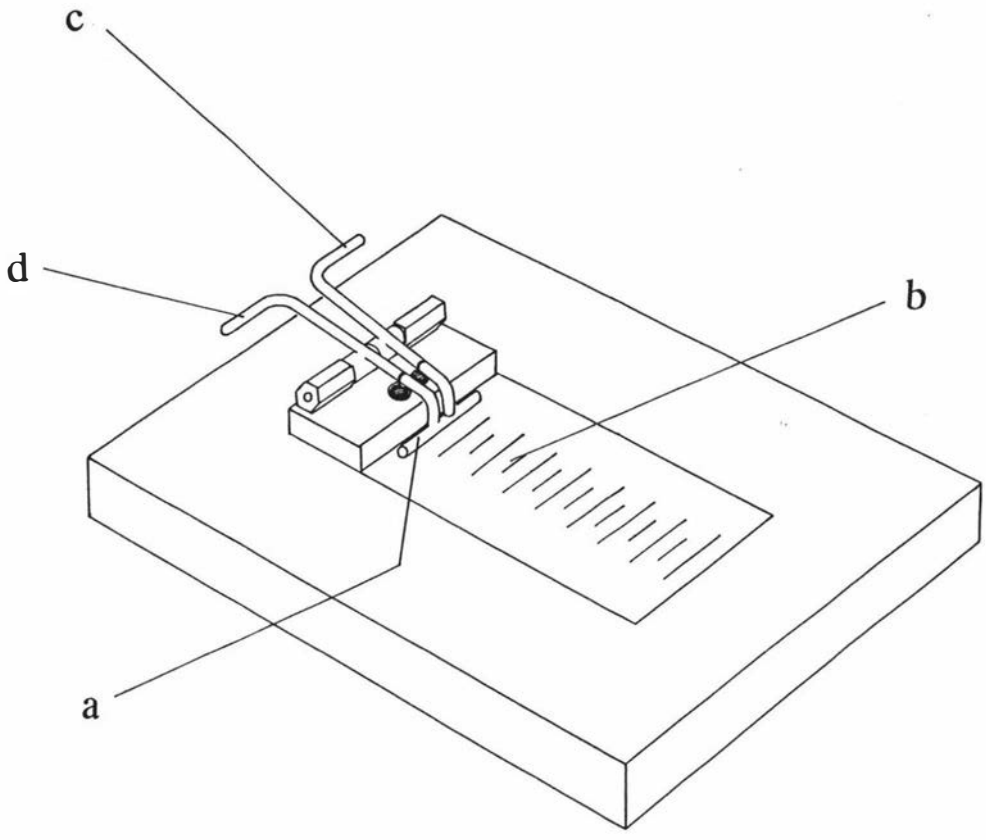


Figure 3.2 A device for measuring fur length. Small staples of fur were clamped beneath the bar (a). Guard hair length was read off the graduated scale (b). When the stronger spring was released by depressing the lever (c), guard hair could be removed while the staple was held in place under the pressure of the weaker spring attached to the lever (d) and bar. Underfur lengths were then measured.

3.6 Summary of trials, animals and methods

The history of each captive animal used in the course of the fur growth study is summarized in table 3.2. Animal numbers beginning "F" represent female possums. Those beginning "M" represent males. Age was subjectively assessed on the basis of body size and testis size. Aging of 26 possums captured at Keebles Bush reserve was performed by tooth sectioning and cementum ring staining (Pekelharing 1970), as a guide to subjective assessment. Animals classed as juveniles in table 3.2 were likely to be 10 to 18 months old at the start of observations. Possums F02 and M08 are listed in both groups A and B, since they were biopsied as well as dyed. Three of the females raised young during part of the observation period. These are indicated with a "+" in table 3.2. Young were weaned when their body weight exceeded 2.0 kg.

Table 3.2 Use of animals

Animal number	Age at start (estimate)	Start date	Finish date	Cause of death
Group A: Fur and skin sampled for seasonal and developmental studies, caged under natural light and temperature.				
F02	Adult	25-3-85	10-8-85	hypothermia after fur dyeing
F11+	Juvenile	25-3-85	11-5-87	euthanased at end of study
F20	Adult	25-3-85	19-9-86	pylonephritis, ulcerative gastritis
F22+	Juvenile	29-7-85	30-6-86	escaped
F28	Pouch Young	27-5-85	5-1-87	pulmonary oedema
F42	Pouch Young	21-10-85	11-5-87	euthanased at end of study
M04	Juvenile	25-3-85	9-9-85	suffocated under anaesthesia
M05	Adult	25-3-85	11-5-87	euthanased at end of study
M08	Juvenile	25-3-85	20-4-87	euthanased, weight loss, calcinosis
M21	Adult	25-3-85	30-9-85	escaped
M36	Juvenile	23-12-85	7-4-86	suffocated under anaesthesia
M86	Juvenile	28-4-86	11-5-87	euthanased at end of study
M88	Juvenile	28-4-86	11-5-87	euthanased at end of study
Group B: Dyed or clipped**, caged under natural light and temperature.*				
F02*		as above		
F10*	adult	10-8-85	20-4-87	euthanased at end of study
M06**	adult	25-3-85	24-7-85	euthanased at end of study
M08*		as above		
M09*	adult	10-8-85	20-4-87	euthanased at end of study
Group C: Fur and skin sampled and autoradiographed for studies of growth following epilation; controlled temperature , 12L:12D photoperiod.*				
F09*	Juvenile	2-9-85	24-7-87	euthanased at end of study
F13*+	Juvenile	2-9-85	24-7-87	euthanased at end of study
M12	Juvenile	2-9-85	12-1-87	euthanased at end of study
M25	Juvenile	2-9-85	25-6-86	strangulated colon

Chapter 4.
PRELIMINARY STUDIES OF THE SKIN AND FUR OF POSSUMS

4.1 Introduction

Possum fur is the basis of a long established industry in New Zealand, yet little attempt has been made to describe fur growth, coat structure, or the relationship between follicle and fibre type. Brief comments on fibre characteristics were made by Toldt (1935), Bolliger and Hardy (1944) and Brunner and Coman (1974). Hardy (1947) described follicle group arrangement in adult possums, and pointed out the need to interpret adult follicle structures in terms of development. Follicle development was described by Gibbs (1938) and Lyne (1970a), as outlined in section 2.1.4. Hardy (1947) also drew attention to the importance of relating follicle types in the skin to fibre types in the pelage, and recommended the use of microdissection to verify her proposition that central primary follicles grow guard hairs and lateral clusters of follicles produce underfur.

The main purpose of these preliminary investigations was to describe the structure and growth of the coat, fibre, and hair follicles of possums as a foundation for further study. In particular, criteria for measurement of follicle activity and relative follicle population size, and a comparison of spontaneous and induced fibre growth were required for the subsequent investigation of seasonal pelage change. A greater understanding of the fibre grown by possums might also be of benefit to the New Zealand fur industry, especially if further steps are taken toward farming of this species.

4.2 A comparison of the fur of some marsupials

Aim

In the absence of published information on comparative coat characteristics of marsupials, a brief examination of the pelage of possum species was made to describe variation in fur length between congeneric species

of possums, and to search for evidence that the growth and type of fur in brushtail possums present in New Zealand differs from other marsupials.

Method

Coat lengths of three *Trichosurus* species were observed from specimen skins at the South Australian Museum (Adelaide, South Australia). The longest crimped guard-hairs from the mid dorso-lateral region (site I, figure 3.1) were measured on the skin using a ruler. Coats of these and other phalangers were compared, and examined for growing fibres and patches of fur such as is common in New Zealand brushtail possums.

Results and discussion

Table 4.1 Fur length in three possum species

Species	Location (Aust. State)	Mean (mm)	Standard deviation	Sample size
T. vulpecula	SA	29.0	4.9	7 (4♂,3♀)
T. caninus	NSW, Vict.	41.6	2.5	7 (3♂,4♀)
T. arnhemensis	NT, WA	19.7	2.5	3 (2♂,1♀)

Fur length measurements summarized in table 4.1 suggest that the fur of the common brushtail (*T. vulpecula*) is longer than in that of the smaller, northern brushtail (*T. arnhemensis*) ($P<0.05$), but shorter than that of the similar sized mountain brushtail (*T. caninus*) ($P<0.01$) , with which *T. vulpecula* is sympatric over part of its range in Australia. In both *T. vulpecula* and *T. caninus*, dark hairs could be found growing through fully grown fur indicating a diffuse moult pattern. In addition, patches of fur loss and regrowth (like those depicted in plate 4) were evident in both species.

The coats of other marsupials of the families Phalangeridae, Petauridae, Macropodidae, and Peramelidae were also examined. Specimens of the ringtail possum, *Pseudocheirus perigrinus*, had shorter, softer fur, but like *Trichosurus*, showed fur growth in patches. No bands of moulting fur, nor marked variation

attributable to seasonal coat change were evident in the skins of any of the marsupials examined.

There was, therefore, no evidence from museum material briefly examined that hair growth and replacement in the common brushtail possum is dissimilar at a gross level to that of other medium sized Australian marsupial species.

4.3 Moult patterns in possum pelts; observations of fur graders and trappers.

Aim

Information from professional fur handlers regarding the timing and topography of moult in possums was compiled as a basis for further studies involving moult patterns.

Method

A chart showing the supposed seasonal changes in pigmentation ("moult") patterns on the flesh side of possum pelts was constructed with directions from an experienced fur grader, Mr D. Campbell of New Zealand Fur Auctions, Wellington. Comments on a draft of the chart were then received from other graders and trappers. Further comment was sought on the final chart, presented in the N.Z.O.F.P.A. magazine, "Fur Facts" (Campbell and Nixon 1985).

Results and discussion

The chart compiled from comments of fur graders and trappers and presented as a provisional description of possum moult sequence, is shown in figure 4.1.

Patterns occur in "moulting" pelts because active follicles contain melanotic melanocytes and pigmented fibre, but no melanin occurs in quiescent follicles (Gunn 1933, Stevenson 1962). Dark colour is therefore directly associated with hair growth, rather than shedding.

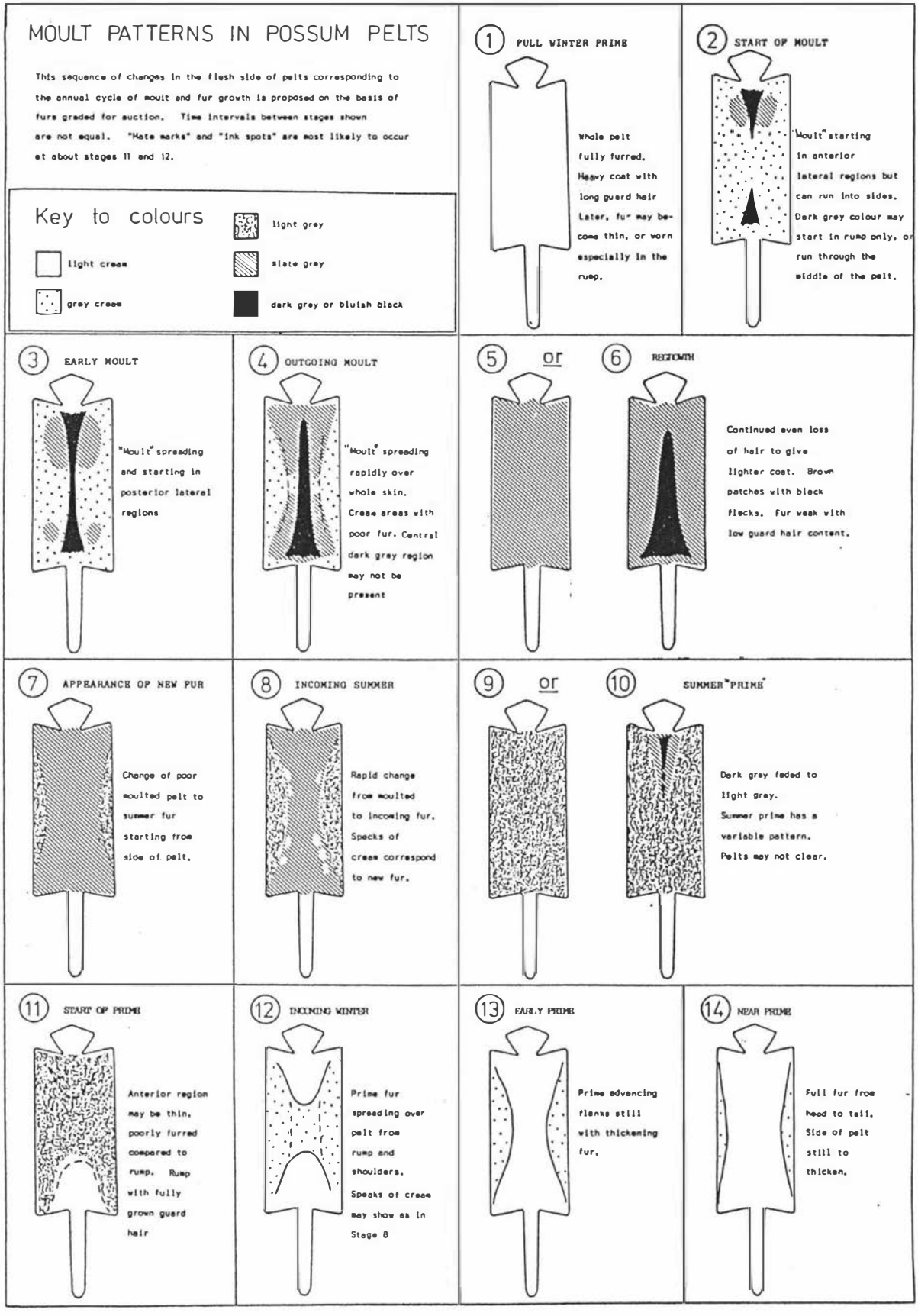


Figure 4.1 Chart of "moult" in possums. This sequence of pigmentation patterns was suggested by fur graders and trappers.

The initial interpretation placed on the sequence of patterns, stated by Campbell and Nixon (1985), was that some waves passing over the animal's body were evident. However, subsequent observation of pelts suggest that the moult of brushtail possums is of the diffuse type. Borders between areas of differing pigment intensity are not as distinct as shown in figure 4.1, and pelts represented by patterns 11 to 14 in particular, are very lightly pigmented and can not be construed to be part of a wave pattern. The typical appearance of a "moulting" pelt is shown in plate 1. This diffuse seasonal growth pattern should be distinguished from the patches of fur loss and regrowth known in the opossum fur trade as "ink spots" (plate 2).

The chart (figure 4.1) reflects the view, widespread amongst participants in the fur industry, that flesh side pigmentation of the pelt, fur quality, and time of year are closely associated. Dark "moult" patterns are reported to occur in late spring, while "prime" pelts (from which pigment is absent) reputedly occur in winter. These moult patterns are used by trappers as a guide to trapping seasons (Comer 1984), and by graders as a major indicator of pelt quality.

4.4 Epilation of possum skin

Aim

It is well known amongst trappers that possum fur is easily lost from the skin of freshly killed animals, and that fur must be left to "set" prior to skinning. There is also anecdotal evidence that fur is less readily lost from calm, unstressed animals (V. Comer pers. comm.). An attempt was therefore made to quantify the force required to pluck fibres under various conditions, and describe the microanatomy of the separation of fibre and skin.

Method

The force required to pluck possum fibres was measured using a modified spring balance. Small patches of fur from the mid side of a grey male possum were glued between layers of card. A clamp was fitted to the balance so that the glued fibres could be held and extracted together by pulling the balance upward. A rider on the barrel of the balance indicated the maximum force required to extract the fibres. The plucked fibres were then mounted on a microscope slide

and counted, so that force per fibre could be calculated. Fibres were plucked by this means from a single grey male possum while it was; (1) restrained in a sack, (2) anaesthetized with 1.2 ml/kg i.m. Saffan, (3) immediately after death by bleeding (a killing method used by possum trappers), (4) five minutes after death, and (5) 24 hours after death with carcass stored at 5°C. The tensile strength of three samples of possum fur was also measured using the modified spring balance, and the ends of fibres glued to card.

The effects of other anaesthetic agents: ketamine (50mg/kg), pentabarbitol and inhaled chloroform, on plucking force were also examined. Possums captured from Keebles Bush Reserve were anaesthetized, and the force required to extract fibres by hand from approximately 1 cm^2 of skin was subjectively assessed. At the conclusion of these experiments, all possums from which fibre was plucked were euthanased while under anaesthesia.

Plucked fibres and sections of epilated skin were stained with Mayer's haemalum, eosin and picric acid, and compared with sections of intact skin.

Results and discussion

The ease with which possum fur can be plucked varies according to physiological state (table 4.2). Fur is readily plucked in animals exposed to the stress of normal handling, but is retained firmly in possums anaesthetized with saffan, ketamine, or pentabarbitol, but not inhaled chloroform. The force required to pluck fibres decreased markedly immediately on death by either bleeding or pentabarbitol overdose.

The tensile strengths of three samples of possum fur were 1.8, 1.9 and 2.5 g/fibre (mean 2.1 g/fibre).

Table 4.2 Force required to extract possum fibres

	State of possum	Plucking force (g/fibre)
(1)	Restrained and conscious	0.65
(2)	Saffan anaesthesia	0.88
(3)	Immediately after death	0.17
(4)	Five minutes after death	0.05
(5)	Twenty four hours after death	0.35

Plucked fibres part from the skin in possums by separation of cells of the external root sheath. In telogen follicles, a covering of epithelial cells remains with the club end of the fibre (plate 15). Some external root sheath, skin glands, the hair germ and dermal papilla remain in the skin (plate 16).

4.5 Fur length variation between body regions.

Aim

Obvious variation in fur characteristics exists between body regions of possum skin. Measurements were made in order to quantify this regional variation.

Methods

Skins were collected from 93 possums, 49 females and 44 males, captured at Keebles Bush Reserve over two years as described in section 3.1. Fur was sampled at four sites, as shown in figure 3.1, and the length of longest crimped guard hairs was measured, as described in section 3.5.

Results and discussion

Coat lengths (length of longest guard hairs) at each of four sites in 94 possums are listed in table 4.3. The coats of females were longer than those of males sampled from the Keebles Bush population ($P<0.01$). There were no significant differences in coat length between grey and brown colour phenotypes ($P=0.60$).

Table 4.3 Fur length at four body sites.
(mean (mm) ± standard deviation)

	Body Site (figure 3.1)			
	I	II	III	IV
Females	37.0 ±2.3	35.6 ±2.0	40.6 ±5.1	35.4 ±2.8
Males	33.1 ±2.1	31.8 ±2.5	37.2 ±5.3	33.1 ±2.8
Both sexes	35.1 ±2.9	33.8 ±2.9	39.0 ±5.4	34.8 ±3.2

A cephalo-caudal gradient in the length of pelage is evident in possum skin. Fur was generally longest over the rump. However the longest fibres from this region were very often broken, and in some cases worn to the extent that they are shorter than fur of anterior regions. This phenomenon contributed to the higher standard deviation for site III.

4.6 Fibre types and coat structure

Aim

Possum fur is composed of fibres of varying size and form. The purpose of this preliminary study was to describe and classify these fibres, relating them to follicle types, and to indicate their relative abundance in the coat. This information was required for development of a simple fur measurement procedure.

Methods

Two follicle groups containing monotrich type fibres and six groups in which crimped guard hairs were the longest fibres were dissected from the mid dorso-lateral site of the dried pelt of two grey male possums. The isolated groups were examined under dissection microscope to determine the follicle types from which corresponding fibre types originate. The monotrich groups were then histologically processed as described in section 3.3. The six guard hair groups were mounted with "superglue" on a 7 x 8 cm perspex sheet. A

fibre type and length distribution was then obtained by stretching fibres individually with forceps and measuring length at the fibre tip on a graduated scale attached to the perspex. Each fibre was removed with a blade after measurement.

Results and discussion

Classification of fibre types

Fibres composing the body pelage of possums are of intergrading form and length, but can be classified into three main types. General characteristics of each type are given in table 4.4. Intermediates between monotrichs and guard hairs are long, wavy, densely pigmented fibres with no subapical band. Intermediates between guard hairs and underfur have smaller awns and are shorter than guard hairs.

Table 4.4 Characteristics of possum fibre types

Fibre type	Follicle type	Approx. Length (mm)	Maximum Diameter (um)	Crimp	Pigmentation
monotrich	central primary	40-50	70	straight	heavily pigmented
guard hair	central and some lateral primaries	30-40	50	crimped	non-pigmented subapical bands (greys)
underfur	derived	10-20	20	crimped	light pigment and banding

In all types, the proximal ends of fully grown fibres are non-medullated and unpigmented 1 to 3 mm from the club end. Diameters in this short region are 10 to 14 μm. Over most of their length, underfur and guard hairs are of uniform diameter, 12 to 18 μm, with a simple serial ladder medulla. In this middle region, possum fibres are approximately round in section, with prominent diamond petal type cuticle scales as shown by Brunner and Coman (1974). Melanin pigmentation occurs mainly in the distal half of the fibre, being most intense toward the tip. Guard hairs and monotrichs have a broad region of

maximum diameter (plate 12), which is lens or "D" shaped in section. In the guard hair of grey possums pigmentation in this region is interrupted to give a subapical band. All fibres taper toward a non-medullated tip of approximately 2 μm diameter.

Dissection and microscopic examination of fibres originating from isolated follicle groups verified that the largest fibres, i.e. monotrichs and longest guard hairs, are produced by central primary follicles. Histological sections of dissected monotrich follicles corroborated the observation of Lyne (1970a) that groups containing these follicles comprised larger numbers of associated follicles and follicle bundles than surrounding groups. The two groups had six and eight bundles. Smaller fibres (underfur, some guard hairs, and intermediates) grow from follicles of lateral bundles.

Coat structure

The fibre type composition of the coat determined from six follicle groups at the mid dorso-lateral site is displayed in figure 4.2. Fibres were arbitrarily classed into four types according to size of the broad distal region. The histogram shows that underfur fibres are the most numerous with a modal length of 14 mm, and also illustrates the intergrading of fibre types. In the sample of six groups, there were 18 guard hairs, i.e. 3.0 guard hairs per group.

The coat of possums thus has a composition similar to that found in many eutherian fur bearers. For example, Robinson (1958) described a comparable array of fibres in rabbits.

4.7 Fibre diameter

Aims

Fibre diameters were measured to characterize possum fur, compare methods of measurement, and determine the usefulness of fibre diameter as an indicator of seasonal fibre growth.

Method

Clipped fur samples were cut at 6 and 8 mm from their proximal end with parallel mounted razor blades, to give 2 mm lengths of fibre. These subsamples

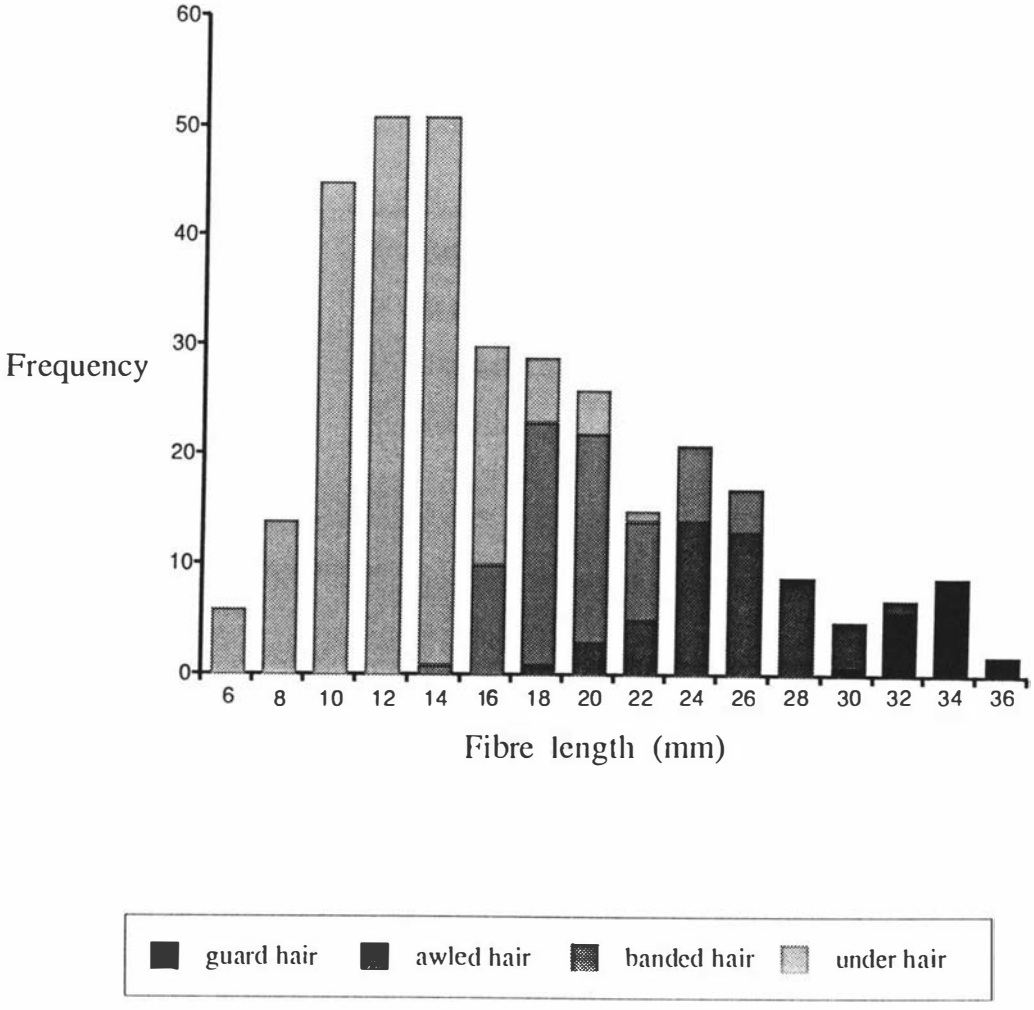


Figure 4.2 Fibre length distribution. Fibres are arbitrarily classed by shape and size; (1) *under hair,* short pigmented curled tip less than 2 mm long with no band, (2) *banded hair,* tip 2-3 mm with small unpigmented band but no thickening toward the tip, (3) *awl hair,* 3-6 mm of tip thickened and banded, (4) *guard hair,* large thickened tip 7-10 mm. (Note: these dimensions vary between individual possums, and between body sites.)

were taken so as to include regions of uniform diameter and simple ladder medullation of all fibre types, but exclude tips and nonmedullated basal portions of fully grown fibres. When new fibres are growing through the coat, greater spread in the distribution could be expected, due to sampling of fibre tips (see figure 4.3).

Cut samples were washed in petroleum ether. Fibre diameters were then measured using a projection microscope, or a fibre fineness distribution analyzer (F.F.D.A.).

Fur samples from the body sites I to IV shown in figure 3.1 were measured by projection microscope. Fourteen samples were measured by both F.F.D.A. and projection microscope as a comparison of methods. Fibre diameters of 33 fur samples from a captive male possum were measured by F.F.D.A. Corresponding skin samples were histologically processed (as in section 3.3) and hair follicle activity determined (as in section 3.4). Correlations between fineness parameters and follicle activity were determined.

Results and discussion

Examples of fibre diameter measurements (samples taken during three winter months of two years from a single possum) obtained using both F.F.D.A. and by projection microscope are shown in table 4.5. Mean diameter measured by either method did not significantly differ between years. Frequent adjustments to the calibration of the F.F.D.A. were necessary during operation. Results obtained by this method must therefore be treated with caution.

Table 4.5 Diameter of possum fur measured by two methods (μm)

Sample date	Projection microscope Mean	s.d.	n	F.F.D.A. Mean	s.d.	n
27 May 1985	15.87	2.21	217	14.20	3.22	1004
17 June 1985	16.53	2.95	247	16.55	2.39	1014
8 July 1985	16.22	2.54	209	17.11	3.43	548
9 June 1986	16.02	2.51	241	16.51	2.09	1034
30 June 1986	16.37	2.93	235	17.45	2.85	1210
21 July 1986	16.43	3.28	234	17.11	2.56	964

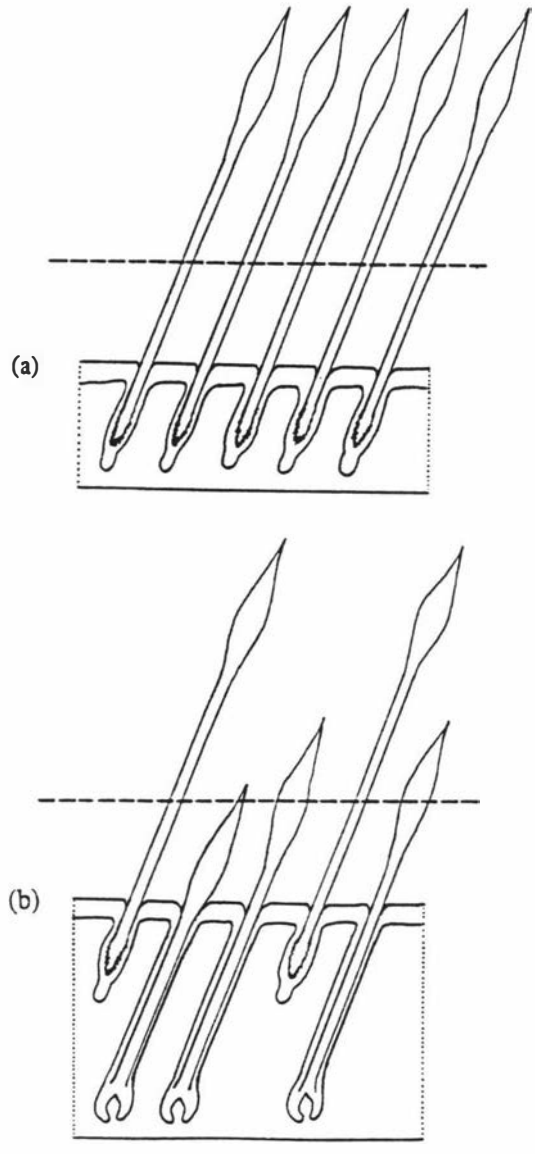
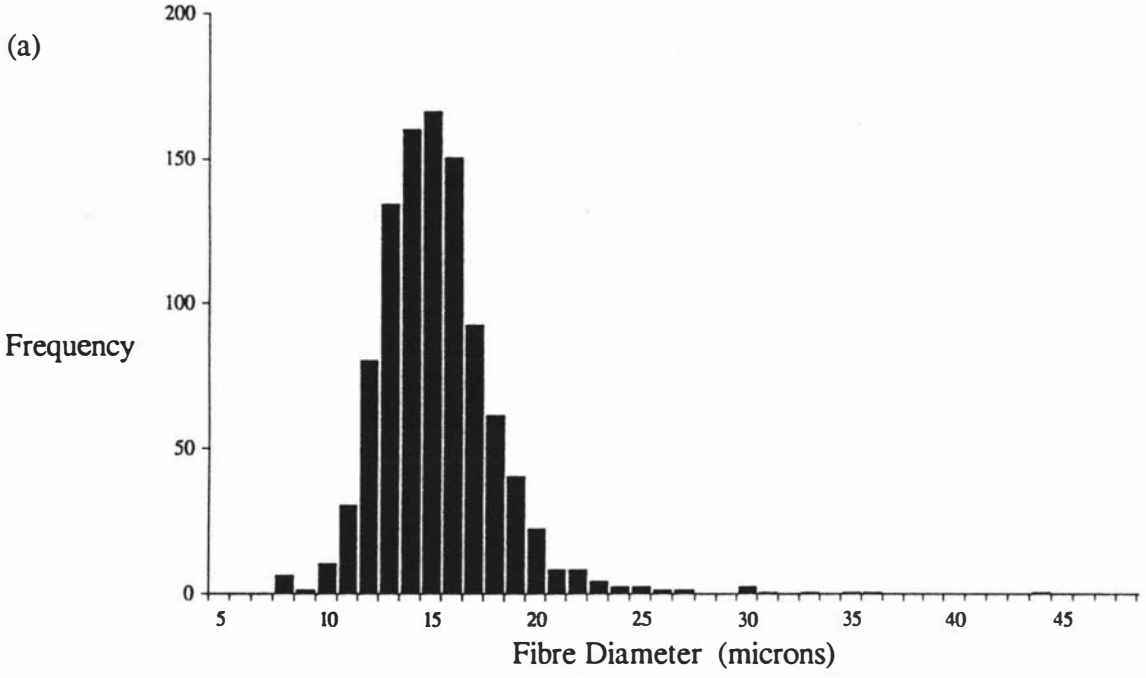


Figure 4.3 Basis of the relationship between fibre growth and diameter. Diagrams represent hair follicles in skin and projecting fibres. (a) A fur sample composed of fully grown fibres has low variation in diameter when measured at the level indicated by the dashed line. (b) A fur sample containing some growing fibres has higher variation when measured by the same method.



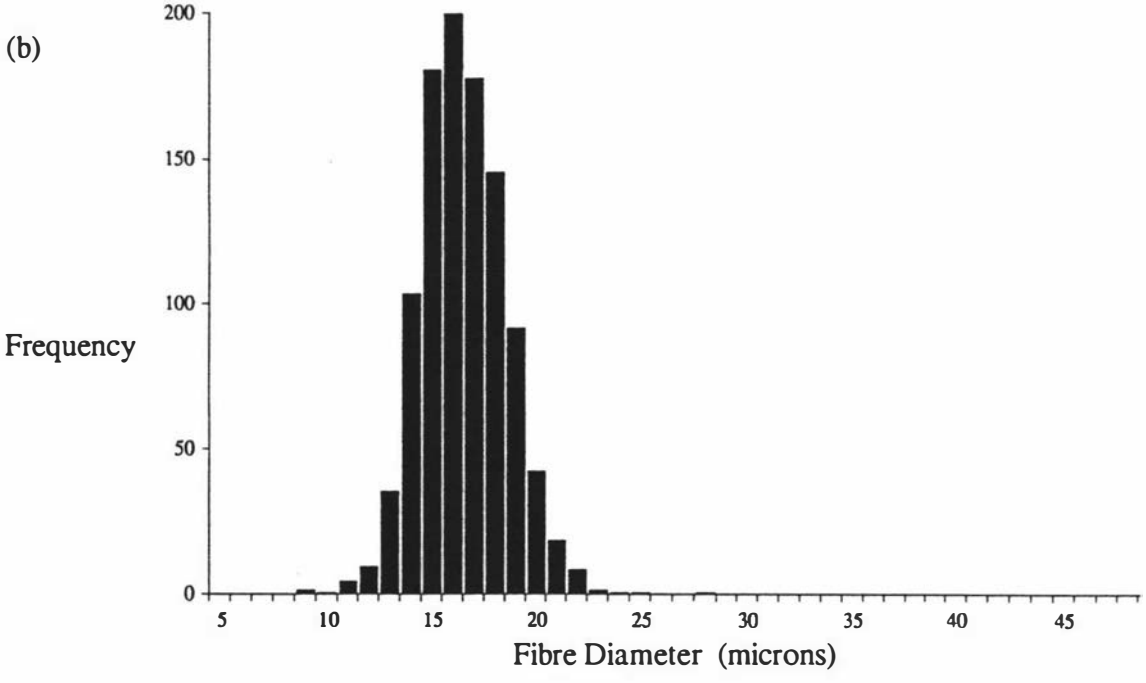


Figure 4.4 Examples of fibre diameter distributions. (a) Summer; high follicle activity (15.9 percent anagen). (b) Winter; low follicle activity (zero percent anagen).

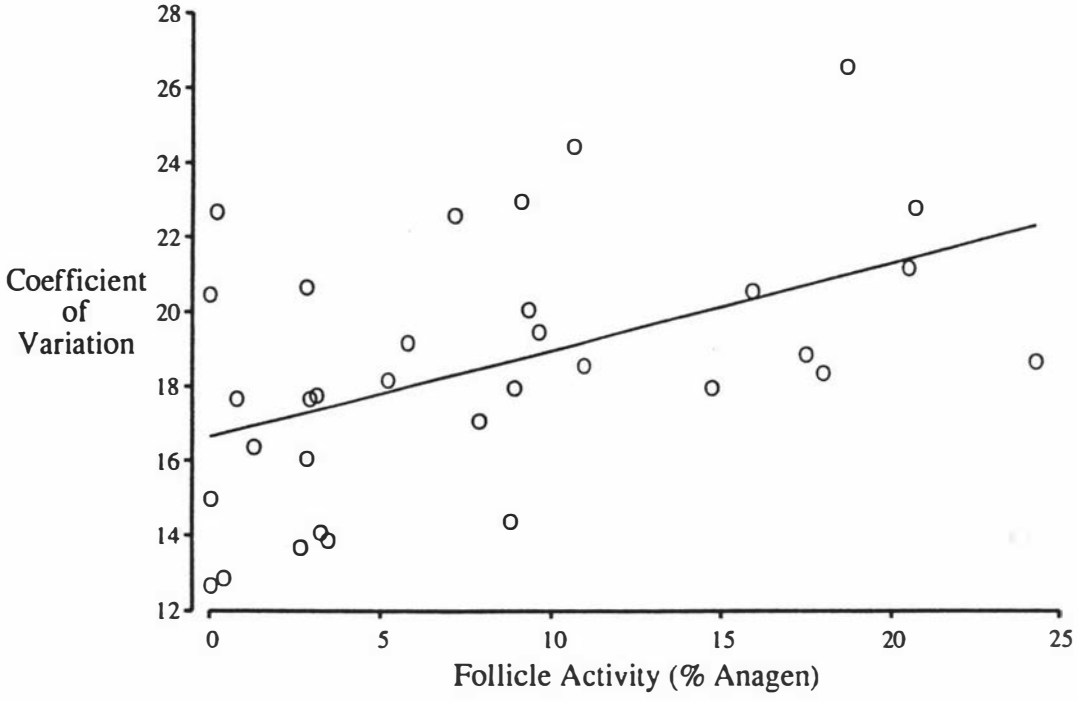


Figure 4.5 Scatterplot of follicle activity with coefficient of variation for fibre diameter. (R=0.45).

Figure 4.4 shows examples of fibre diameter distributions obtained by F.F.D.A. from samples taken during periods of high and low follicle activity. In the December sample follicle activity was 15.9 percent anagen, and in June follicle activity was 0.0 percent anagen. These distributions had similar modes, since even at the higher growth level, most fibres sampled were fully grown.

The relationship of fibre diameter with seasonal growth was further analysed by correlating diameter parameters with follicle activity. For mean diameter and follicle activity, the correlation coefficient was 0.322 ($P<0.05$, N.S.). For coefficient of variation and follicle activity the correlation coefficient was 0.454 ($P>0.05$). There is thus a slight but significant change in the fibre diameter distribution as a result of some coarser and finer portions of the distal ends of fibres being present in the middle layer of the coat during periods of growth. A scatterplot of this relationship is shown in figure 4.5.

4.8 Fibre growth rate

Aim

A brief study was conducted to compare the rate of spontaneous fur growth with that induced by epilation. This comparison was made to test the assumption that duration of induced growth could be used to estimate duration of spontaneous growth (sections 5.3.6.1 and 5.3.7).

Methods

Clipping

A small patch of fur was clipped from a single grey male possum while under saffan anaesthesia. An area 2 cm by 8 cm at the right mid dorso-lateral position (the site outlined by a broken line in figure 3.1), was removed as close to the skin as possible with scissors. Skin and fur samples were taken (as in section 3.2) at the time of first clipping, and then at approximately six day intervals for 121 days. The lengths of monotrichs, guard hairs and underfur were measured from fur samples (as in section 3.5). Skin biopsies were histologically processed (as in section 3.3) and follicle activity determined (as in section 3.4).

Autoradiography

Two grey female possums maintained under 12L:12D photoperiod and 12°C temperature were plucked at the left mid dorso-lateral site (figure 3.1). The animals were then given weekly or two-weekly injections of 10 μCi of ^{35}S cystine via veins in the ear. After 84 days, the animals were killed and fibres sampled from plucked and non-plucked (spontaneously growing) mid dorso-lateral areas of the skin. The fibres were then studied by the method of Downes and Lyne (1961), and Downes *et al* (1967). Growth rates of guard hairs were calculated by measuring distances between labeled points on autoradiographs.

Results and discussion

The growth of three fibre types following clipping is illustrated in figure 4.6. The time taken to reach terminal length was 62 days. This is not directly comparable with the value of 99.4 days obtained from plucked possums (section 5.3.6.2), as some hair follicles (19 percent) were already active at the time of clipping and these probably grew the fibres measured.

Unlike the response in epilated skin, follicle activity in clipped skin remained at a constant low level. From the time of clipping to 121 days after clipping follicle activity amongst bundle (compound) follicles ranged from 12 to 22 percent. This suggests that follicles of this type were not induced into a new cycle by clipping, and that many clipped ends of fibres remained long after full length fur was regrown. However, amongst central primary follicles alone, follicle activity increased to a peak of 100 percent (n=6) at 32 days. These follicles, growing guard hair, therefore could have been induced into a new hair cycle, possibly by the mechanical forces of clipping (Ghadially 1958).

Measurements from autoradiograms showed that growth rates of guard hairs varied along the length of the fibre. Growth rates between points on the middle sections of fibres grown at 5 to 7, 7 to 8, and 8 to 10 weeks after plucking differed significantly ($P<0.001$). Increase in length was most rapid over the part of the fibre with a simple ladder medulla below the broad shield region. This variation in rate was similar to that described in rats (Priestley 1966, Hale and Ebling 1975). However it contrasts with the finding of Lyne and Downes (1972), who, using the same technique on a juvenile possum stated that "most of the hairs of the midback grew at about the same rate during any given period, but there was accelerated growth during early stages of the cycle".

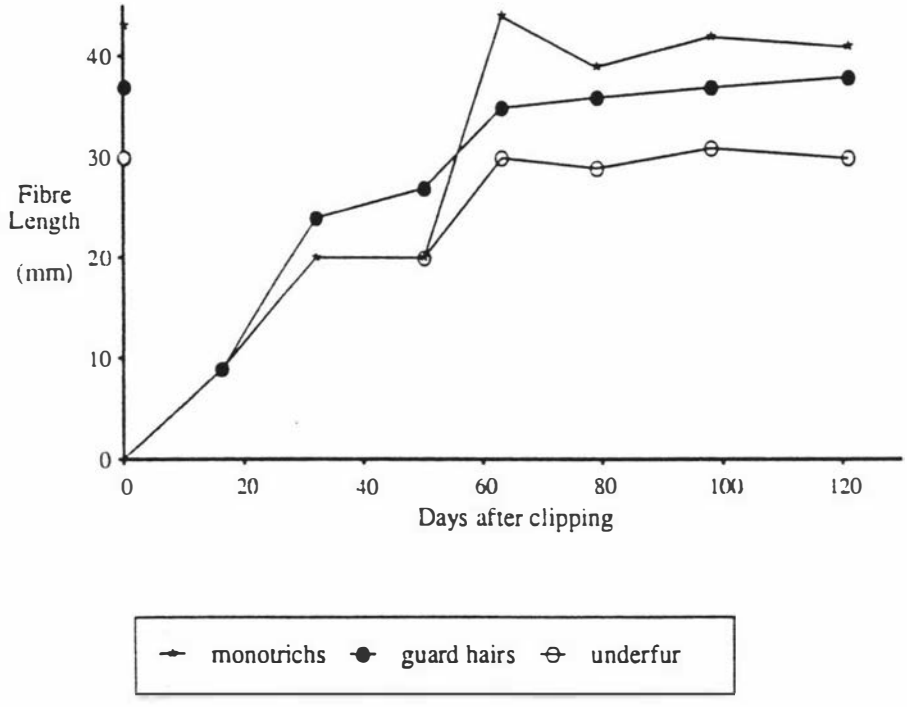


Figure 4.6 Fibre growth after clipping.

Such variation in length growth rate requires that measurements be taken from equivalent parts of the fibre in the experiment to compare induced and spontaneous growth. Since spontaneously active follicles grew fibres in an unsynchronized manner, maximum growth rates for each fibre were compared. Mean values for both types of growth are shown in table 4.6. SED was 0.007, i.e. induced fibre growth is more rapid than spontaneous fibre growth ($P<0.001$).

Table 4.6 Mean maximum fibre growth rates determined by autoradiography (mm/day)

Growth type	Mean	s.d.	n
Pluck induced	0.634	0.035	53
Spontaneous	0.584	0.031	30

All guard hairs grown in response to epilation received their first radioactive label at the third or fourth injection, at 27 and 42 days respectively. That is, none of these fibres began growing before the second injection at 14 days, and some did not begin until after 27 days. Silver *et al* (1969) noted histological features in early post-plucking follicles attributable to repair of damage, and Johnson and Ebling (1964) suggested that plucking in late anagen interferes with inductive events so as to delay eruption of the fibre. A similar initial regenerative period would seem likely in possums, especially given that many epithelial cells are removed with the plucked club.

Some differences between spontaneous and induced growth are therefore apparent. It is not possible to determine from these results whether the duration from initial stimulus to club formation in pluck induced hair growth is longer or shorter than in spontaneous hair growth.

4.9 Follicle types and follicle group structure

Aims

The aims of this preliminary study were to describe aspects of the histology of possum hair follicles and follicle groups, and to identify characteristics that could be used in a procedure for measuring follicle activity.

Methods

The structure and staining properties of hair follicles and follicle groups were observed in sections of skin samples collected as described in section 3.2, and processed as described in section 3.3. A count was made of the number of bundles in 345 follicle groups from 20 possums (10 of each sex) which ranged in weight from 1.25 to 3.40 kg. Histological sections were stained using Mayer's haemalum and eosin, sacpic (Auber 1952, Ryder and Stephenson 1968), Masson's trichrome, and Verhoeff's elastin stain (Humason 1979).

Results and discussion

Hair follicles of possums are arranged in groups (see plates 9 to 11). Table 4.7 shows the composition of sampled follicle groups. Groups generally comprised three to five bundles and a central primary follicle, as previously reported (Hardy 1947, Lyne 1970a). Twenty one percent of follicle groups had no solitary (unbranched) central primary. Some follicle groups centred around a supposed monotrich follicle, were found to be characterized by central primary follicles with a larger club end or fibre, more circular arrangement of bundles, more bundles per group, and fewer bundles in surrounding groups.

Table 4.7 Follicle group composition (345 groups)

Number of bundles per group	Unbranched central follicle absent. (% frequency)	Unbranched central follicle present. (% frequency)
1	0.3	0.0
2	2.6	1.7
3	8.7	18.6
4	8.1	47.0
5	1.2	9.3
6+	0.0	2.6

The follicle bundles, or compound follicles, are compact clusters of follicles with conjunct external root sheaths and a common hair canal. Therefore, at the level of sebaceous gland, the boundaries of individual follicles can not be easily defined (see plates 9 to 11). Some follicles with an empty follicle lumen were found, and were assumed to be naturally shed. However these were relatively rare, identified in 29 of 1225 follicle groups (2.4 percent) from 102 possums.

Numerous mast cells were found within the dermis associated with hair follicles (plate 13). A network of elastic fibres was shown in the dermal connective tissue using Verhoeff's elastin stain (plate 14). These were present throughout the dermis, but were most concentrated in the middle reticular layer, surrounding hair follicle groups. Elastic fibres were mostly aligned in the same plane as the skin surface, perpendicular to hair follicles.

Active (anagen) follicles are approximately two to three times longer than quiescent (telogen) follicles (see plate 11). Distally, they possess melanotic melanocytes, germinal epithelial cells and bulbous external root sheath surrounding the dermal papilla. An internal root sheath, staining with eosin or safranin is usually visible in transverse sections made between sebaceous gland and keratogenous zone. The most distinctive feature of active follicles in transverse sections is the growing fibre. The range of fibre structures corresponds to that described in section 4.6. Resting follicles generally consist of an external root sheath two to three cells thick surrounding a club end. The club end is spear-shaped in longitudinal section and round in cross section, with a comparatively smooth (i.e. not brush-like) surface. Below the club end the external root sheath terminates in a hair germ, seen as a cluster of cells of low cytoplasmic volume.

4.10 Estimates of follicle number

Aim

The purpose of this preliminary study was to select a measure of relative follicle number which is independent of changes in skin area. Since follicles develop in groups, and the absolute number of groups can be assumed to remain constant (Hardy and Lyne 1956a, Lyne 1966), the number of follicles per group (group size) could be used as a parameter of relative follicle number. Alternatively, the number of follicles per lateral bundle (bundle size) could be used.

Method

Skin samples collected from 14 possums as described in section 3.2 were processed as described in section 3.3. Transverse sections of hair follicles at the level of the sebaceous glands were used to obtain counts of group size and bundle size. Coefficients of variation for group size and bundle size were then compared.

Results and discussion

Group size (CV 27.7 percent) was more variable than bundle size (CV 17.7 percent) ($P<0.001$). Bundle size may therefore provide the more reliable measure of changes in follicle numbers. Furthermore, changes in bundle size directly represent compound follicle development (Hardy and Lyne 1956a, Lyne 1957a, 1966), and the processes that underlie seasonal change in pelage density in some other species, i.e. formation and regression of derived follicles within the bundle (Rougeot *et al* 1984b).

Bundle size does not take account of unbranched central primary follicles. The formation of new compound follicles by branching from central primaries could increase the follicle population yet result in reduced mean bundle size measured by this method. However, unbranched central primary follicles remain common in animals with large mean bundle size. Also, unbranched primary follicles represent only a small proportion of total follicle population. From skin samples of 15 possums, 137 of 4704 follicles (3 percent) were not accompanied by derived follicles.

4.11 Development of pelage and follicle groups

Aim

The sequence of follicle initiation was described by Lyne (1970a), however the relationship between follicle development and acquisition of adult pelage remains unclear. A brief study of events which follow early follicle development was made as an aid to understanding the skin and fur of mature possums.

Methods

Mid dorso-lateral skin samples were taken from 35 pouched young of female possums captured at Keebles Bush Reserve, and processed as described in section 3.3. Estimates of age were made from measurements of head length (Lyne and Verhagen 1957).

Skin biopsies and fur samples were taken at three weekly intervals from the mid dorso-lateral region (as in section 3.2) of two female possums, which were raised in captivity from pouched young. The animals were aged by head, tail and body length measurements (Lyne and Verhagen 1957) when first handled as pouched young. Samples were taken once the animals were of a size which allowed repeated skin biopsies (body weights; 1.05 kg, 0.40 kg). Guard hair and underfur lengths were measured from fur samples (as described in section 3.5). Follicle activity and bundle size were determined from histological sections of skin (as in sections 3.3 and 3.4).

Results and discussion

The early sequence of follicle initiation and group development in possums from the Manawatu conformed to that described by Lyne (1970a). Compare figure 2.1 and plates 5 to 10.

Lengths of longest guard hairs and underfur for one animal (F42) are shown in figure 4.7(a), and compared with hair follicle activity in figure 4.7(b). In the other animal (F28), fibres had reached full length (guard hair 33.4 mm) by the time of the first sample 31 weeks after birth. Possum F42 attained mature coat length between 27 and 33 weeks after birth, during which time both central primary and total follicle activity declined markedly. This demonstrates

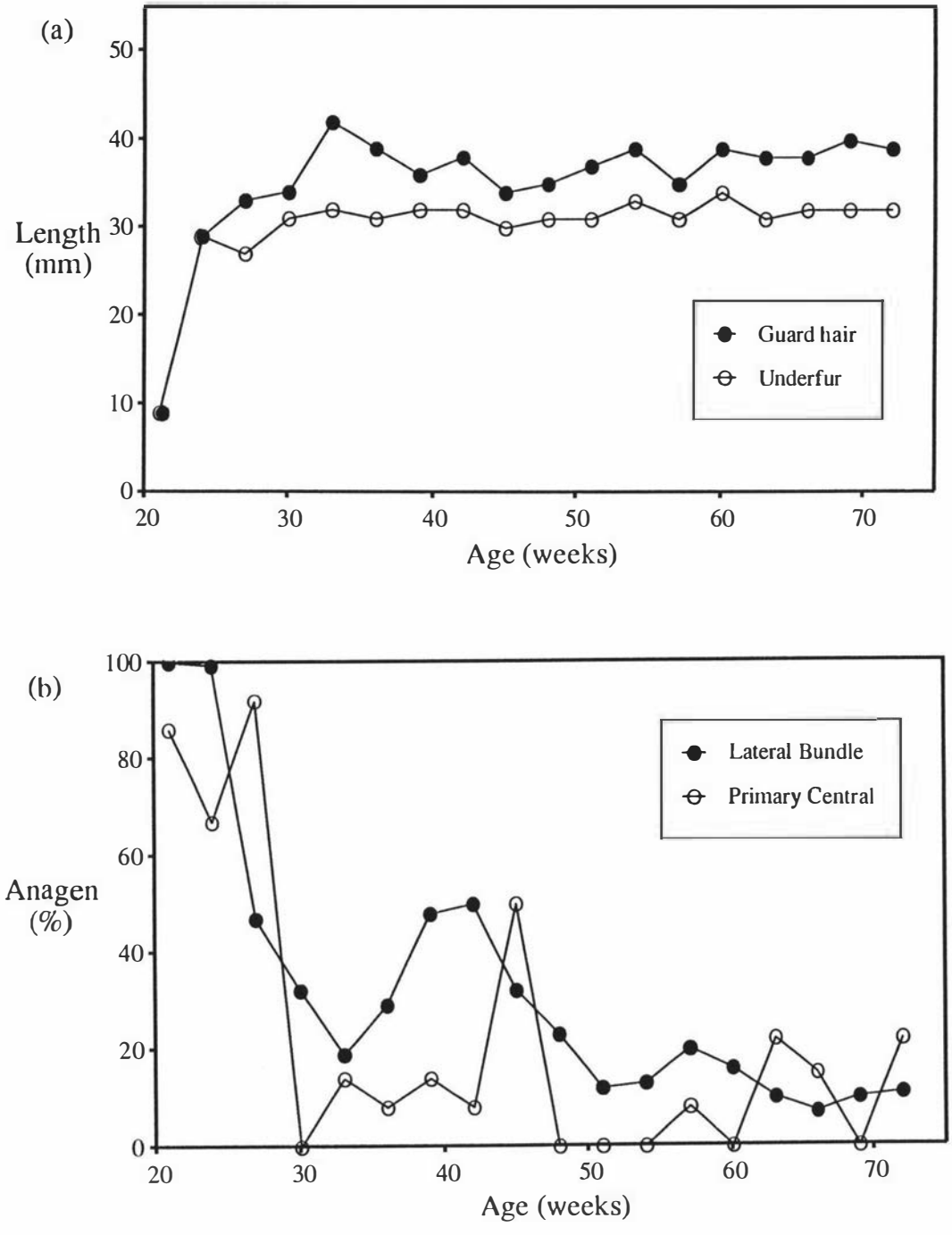


Figure 4.7 Fibre growth during development of possum pelage. (a) Fibre length. (b) follicle activity.

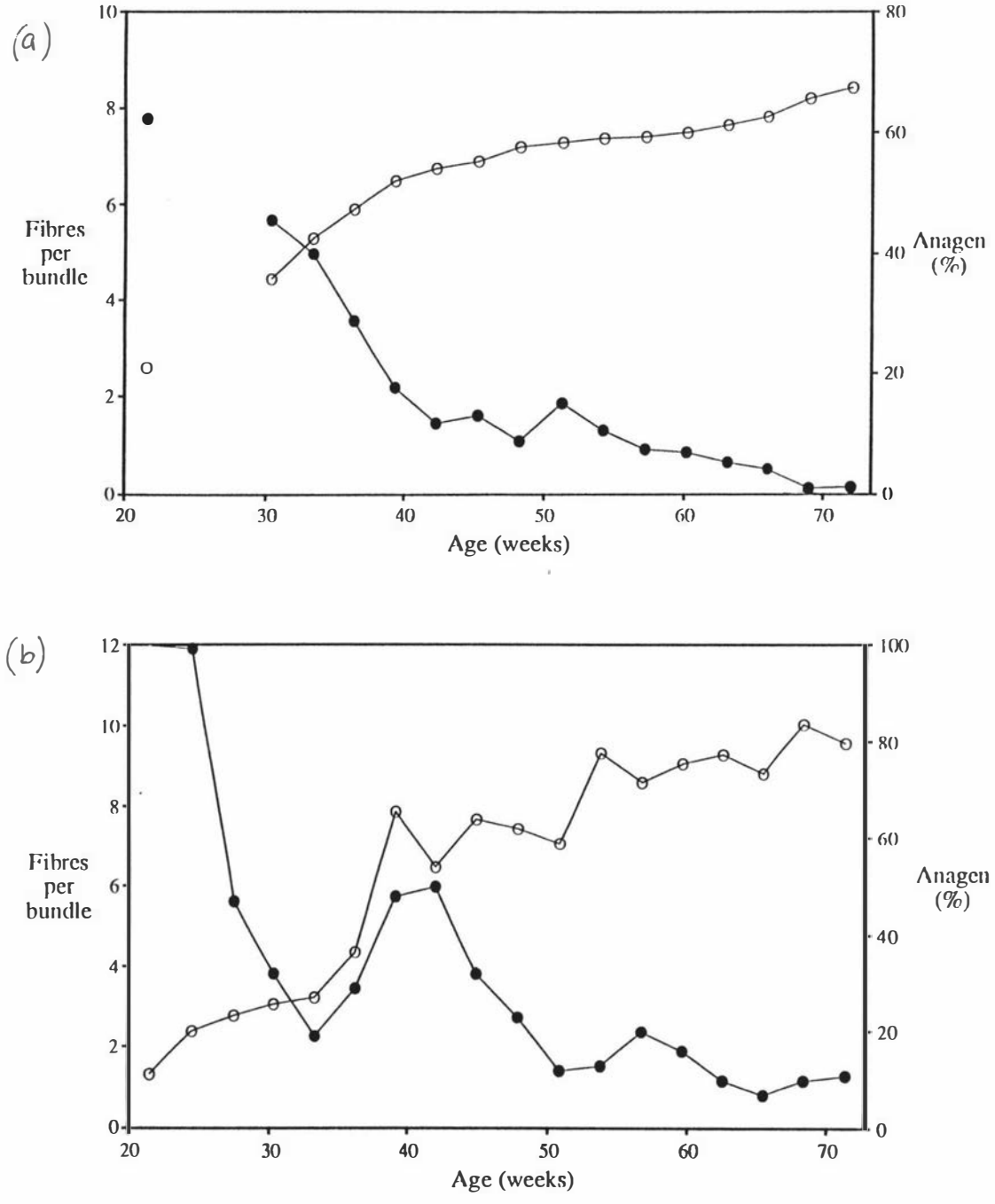


Figure 4.8 Bundle formation and follicle activity. -o- bundle size, -●- follicle activity. (a) Possum number F28. (b) Possum number F42.

the synchronized end of growth of the first full sized hairs. Therefore, subsequent activity in primary follicles represents further cycles with fibre replacement.

On the other hand, activity measured across all follicle types included development of new follicles. This continued initiation of new follicles, manifested in follicle activity and increase in group size, is more clearly illustrated in figure 4.8, showing bundle (compound follicle) size and bundle follicle activity. At about 20 to 22 weeks bundle size was about 2, i.e. compound follicles generally comprised only one original and one derived follicle. The steepest increase in bundle size, accompanied by a peak in activity, occurred between 23 and 40 weeks, and marks the rapid formation of the adult group pattern as it has been previously described (Hardy 1947, Lyne 1970a). By 45 weeks in both animals, activity had declined, as did rate of increase in bundle size. However, bundle size continued a gradual increase throughout adult life in both cases.

Some differences between the two possums may be noted. F42 (figure 4.8(*b*)) appeared to exhibit waves of activity and corresponding change in bundle size, whereas F28 (figure 4.8(*a*)) underwent smoother changes in both development and follicle activity. When sampling was begun at 21 and 22 weeks of age, bundle development was more advanced in F28, but F42 gained derived follicles at a greater rate and attained higher bundle size.

Pelage of mature appearance was thus acquired at about 30 weeks, after initiation of the first derived follicles, but before the compound follicles were well developed. Follicle branching was most frequent between about 30 and 45 weeks, and continued into adult life. This suggests that the formation of new follicles, which in most species is considered to cease early in life (Billingham 1958) might be important in seasonal cycles of adult possums, as proposed by Lyne (1957a) for bandicoots.

4.12 Conclusions

These preliminary studies provided some guidelines for procedures for the study of seasonal hair growth in possums.

(1) Brushtail possums, and probably other marsupials, have a diffuse seasonal moult pattern. However, under certain conditions fur is also easily lost and regrowth initiated through plucking. When sampling skin to measure seasonal growth and follicle numbers, some care must be taken to avoid previously plucked sites.

(2) A cephalo-caudal gradient of fur length and fibre form was described. The mid dorso-lateral position near the middle of this gradient is therefore considered suitable as a representative site for sampling of skin and fibre.

(3) Guard hair length can be readily measured from clipped staples of possum fur. Underfur length can also be determined by removing guard hairs from the staple, although this procedure is complicated by the presence of intermediate fibre types.

(4) Fibre diameter is more variable in samples from growing pelage. However, this effect is slight since only a small proportion of the fibres grow at one time. Fibre diameter is therefore not a suitable estimator of seasonal fibre growth.

(5) Rates of spontaneous and pluck induced fibre growth are significantly different, but there may be a period of follicle repair after plucking before the appearance of the new fibre. Epilated possum skin contains tissue elements necessary for hair growth.

(6) Individual follicles are difficult to distinguish because of close packing of follicles within the bundle. Shed empty follicles appear to be rare. To simplify the follicle scoring procedure, the number of follicles can be equated to the number of fibres, assuming that the old club fibres are shed close to the time of the appearance of new fibres.

(7) Criteria for scoring of follicle activity from histological sections can be based on follicle and fibre structure. Active (anagen) follicles possess an eosinophilic internal root sheath. Sectioned fibres growing within anagen follicles are pigmented, medullated, and possess cuticle scales. Quiescent (telogen) follicles feature a club end, and the above characteristics are absent. Shorter duration intermediate states (catagen and proanagen) are rarely seen, and can be assigned to either active or quiescent categories.

(8) Quantitative analysis of possum hair follicle populations can be achieved by counting follicles on a group by group basis, recording bundle size as an estimator of relative change in follicle numbers.

(9) An estimate of follicle population size is particularly important in the study of seasonal hair growth in possums because derived follicles continue to develop after acquisition of the adult pelage.

Chapter 5
SEASONAL CHANGES IN HAIR FOLLICLES OF CAPTIVE POSSUMS

5.1 Introduction

Many mammals replace all or most fibres of the adult pelage annually or biannually. In some species, this results in a seasonal change in appearance and properties of the coat (Johnson 1977b, 1984). In examples such as the red fox, this is achieved by different follicle types becoming active and shedding at different times, changing the fibre type composition of the coat (Maurel *et al* 1986). In others such as the ferret and the mink, it is claimed that follicle and fibre density change seasonally (Harvey and MacFarlane 1958, Rougeot et al 1984b). Pelage changes in marsupials are less well understood. Dawson and Brown (1970) reported seasonal variation in coat depth and insulation in macropods, and from examination of hair follicle groups, Lyne (1957a) suggested that follicle branching plays a role in hair replacement in bandicoots. However, no marsupials have been studied using repeated sampling and histological methods which are necessary to accurately describe the behaviour of hair follicles over time, as advocated by Ryder (1965) and Ling (1970). Development of hair follicles and follicle groups has been described in possums (Gibbs 1938, Lyne 1970a), but little is known about their hair replacement.

In this study, seasonal hair growth in captive possums was described by sampling skin and fur at regular intervals to show annual patterns of follicle activity and follicle group structure. A simple model of follicle population dynamics was used to account for fibre shedding, and thereby estimate the proportion of the coat which was replaced annually.

5.2 Methods

5.2.1 Follicle activity and changes in the compound follicle

An initial group of eight possums (group A) was captured and maintained under conditions of natural light and temperature as described in section 3.1. Animal weights and weekly maximum and minimum temperature inside the enclosure were recorded, also as described in section 3.1

Fur samples and skin biopsies were taken under saffan anaesthesia at three weekly intervals over a 26 month period, as described in section 3.2. Skin tissue was processed as described in section 3.3, and transverse sections of hair follicles scored as in section 3.4. Raw data processed with the computer programme listed in appendix I gave: (1) percentage of follicles in anagen as a measure of the seasonal pattern of fibre growth, and (2) average number of follicles per bundle or compound follicle as a measure of change in the follicle population.

5.2.2 Determination of the duration of anagen

The duration of anagen and fibre growth rate following epilation was measured in a second group (group B) of four possums, two of each sex, which were captured and maintained as described in section 3.1. Three trials were conducted in a controlled environment room, with a photoperiod of 12 hours light and 12 hours dark (12L:12D). Animals were exposed to this photoperiod and a constant temperature of 15°C for 39 days before the start of the first trial on 2 September 1985. Temperatures during trials one to three were held at 15±1°C, 25±2°C and 5±2°C respectively.

For each trial, an area of fur 2cm x 8cm was plucked[1] from the mid dorso-lateral region of each possum, as shown in figure 3.1. Alternate sides were used in each trial, and care taken to avoid previous sample sites. Fur clips and skin biopsies were taken as described in section 3.2. from the epilated area at three weekly intervals, until regrowth of the fur was complete. Two biopsies were taken at the start of each trial; one from epilated skin to determine the completeness of hair removal, and one from the adjacent non-epilated skin to determine the starting activity level. Data from successive samples were analysed to show fur growth rate and a curve for recovery of follicle activity. From the latter, a value for median duration of anagen was derived.

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1 Plucking of fur from possum skin is not abnormally stressful for the animal as the fur is easily lost during handling.

5.2.3 Inference of hair shedding and replacement from follicle population dynamics.

A model of the hair follicle/fibre population was developed from the relation:

$$N_{t1} = N_{t0} + b - d$$

where: N_{t0} = initial fibre population size
N_{t1} = fibre population size at time t1
b = new fibres recruited (growth)
d = fibres lost (shedding)

Using a derivation of this model, and parameters obtained as described above (namely: follicle activity, duration of anagen, and changes in the follicle population indicated by composition of the compound follicle) approximate levels of shedding and annual hair replacement were determined. Histological investigation (section 4.3) indicated that the number of fibres closely approximated the number of follicles, and that the number of fibres/follicles per bundle is a suitable estimator of relative fibre /follicle population size.

5.3 Results

5.3.1 Body weight and health of captive possums

There was a general tendency for body weight of captive possums to increase during the observation period (figure 5.1). Some body weight gains were due to normal growth and maturation, as young animals were generally selected for study. At post-mortem examination healthy animals were noted to have extensive sub-cutaneous and visceral fat deposits. Some animals showed weight loss, and two (F20 and M08) became emaciated. Faecal parasitology was negative for coccidia cysts and worm eggs for these animals. *Campylobacter* organisms were cultured from the faeces of M08. Diagnoses at post-mortem examination were: pulmonary oedema (F28), chronic pylonephritis and ulcerative gastritis (F20), and arterial calcinosis (M08). Fates of all animals

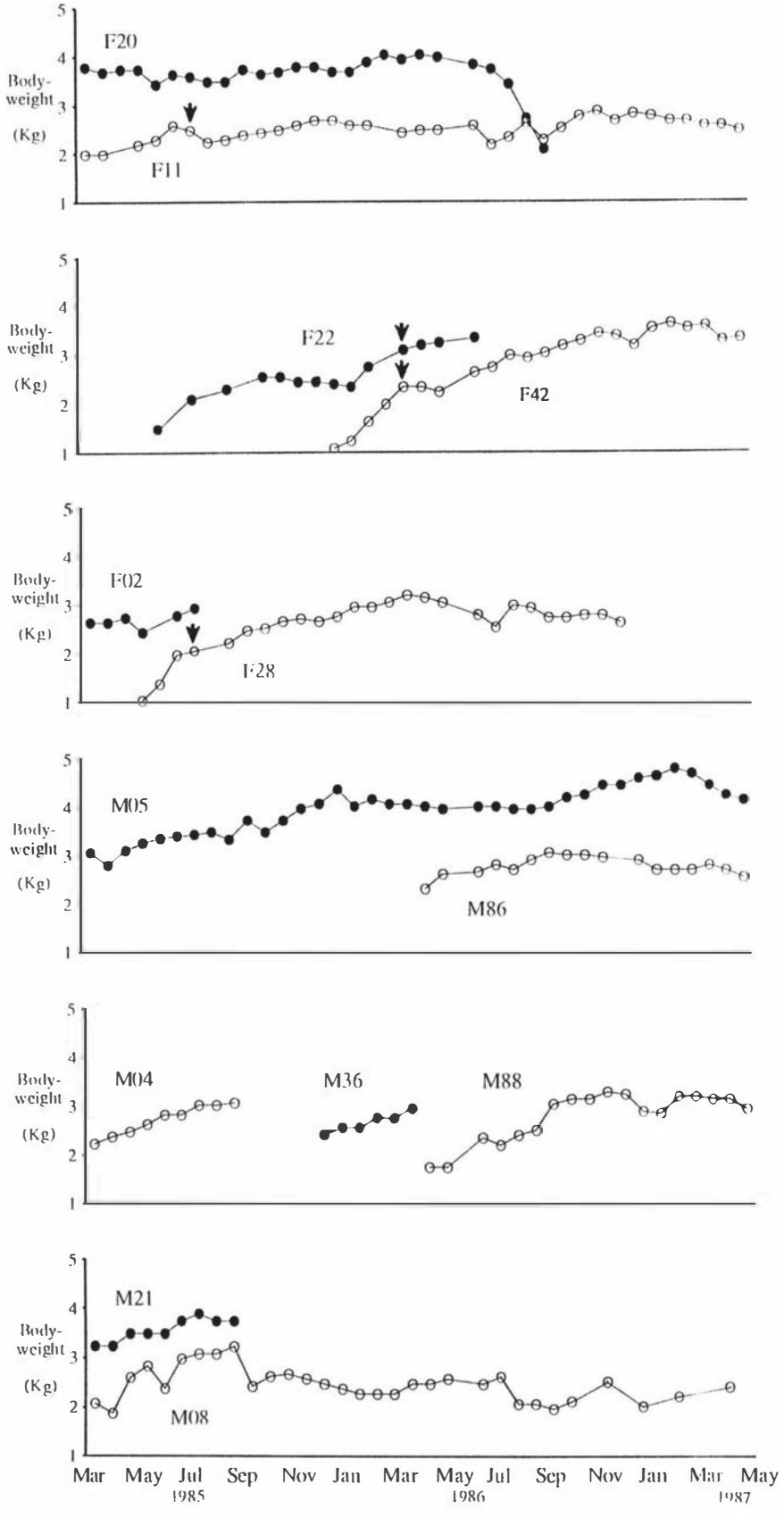

Figure 5.1 Body weights of captive possums. Arrows indicate dates of removal of young, F28 and F42, from mothers, F11 and F22 respectively.

are summarized in table 3.2. Periodic skin sampling was continued from animals in poor condition, but data excluded from analyses. Reduction in body weight invariably coincided with a decrease in hair follicle activity.

5.3.2 Environmental temperature

Weekly maximum and minimum ambient temperatures in the cage enclosure are represented in figure 5.2. Marked seasonal periodicity is apparent.

5.3.3 Hair follicle activity

Figures 5.3 and 5.4 show the general annual patterns of hair follicle activity observed in female and male possums respectively. Activity is expressed as mean percent follicles in anagen, with the upper graph (a) of each figure showing values obtained for bundle (compound) follicles, and the lower graph (b) values for primary central follicles. Since the latter type comprise a very small proportion of the follicle population, activity of bundle follicles (graph (a)) closely resembles total follicle activity. Since small numbers of central primaries were scored per sample large errors in estimates of activity for this follicle type are likely. There is some indication that percent anagen in primary follicles is slightly higher, and peaks occur later, than in bundle follicles.

The results show three noteworthy features of hair growth in possums. First, peak hair follicle activity levels are very low. In no mature individual did follicle activity observed at the dorso-lateral site exceed 40 percent. Second, seasonal trends in follicle activity are evident. In both sexes mean activity maxima occur in December-January, and minima during April-May. Third, there is considerable individual variation in annual patterns of activity, indicated by large error bars. That is, the timing of hair growth is not closely synchronized between individual possums. Individual females exhibited peak activity in November and December, with one case in July. Activity peaks of individual males were observed between July and January. Valid statistical tests using the summary data shown in figures 5.3 and 5.4 are not possible because individual animals are not equally represented in each month.

Individual variation is more clearly demonstrated in graphs (a) to (d) of figures 5.5 and 5.6. These figures depict the annual pattern of hair follicle behaviour in three possums of each sex. One of each is shown for two consecutive years, i.e. graph (b) continues graph (a) in both figures. Not only do

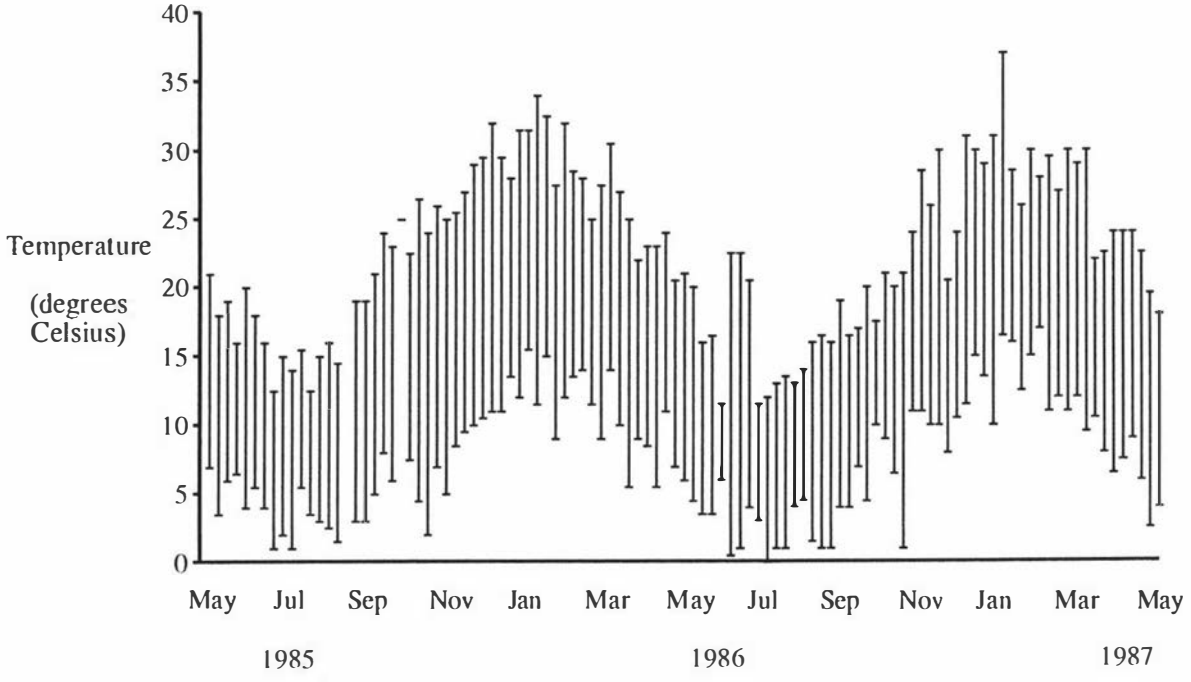

Figure 5.2 Variation in temperature within the possum cage enclosure. Vertical bars connect weekly minima and maxima.

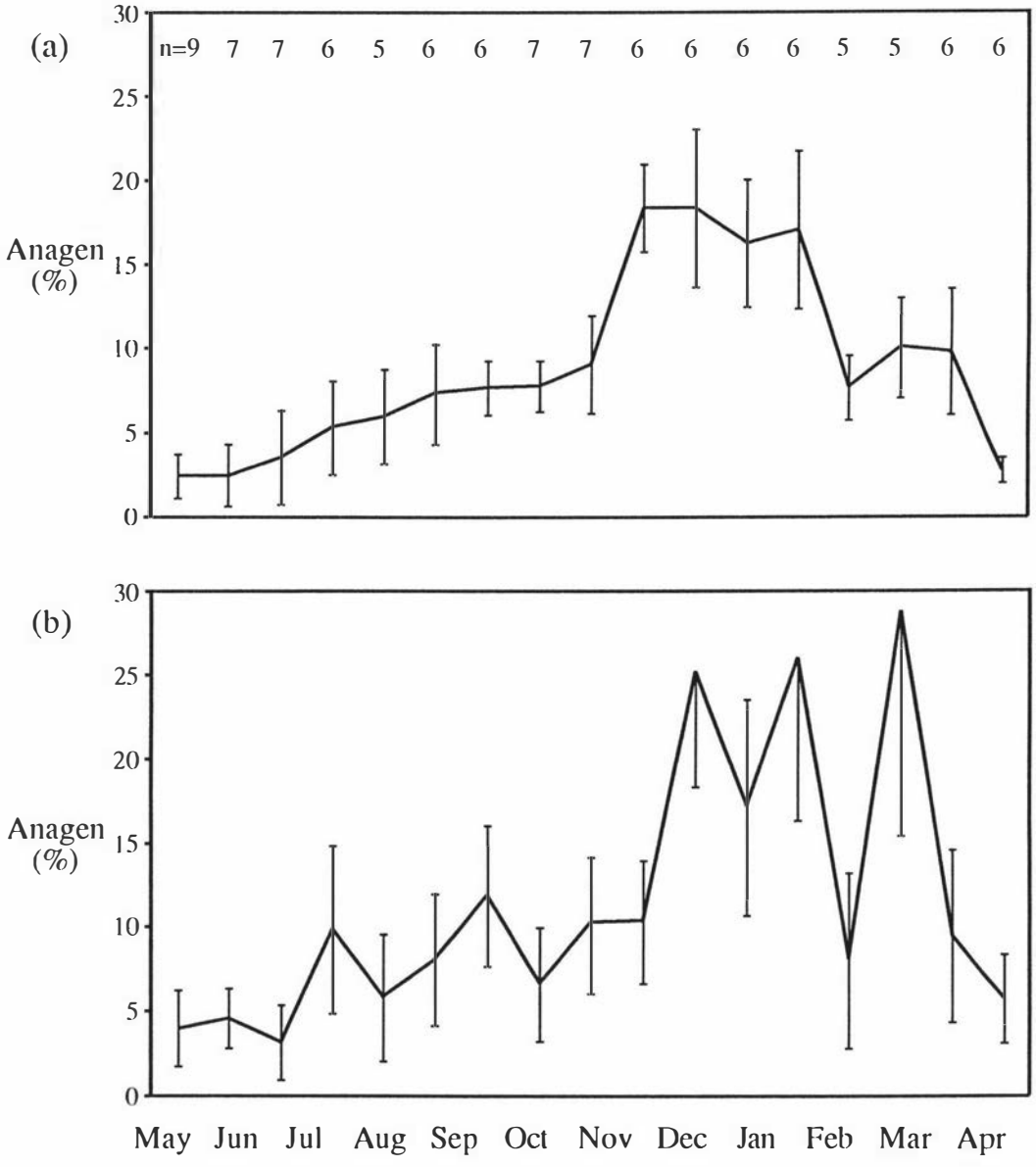

Figure 5.3 Hair follicle activity in captive possums; females. (a) bundle follicles (compound follicles) (b) central primary follicles. Data from six individuals sampled discontinuously over 25 months are combined to show a general annual pattern. Connected points are means of activity for the given number of possums sampled at each time (n). Bars indicate the S.E.M.

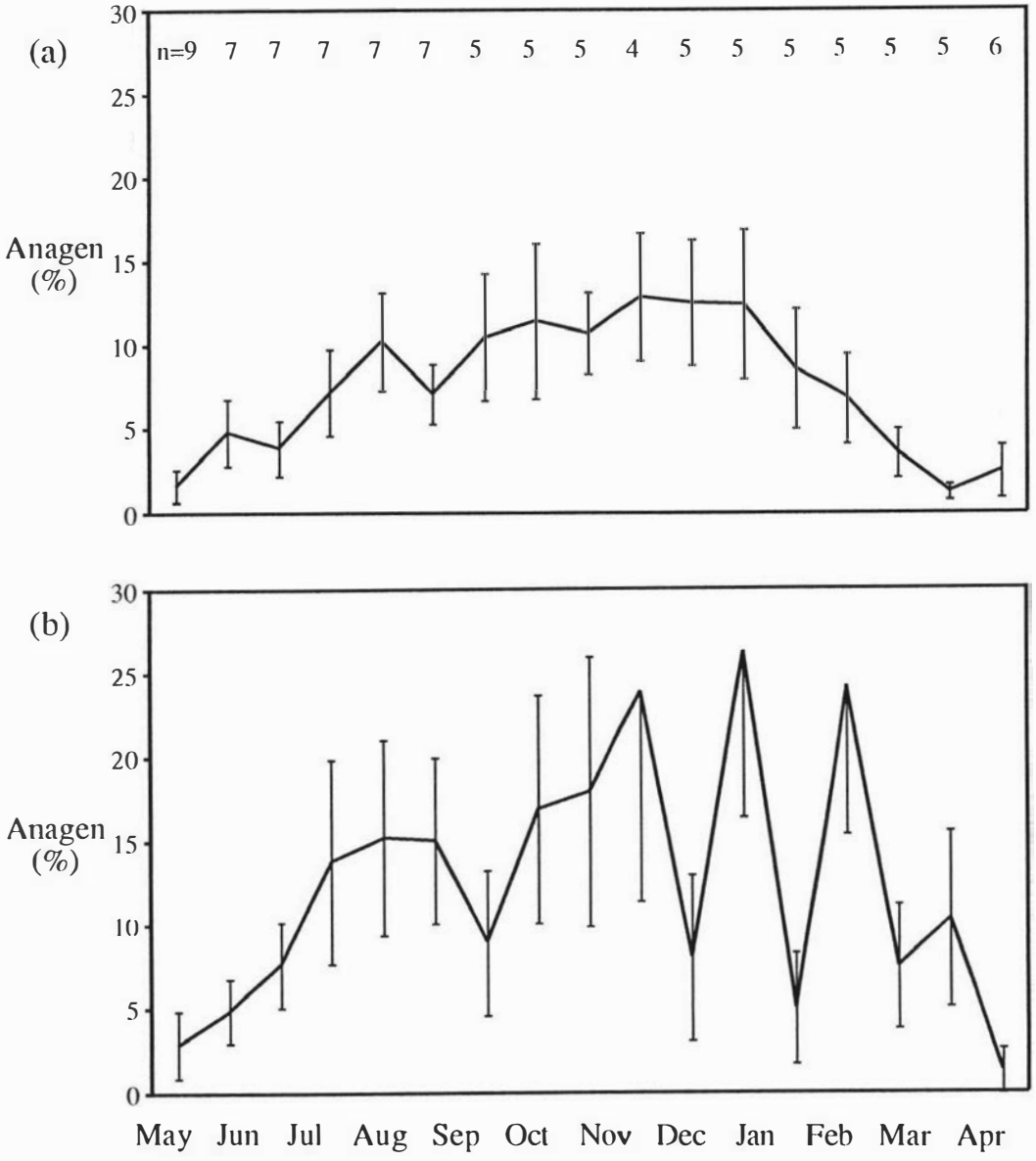

Figure 5.4 Hair follicle activity in captive possums; males. (a) bundle follicles (compound follicles) (b) central primary follicles. Data from seven individuals sampled discontinuously over 25 months are combined to show a general annual pattern. Connected points are means of activity for the given number of possums sampled at each time (n). Bars indicate the S.E.M.

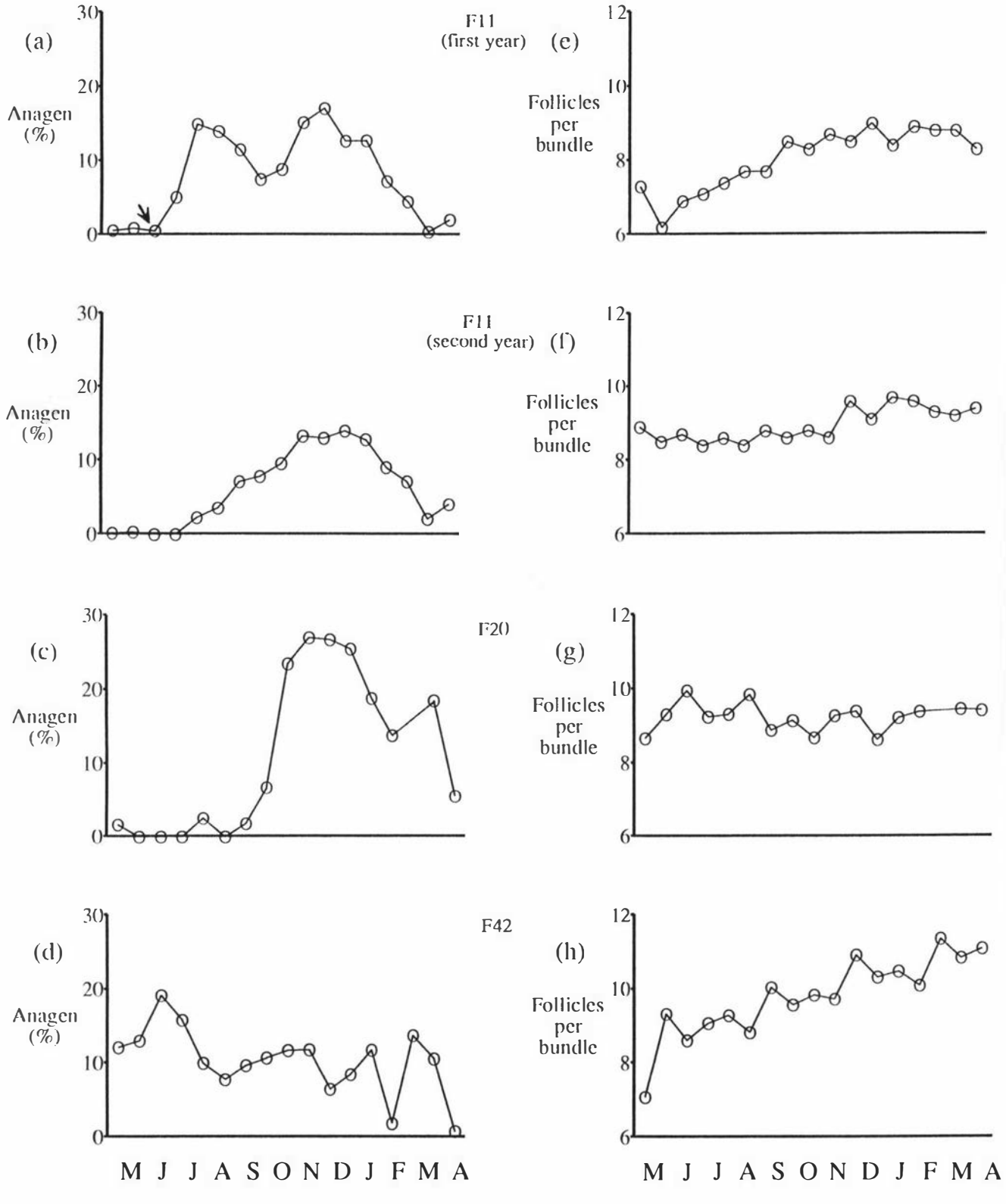

Figure 5.5 Hair follicle activity and bundle size in individual possums: females. Arrow on graph (a) indicates time of removal of young.

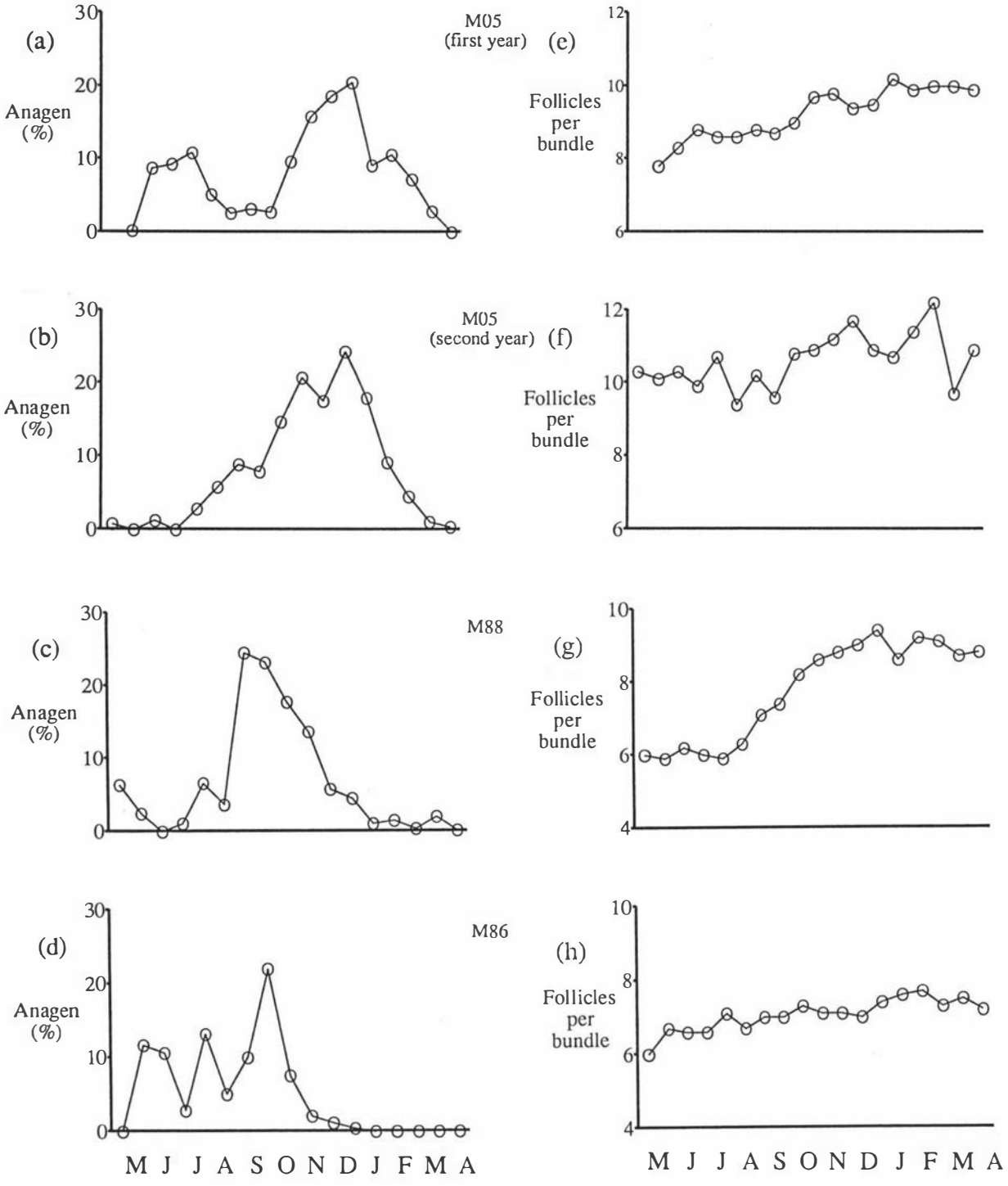

Figure 5.6 Hair follicle activity and bundle size in individual possums: males.

individuals differ in the timing of hair growth, but different patterns were observed within the same individuals in successive years. In both F11 (figure 5.5 (a) and (b)) and M05 (figure 5.6 (a) and (b)) follicle activity was bimodal in the first year of observation and unimodal in the second. The similarity of these two examples appears coincidental as other cases showed alternative patterns over the same time. Also, possum F11 was separated from her young on 7 April of the first year (indicated by an arrow on figure 5.5).

Follicle activity patterns were observed in two female possums raised entirely in captivity. At maturity, one of these (not shown) exhibited low activity between March and June and higher levels from September to December, in a similar manner to possums captured from the wild. The other captive raised female showed an irregular pattern (illustrated in figure 5.5 (d)) maintaining activity at approximately 10 percent over most of the year.

5.3.4 Changes in the compound follicle

Also shown in figures 5.5 and 5.6 are estimates of the mean number of follicles counted per bundle (compound follicle). In all cases except F20, an increase in follicles was observed, indicating the addition of derived follicles to the compound follicle during adult life. Greater increases in bundle size occurred in animals judged or known to be in their second year (figure 5.5 (e) and (h), figure 5.6 (g) and (h)) than in older animals (figure 5.5 (f) and (g), figure 5.6 (f)).

Marked increases in bundle size generally, but not always, corresponded to times of higher follicle activity.

5.3.5 Fur Length

Lengths of guard hair and underfur are shown in figure 5.7. No obvious or consistent annual cyclic changes in fur length are apparent. In addition, no seasonal changes in coat structure or pelage density were noted amongst caged possums. No appreciable quantities of shed fur were observed to accumulate in cages.

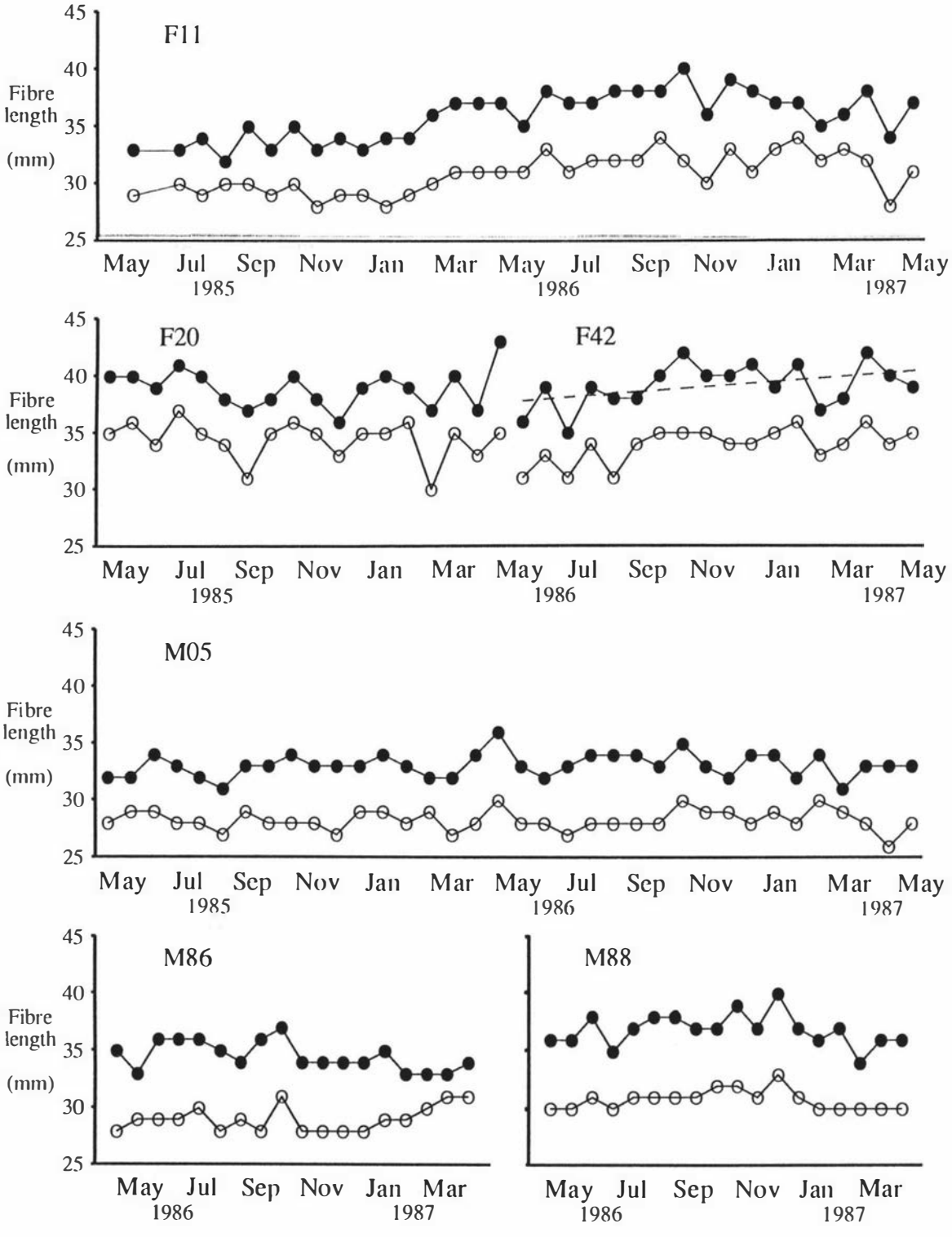

Figure 5.7 Fibre length in individual possums. -●- guard hair, -o- underfur. The bottom two graphs cover the same time period.

5.3.6 Fibre growth initiated by plucking

5.3.6.1 Duration of anagen

Follicle activity during the 21 weeks following epilation is shown in figure 5.8. Similar sigmoid curves were exhibited in each of the three trials conducted at different temperatures (25°C, 15°C, 5°C). Follicle activity approached the resting level measured at the start of each trial, but did not generally reach nil activity by 21 weeks.

Median duration of hair follicle activity following plucking was determined by reading the time corresponding to 50 percent anagen from the graphs. Values for each animal in each trial are listed in table 5.1.

Table 5.1 Median duration of anagen (weeks)

Temperature (°C)	Possum number F09	F13	M12	M25	Mean
25	12.5	11.8	12.0	10.5	11.7
15	13.0	11.5	12.8	10.0	11.8
5	12.0	11.5	10.8	*	11.4
Mean	12.5	11.6	11.9	10.3	11.7

*M25 died of acute intestinal torsion before the experiment was completed.

There was no significant difference in median duration of anagen between trials ($P>0.1$). The overall mean ± standard deviation was 11.67 ± 1.04 weeks (or 82 days). Duration of anagen differed between the four animals ($P<0.05$). The number of follicles per bundle also showed a gradual recovery up to approximately 12 weeks. Since follicle bundle size had returned to initial value by 12 weeks ($P<0.05$) no correction for unobserved follicles was applied to duration of anagen.

5.3.6.2 Fibre Growth

Lengths of longest guard hairs following epilation are depicted in figure 5.9. The length of time taken for each possum in each of the three trials to regrow its fur was determined from these growth curves.

Terminal fibre length (Y_{max}) was estimated from repeated measurements of fur samples taken at the beginning and end of each trial. Remaining growth

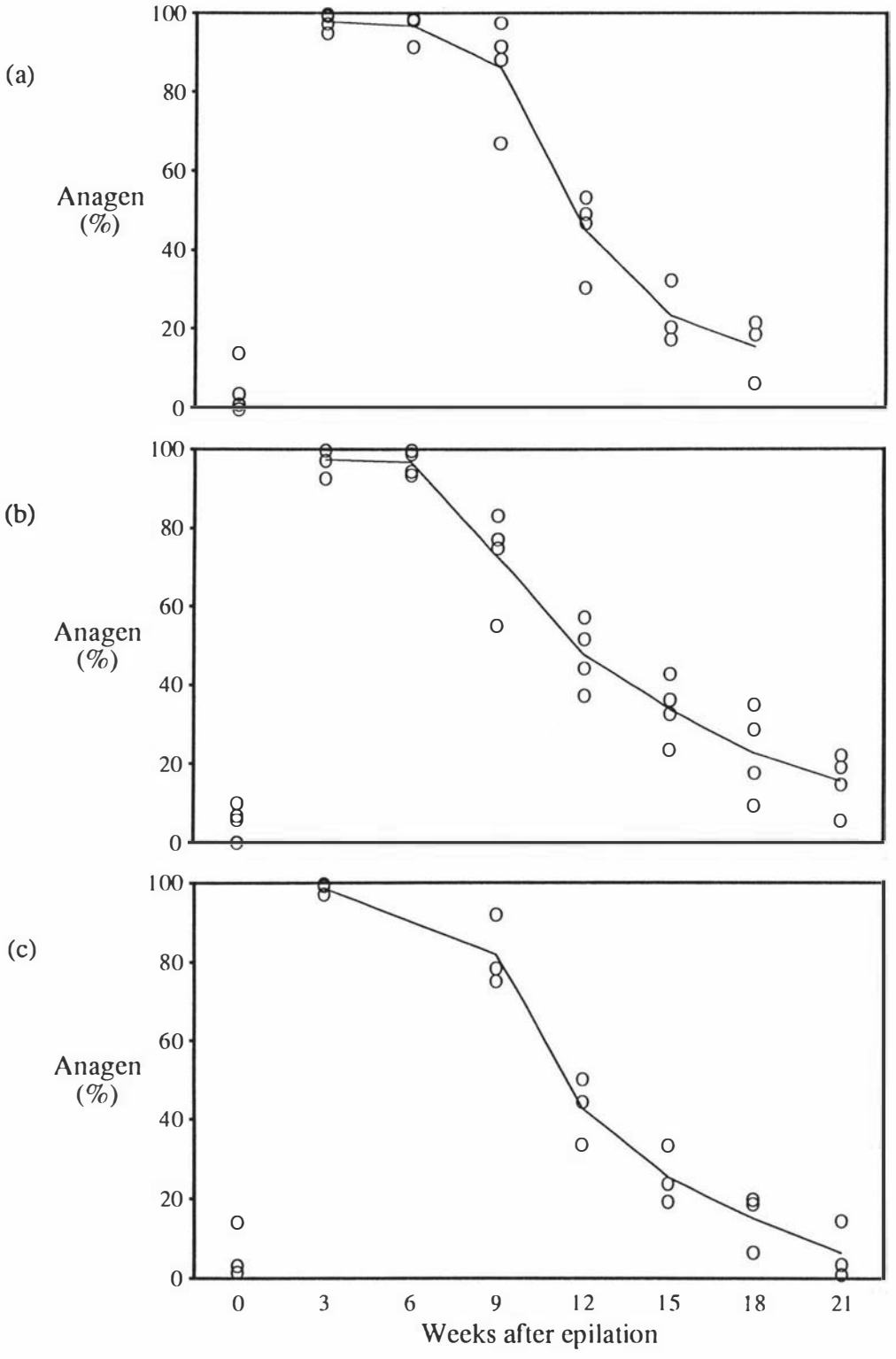

Figure 5.8 Hair follicle activity induced by epilation at three ambient temperatures. (a) 25°C, (b) 15°C, (c) 5°C.

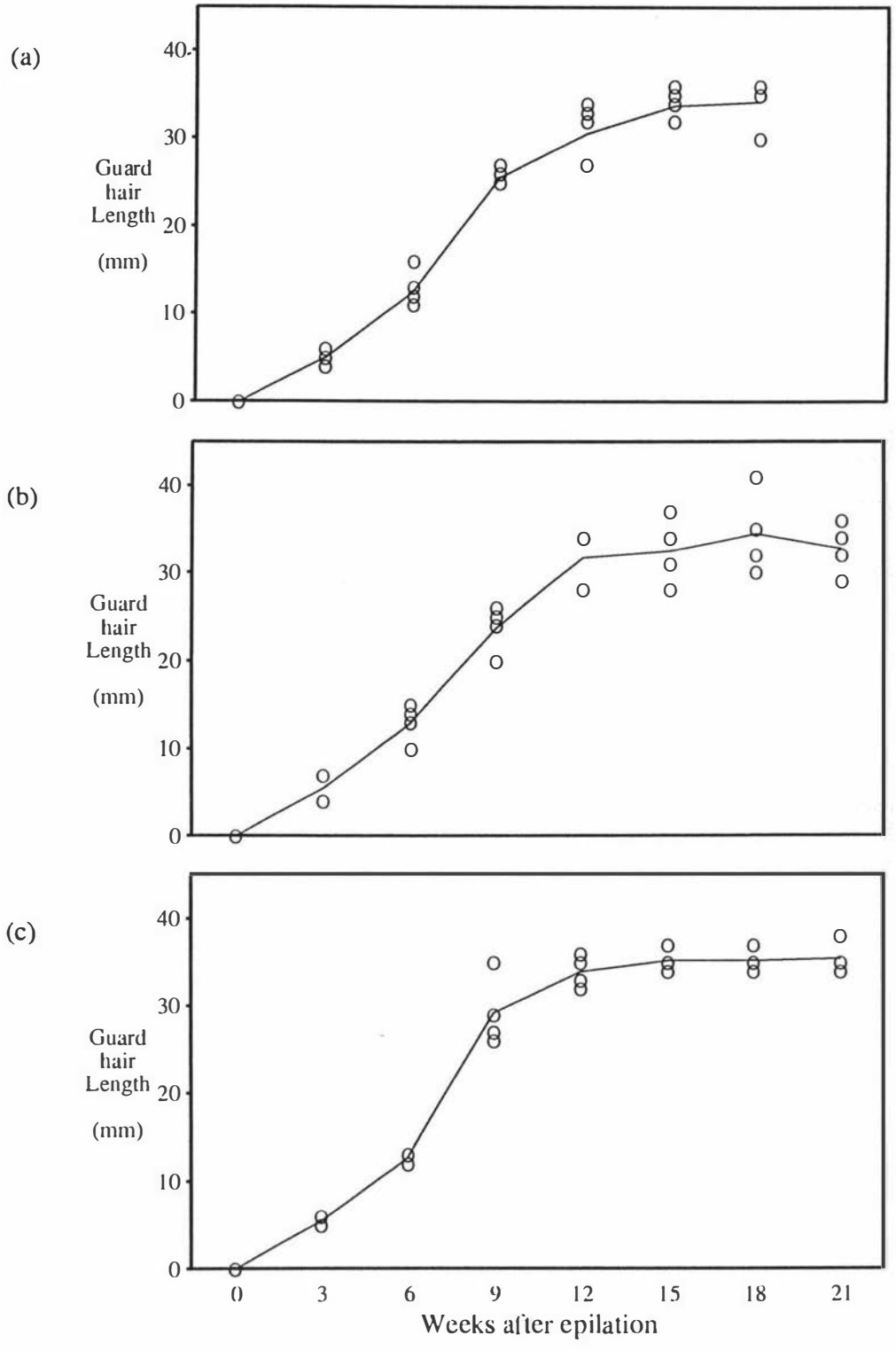

Figure 5.9 Growth of guard hairs following epilation. (a) 25°C, (b) 15°C, (c) 5°C.

(Y_{max} - Y_i) was calculated for samples from 6 to 18 weeks. A log-transformation was performed on these data and a least squares regression line fitted.

An exponential model was then fitted to ascertain the time taken for guard hairs to grow within 90 percent of full length (Y_{90}):

$$(Y_{max} - Y_{90}) = a.e^{-bt_{90}}$$

$$t_{90} = \frac{\ln a - \ln (Y_{max} - Y_{90})}{-b}$$

where: ln a and -b are the intercept and slope regression coefficients.

Y_{max} and t_{90} calculated for each animal in each trial are shown in table 5.2 There was no significant difference in growth times of guard hairs between trials (P>0.1). The grand mean ± standard deviation was 14.2 ± 1.8 weeks, or 99.4 days.

Table 5.2 Terminal length (millimetres) and growth time of guard-hairs (weeks)

Possum number	25°C Y_{max}	t_{90}	15°C Y_{max}	t_{90}	5°C Y_{max}	t_{90}
F09	39.4	16.3	38.3	12.2	36.8	13.9
F13	39.7	17.0	34.0	16.5	39.9	13.8
M12	37.8	14.4	36.6	13.3	37.3	13.2
M25	34.7	14.5	32.0	11.2	*	*
Mean		15.5		13.3		13.7

5.3.7 A model of hair follicle population dynamics

Given the occurrence of: (i) new follicle formation in the skin of mature possums (section 5.3.4), and yet (ii) comparatively low levels of growth (section 5.3.3), *how much, if any, of the growth is attributable to seasonal replacement of the coat?* That is, what proportion of the observed follicle activity represents neogenic follicles, and what proportion results from shedding and regrowth of fibres from existing follicles?

To address this question, a population model was formulated to describe changes in hair fibres/follicles:

$$N_{t1} = N_{t0} + b - d$$

$$\frac{d}{N_{t0}} = 1 - \frac{N_{t1}}{N_{t0}} + \frac{b}{N_{t0}}$$

Where:

N_{t0} is the number of fibres at time t0
N_{t1} is the number of fibres at time t1
b is the number of fibres recruited (growth)
d is the number of fibres lost (shedding)

and:

$$\frac{b}{N_{t0}} = \frac{a(t1-t0)}{100.L} = \text{recruitment relative to initial fibre population}$$

Where:

a is the mean percent anagen over time interval from t0 to t1
L is the duration of anagen

The calculated value d/N_{t0} then, is shedding relative to the initial follicle population, or, the proportion of fibre replaced over time interval from t0 to t1. Since N_{t1}/N_{t0} is a proportion, the number of fibres per bundle can be used as an estimator of relative follicle population size. Initial and final bundle sizes were calculated from the average of three consecutive samples. Mean percent anagen, a, can be calculated from three-weekly samples throughout the year. The median duration of anagen derived in section 5.3.6 was used for the value L in the model.

Recruitment ($b/N_{t0} = a(t1-t0)/100.L$), relative change in fibre population (N_{t1}/N_{t0}), and shedding as a proportion of the initial fibre population (d/N_{t0}) were calculated for each of the eight possum-years previously described. These values, listed in Table 5.3, are crude estimates of actual annual changes in the hair follicle population in each case, but serve to illustrate the highly unusual nature of hair replacement in possums.

Table 5.3 Annual hair replacement

Possum number	$\frac{a(t0-t1)}{100.L}$	$\frac{N_{t1}}{N_{t0}}$	$\frac{d}{N_{t0}}$
F11 (1st year)	0.36	1.27	0.08
F11 (2nd year)	0.28	1.09	0.19
F20	0.48	1.00	0.48
F42	0.46	1.35	0.11
M05 (1st year)	0.36	1.33	0.03
M05 (2nd year)	0.36	1.08	0.28
M86	0.23	1.18	0.05
M88	0.30	1.50	-0.20
Mean	0.354	1.225	0.128

A small proportion (0.23 to 0.48) of new fibres was grown in one year relative to the initial fibre population size, and a highly variable proportion of these appear to be the product of newly developed follicles. (A value for change in follicle population of 1 means that all hair growth was replacement, whereas values greater than 1 indicate some growth attributable to neogenesis.) The proportion of pelage shed and replaced (indicated in the far right column of the table) was low. In no case did this exceed 0.5, or 50 percent of the initial fibre population. A negative shedding value obtained for possum M88 indicates error in values for model parameters. When older animals (F11 second year, F20, M05 second year) are compared with animals judged or known to be in their second year of life (F11 first year, F42, M86, M88) shedding, or actual replacement, was greater, but total amount of hair growth about the same as in older animals.

5.4 Discussion

Repeated observations of the skin of possums have revealed some unusual characteristics of hair replacement. This study has shown, first, that new follicles continue to develop in the coat of sexually mature possums; second, that rates of hair replacement are comparatively low; and third, that although a seasonal pattern of hair growth is apparent, variation between individuals in timing is high.

5.4.1 Neogenesis

The increase in the number of follicles per bundle observed in the skin of adult possums signifies that follicle neogenesis occurs as a normal process in this species. Bundles are compound follicles, composed of either primary lateral or secondary follicles sharing a common hair canal. In general, new follicles are added to compound follicles by branching (Lyne 1966), with each derived follicle possessing a separate dermal papilla.

Neogenesis in older animals has generally been considered rare, occurring only in special circumstances such as antler growth and wound healing (Billingham 1958, Lyne and Brook 1964). However, additions of derived follicles to groups have also been described in another marsupial, the bandicoot, *Perameles nasuta* (Lyne 1957a). Lyne reported increases in the number of follicles per group, while the number of epidermal orifices and original follicles per group remained constant in bandicoots up to one and a half years of age. Lyne described the branching process from histological study with a limited number of repeat observations, and suggested that the addition of derived follicles is integrated with hair replacement. Hence, these two distantly related marsupials - the possum and the bandicoot - share similar hair replacement mechanisms.

Compound follicles formed by branching are commonly found in all three subclasses of mammals, as pointed out by Lyne (1957a). However, information on development and variation in the number of follicles over time is required to determine whether derived follicles continue to initiate in adults of other species, and hence whether neogenesis is unique to marsupials. Certainly, the only two marsupials to have been adequately described show neogenesis, and there is no existing evidence of this phenomenon occurring in eutherian mammals under

normal circumstances. Indication of whether it is phylogenetically primitive or derived would come from an investigation of monotremes, especially the platypus.

It may be of significance that the greatest increases in follicles per bundle occurred in younger animals (table 5.3) showing more rapid weight gain (figure 5.1), and therefore increasing in skin surface area. Follicle neogenesis has been experimentally induced with varying success in rabbits and sheep by preventing contraction of wounds during healing (Breedis 1954, Straile 1959, Lyne and Brook 1964). These observations might suggest a common mechanism involving growth of skin or follicle density operating to initiate follicle formation. The theory that follicles compete for some limited substrate or space within the skin (Fraser 1951, Fraser and Short 1952) could account for renewed follicle initiation in situations where more of the limiting factor becomes available. Alternatively, continued follicle development in possums could be explained by a reaction-diffusion model applied to follicle formation (Nagorcka and Mooney 1985, Mooney and Nagorcka 1985). This model predicts that follicle pattern formation will arise spontaneously because of inherent instability in the system of diffusing and reacting morphogens (Turing 1952), and is sensitive to the geometry of the skin. Nagorcka and Mooney (1985) claimed that their theory encompassed termination of follicle initiation and neogenesis, explaining them in terms of levels of mitotic activity and diffusion distances of morphogens required for spontaneous pattern formation. However, if follicle branching continues in possums because of continued growth of the skin, then further explanation is required for animals which continue to grow rapidly after follicle formation ceases, as in humans (Szabo 1957, cited in Billingham 1958).

Follicle branching in possums should not be confused with seasonal change in compound follicles reported in some eutherian mammals. In Angora rabbits (Rougeot and Thebault 1983), and mink (Rougeot *et al* 1984b, Maurel *et al* 1986), some derived follicles have been noted to regress during the spring moult, thus reducing the down density of the summer coat. New follicles form again in autumn to give a thick winter coat. Such marked coat changes do not occur in possums, and increases in the number of derived follicles appear to be permanent. Branching in a compound follicle is also distinct from club accumulation within a single follicle, as claimed for example in rats and mice (Chase 1954).

5.4.2 Hair replacement

The second notable feature of possum fur identified was the low rate of hair replacement. The peak follicle activity is much lower than previously observed in other mammals. In many species, maximum follicle activity at one site may to be close to 100 percent. In the examples of mice (Borum 1954), rats (Johnson 1965c), hamsters (Brust 1962), rabbits (Whiteley and Ghadially 1954), mink (Rust *et al* 1965) and several species of ungulates (Ryder 1966a, 1977, 1978) hair cycles in adjacent follicles of the same type are in close synchrony, and replacement is complete. In other cases, such as the domestic cat (Baker 1974, Ryder 1976) and dog (Al Bagdadi *et al* 1977) follicle activity is less well coordinated. Peak activities of adult animals were observed to be about 50-60 percent and 80 percent respectively, and although seasonal peaks were still evident, a moderate proportion of follicles are active throughout most of the year. Hair replacement in these species is therefore more gradual, but it probably still involves most, if not all, of the coat.

This is not so in possums. The highest hair follicle activity observed during the present study was 40 percent, and was generally much lower. This is not just a lack of synchronization between follicles extending the time of pelage replacement, but the low activity represents much less growth and replacement in possums than has been previously shown in other mammals. The model applied in section 5.3.7 demonstrated that, given the increases in the follicle population and an approximate value for the duration of anagen, the observed activity was insufficient to replace the coat in one year. Indeed, annual replacement estimates for eight individuals averaged 0.13, and did not exceed 0.48. That is, on average amongst animals of differing sex and age, only 13 percent of the initial fibres present in their coats were shed and regrown in the course of one year.

The negative value obtained for one possum means that more fibres (and follicles) appeared to be added than could be accounted for by growth. This is not possible and is an indication of sizable errors in the values for parameters used. Errors could arise from variation between biopsy sites in both bundle size and activity, and inaccurate estimates for duration of anagen. A mean value obtained from the four group B animals was used to approximate duration of anagen for each possum of group A (as it was not feasible to perform tests on

each animal). However, there was significant variation between the individuals of group B. It should also be noted that the time taken for the fastest growing guard hairs to approach full length (99 days) was longer than the estimate of median duration of anagen (82 days) for all follicle types, indicating differences in duration of anagen between follicle types.

Also, measurements were made from follicles induced into growth by epilation, but investigation of the differences between pluck induced and spontaneous fibre growth (section 4.8) indicated that they are not directly comparable. The delay between epilation and labeling of newly formed fibre probably represents a period of reorganization following trauma (Argyris 1962). This would result in an overestimate of the duration of anagen based on plucking. On the other hand, peak fibre growth rates were significantly higher in plucked fibres which would tend to make the estimate of growth time less than in spontaneously growing fibres. Some indication of the sensitivity of the model to errors in the duration of anagen is therefore required. A tabulation of the response of annual replacement to changes in duration of anagen is given in table 5.4, using the mean values for the eight possum years as above.

Table 5.4 Sensitivity of annual replacement to duration of anagen

Percent error in duration of anagen estimate	Duration of anagen (days)	Fibre growth as proportion of initial follicles	Shedding (fibres replaced)
+20	98	0.30	0.07
0	82	0.35	0.13
-20	65	0.44	0.21
-50	41	0.71	0.48

The calculations summarized in table 5.4 show that varying duration of anagen produces sizable changes in the calculation of shedding, but the general conclusion that replacement was very low in possums holds irrespective of the accuracy of the duration of anagen estimate. An error of 50 percent is unlikely (section 4.8), but even with an error of this magnitude, less than half of the initial fibres would have been replaced, given the measured follicle activity and increase in fibre/follicle population.

Therefore, while limited data and oversimplification may make the model imperfect, it is adequate to demonstrate the unusual nature of hair replacement in possums. For comparison, mean values obtained for growth, follicle population change, and shedding as a proportion of the initial fibre/follicle population (i.e. the quantities shown in table 5.3) are listed in table 5.5. with theoretical values expected in adults of annually, biannually and continuously moulting species. (Values for mice are calculated from Borum's (1954) data in which average times between the eruption of hairs of successive generations (G2 to G8) varied from 52.5 to 80.8 days, depending on body site and sex.)

Table 5.5 Theoretical annual hair replacement in described species compared with replacement observed in possums

	Fibre growth as a proportion of initial follicles	Coefficient of follicle popul -ation change*	Shedding (fibres replaced)
Possum (means from Table 5.3)	0.35	1.23	0.13
Annual species (e.g. cat, fox)	1	1	1
Biannual species (e.g. mink, deer)	2	1	2
Continuous species (e.g. mice)	6	1	6

* (A coefficient of 1 indicates no net change in follicle population. A value of 2 would represent doubling of follicle numbers over one year.)

In addition to these calculated values for shedding, however, there was anatomical evidence of some replacement within existing follicles in the fashion normally seen in other species. Some shed follicles possessing an empty, irregular shaped, or closed lumen were occasionally observed. Follicles in this state probably replace shed fibres in the normal manner.

An attempt was made to distinguish primary central follicles from compound follicles. However the tendency for some primary central follicles to form bundles resulted in some ambiguity. Solitary primary central follicles in

anagen (containing a single growing fibre) were nonetheless evidence of normal replacement cycles occurring particularly in this follicle type. That is, almost all of the hair growth represented in figures 5.3(b) and 5.4(b) was guard hair replacement, whereas figures 5.3(a) and 5.4(a) represent both neogenesis and replacement, mostly of underfur. Lyne (1957a) reached a similar conclusion regarding primary central follicles in the bandicoot. It should also be noted that primary central follicles make up a small proportion of the total population (3 percent in adult wild possums examined in section 4.10) which diminishes as derived follicles are added.

With only 0 to 50 percent of the fibres in the pelage being replaced gradually over the year, marked seasonal changes in coat structure would seem unlikely. Greater replacement of guard hairs as described above could result in slight differences in mean guard hair length. But, observations of guard hair and underfur length, and subjective assessment of pelage of captive possums were consistent with an absence of seasonal variation. These findings contradict a popular belief that some change in pelage results in "winter" and "summer" furs in possums. It is possible that the conditions of captivity have acted to diminish seasonal effects, but evidence presented in chapters four and six suggests that hair growth and replacement described here for captive possums is representative of events in the wild.

Lack of seasonal pelage change in possums contrasts with most available descriptions of other species, particularly commercial fur bearers. Pelage changes are most obvious in boreal mammals which alternate their colour (e.g. Rust 1962, Jackes and Watson 1975). Although laboratory rodents show continuous wave replacement, many northern hemisphere mammals including rodents, lagomorphs, carnivores and ruminants already mentioned exhibit seasonal modifications to fibre type composition, density and insulative properties as an adaptation to the environmental temperature variation (Hart 1956, Johnson 1984). Possums apparently do not require such adjustments to survive in the range of habitats that they occupy with considerable success in Australia and New Zealand. This may not be a peculiarity of possums so much as a reflection of the greater research effort on particular northern hemisphere species. Tropical mammals are much less well described, but are generally regarded as having little seasonal variation in pelage (Irving *et al* 1955, Ewer 1972). In some southern temperate zone mammals hair replacement is also

noted to be gradual, for example, guinea pigs (Jackson and Ebling 1971), and South American cameloids (Ryder 1987).

5.4.3 Variability in time of growth

This leads to consideration of the third unusual feature of hair growth in possums - the variability in timing of growth. As shown in figures 5.3 and 5.4, a general seasonal pattern exists. Greatest follicle activity tended to occur in spring-summer, and greatest quiescence in autumn. (The summer growth period is commonly referred to by trappers as the "moult", while pelts with inactive follicles taken during autumn and winter are said to be in "prime". These terms derive from, and describe, pelage changes in northern fur bearers. But, like the notion of pelage change, they are probably inappropriate when applied to possums, since in many possums a very small proportion of the coat may be shed or renewed.)

Examination of follicle activity in the skin of individual possums shows that not all animals follow the general seasonal trend and there is considerable variation in the timing of growth. Peaks in growth occurred from July to January. Annual variation in the timing of growth was also evident. This very high degree of variability made analysis of differences due to sex, reproductive condition and age difficult, and restricted intended experimental study.

Variation in results may also have been increased by differing physiological states. Pregnancy and lactation have been shown to inhibit hair growth in several species (e.g. Nay & Fraser 1955, Corbett 1979). The effects of pregnancy and lactation on hair growth have been difficult to interpret from the two female possums which raised young in the course of this study. One showed a sharp increase in follicle activity following removal of young, whereas the other continued on a trend of declining activity.

In many other species in which moulting is much more synchronized, it has been demonstrated that the initiation of hair growth is primarily cued by photoperiod (e.g. Hart *et al* 1963, Rougeot *et al* 1984a, Duncan and Goldman 1984b). Individual variation in possums thus suggests that hair growth in this species is not controlled primarily by a single environmental factor. In the possums examined, hair growth was not related closely to either photoperiod or ambient temperature. Rather, hair growth showed similar variation to breeding cycles observed in possums. In most wild populations, breeding occurs mostly

from March to June, but the variation is such that individual possums may breed at any time over most of the year (Gilmore 1969, Crawley 1973, Kean 1975, Bell 1981). It may be that hair growth is controlled by a similar physiological mechanism to that of reproduction, which is at present poorly understood (Tyndale-Biscoe 1984).

Finally it is possible that the seasonal patterns of hair growth observed in this study are influenced by the effects of captivity. Circulating hormones of possums have been noted to be affected by the stress of handling and confinement (Curlewis and Stone 1985), and individuals in this study varied in their adjustment to captivity. Those that lost weight and became ill showed marked declines in hair follicle activity. Some verification of results from captive animals is therefore necessary. This was provided by continued sampling of the population which was the source of captive animals, and survey of pelts, reported in chapter six.

Chapter 6
MOULT PATTERNS IN POSSUM PELTS

6.1 Introduction

In most mammals, shedding and regrowth of pelage, or moult, occurs biannually or annually in a topographic pattern characteristic of the species (Ling 1970). Such patterns generally result from the manner in which hair follicle cycles are coordinated (Ebling and Johnson 1964a). Follicle cycles can be synchronized over a small skin area, but out of phase between widely separated regions so that waves of hair replacement appear to progress over the skin. Alternatively, hair cycles of adjacent follicles can be out of phase, which tends to produce a diffuse moulting pattern. Relatively few species, including man, guinea pigs (Dawson 1930) and cats (Baker 1974) have been reported to exhibit diffuse moulting only.

Moult patterns are made visible on the flesh side of animal pelts by pigmentation associated with hair growth. Active follicles usually contain active melanocytes (Breathnach 1971) and pigmented growing hair, whereas resting follicles with fully grown fibres are unpigmented (Gunn 1932, 1933, Stevenson 1962). Areas of skin with a high proportion of active follicles therefore appear dark in relation to those with resting follicles. This feature has been widely used in small and medium sized mammals to describe moult by collecting whole skins at known intervals to show the timing and progression of skin pigmentation (e.g. Ecke and Kinney 1956, Kryltzov 1964, Linde 1963, Stodart 1965, Golley *et al* 1966, Linzey and Linzey 1969, Olsen 1980). Since in some cases these pigment patterns have been shown to correspond with age, methods of aging animals for ecological study have been based on moult (e.g. Shanks 1948, Schofield 1955).

Moult patterns have not been described in any marsupial, although as a preliminary guide to this study, comments on moult from New Zealand fur graders and trappers were collated and summarized in chart form (section 4.3, Campbell and Nixon 1985). In the present study, melanin pigmentation patterns occurring in samples of wild possum pelts were used to describe the timing and topography of hair growth. This survey was initiated when observations of hair

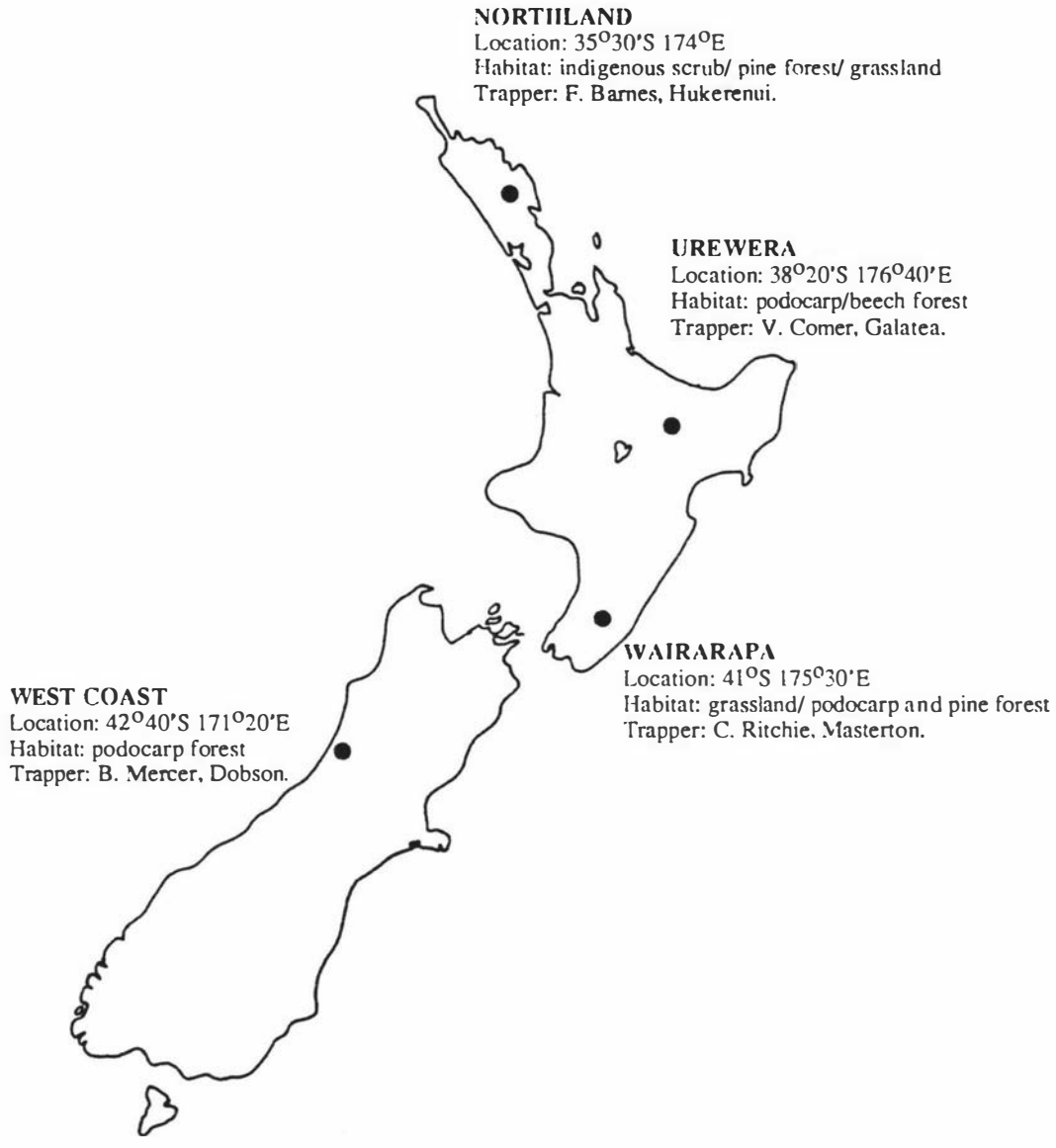

Figure 6.1 Locations within New Zealand of possum pelt sample collection.

follicle activity in a small number of captive possums (chapter five) began to show considerable variation between individuals in the timing of fibre growth. It was therefore of interest to ascertain the variation in timing and pattern of moult which exists within and between wild populations, and how this is related to the captive studies.

The term "moult" in this account is in accordance with common usage, even though comparatively little shedding and replacement may be involved.

6.2 Methods

6.2.1 Pelt material

A total of 2309 possum pelts was collected between February 1986 and June 1987 from four locations each separated by approximately three degrees of latitude (figure 6.1) The flesh sides of pelts were observed and compared with a reference chart of moult patterns. Professional possum trappers from each area provided labelled pelts from the first 50 possums taken over a period no greater than three days, beginning as close as possible to the 15th day of each month. Dependence on commercial trapping operations meant that samples were not obtained for some months outside the normal trapping season. Also, sampling within each location shifted between areas of differing vegetation, altitude and other environmental conditions. Ten items of information were recorded from each pelt as detailed in table 6.1.

The reference chart of moult types (figure 6.2) was formulated so as to include the full range of patterns encountered during an initial period of observation of commercially trapped pelts.

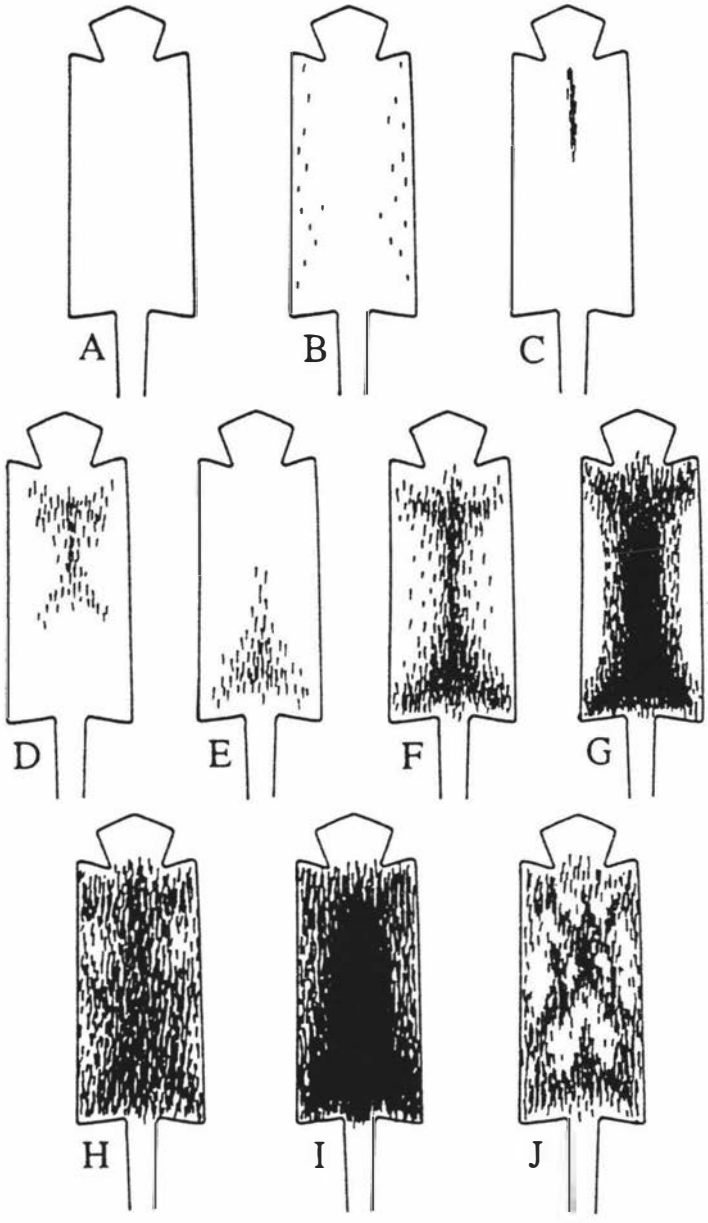

Figure 6.2 Reference moult patterns. Lettered shapes represent the view of the flesh side of dried possum pelts, showing melanin pigmentation due to hair growth.

Table 6.1 Information recorded from possum pelts

Variable	Categories	Criteria
location	Northland Urewera Wairarapa West Coast	(see figure 6.1)
date of capture*		
age*	adult juvenile	) juvenile younger than) about 1 - 1.5 years, by) body size, and testis) and pouch maturation
sex*	male female	
reproductive condition*	dry lactating	) presence of pouched) young, or state of) mammary glands
fur colour	grey (grey/ pale/ redneck) brown (dark/ red-brown) slate	
grade	large 1st, 2nd, 3rd med/large 1st, 2nd, 3rd medium 1st, 2nd+3rd small 4th	)) as graded for sale) at New Zealand) Fur Auctions)
patchy fur growth (see plate 2)	slight moderate extensive	pigment spots <5 cm^2 spots over greater than half of pelt
wounds	absent present	
moult	categories A to J	(see figure 6.2)

* Data provided by trappers.

6.2.2 Dyeing

Four adult grey possums (two of each sex) were maintained under the conditions described in section 3.1. They were dyed on 10 August 1985, when they might be expected to have fully primed coats (Comer 1984). While under saffan anaesthesia, the animals were washed in weak detergent to remove oily

cutaneous secretions and wet the fur. Then the entire coat, except the face, was dyed with "Durafur black" (Chapman and Wheeler 1963). The fur was dried with towels and a fan heater. Replacement of fur was observed until 20 April 1987 by noting change from brown (dyed) to grey (new undyed) coloured fur. One male showed rapid loss of body weight nine weeks after dyeing, but remained alive until completion of observations. One female possum died after washing and dyeing, probably due to hypothermia (table 3.2).

6.2.3 Histological material

In order to calibrate the moult categories with levels of hair growth, samples of skin were taken from the mid dorso-lateral position of 122 possums trapped at Keebles Bush over 25 months. The skin was fixed, processed and embedded in paraffin wax as described in section 3.3. Hair follicles were scored from transverse sections as described in section 3.4, to determine the proportion of follicles in anagen.

6.2.4 Statistical analysis

The moult survey data were analysed using generalized linear models, in which a response variable, Y, is predicted by a linear combination of explanatory variables (Dobson 1983). Goodness of fit is determined from a statistic called the scaled deviance from the maximum model. The scaled deviance has an approximately chi square (d) distribution, with d degrees of freedom. The importance of a term in a generalized linear model can be judged by comparing the scaled deviance of that model with a model from which the term is excluded. The difference in deviance has an approximately chi square (t) distribution, with t degrees of freedom.

The log-linear model for contingency tables is a generalized linear model with a log link function:

$$\log [Y] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots + \beta_n x_n$$

and a Poisson error distribution. This provides tests of independence in multiway contingency tables using the G-statistic (Sokal and Rohlf 1969).

While log-linear analysis is appropriate for count data of the type collected during the moult survey, in most instances higher order interactions were highly

significant, precluding tests of the interactions of interest. Further analysis was possible by fitting generalized linear models to the data in a simplified, binomial form (Dobson 1983). The logit link function was used:

$$\log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots + \beta_n x_n$$

where p is the predicted proportion.

Binomial predictor variables were created from some ordinal level variables by combining categories (as described in section 6.3.4) so they could be expressed as a proportion. This resulted in some loss of information, but allowed a reduction in the dimensionality of the data or a larger number of variables to be included.

Neither log-linear nor binomial statistical methods took account of order of months, so that small differences in seasonal timing could result in large residuals.

6.3 Results

6.3.1 Moult patterns in pelts

Almost all (99.8 percent) of the possum pelts observed could be matched to patterns on the reference chart shown in figure 6.2. These were generally representative of a diffuse pattern. The typical example from a young animal shown in plate 1 is approximately intermediate between categories "F" and "H" of the reference chart. The most common patterns were those with somewhat more intense pigment in posterior and mid dorsal regions ("E", "F", "G", and "I"). Pattern "G" occurred mainly in grey coated possums, as in this colour morph ventral fur is pale. Skin pigment is therefore light on the corresponding lateral margins of the stretched and dried skin. Pattern "D", representing pelts with hair growth concentrated in the anterior region was rare (0.5 percent). Some irregular patterns were classified as pattern "J". These could represent stages of a wave progression, but they occurred infrequently (4.6 percent of observations) and were distributed throughout the year. There was no other

evidence of a sequence of symmetrical bands of skin pigmentation associated with wave moulting. Pattern "H", a comparatively uniform dark pattern was more common in juveniles ($P<0.001$). Of animals classed as juvenile, 22.4 percent showed this type, compared with only 6.2 percent of adults.

6.3.2 Moult exhibited by dyed possums

Confirmation of a diffuse moult pattern was provided by observations of the two dyed possums that remained in good health over the observation period (possum numbers M09 and F10). Both appeared to return gradually and evenly to their original grey colour through hair replacement, and possibly also fading of dye on outer fibres. But both animals retained dyed hairs in their coats for at least 21 months following dyeing.

A third dyed possum (number M08) lost 25 percent of its body weight over three weeks, between 9 and 12 weeks after dyeing. A diagnosis of arterial calcinosis was given for this animal at post mortem. Follicle activity monitored by skin biopsy decreased to zero at the time of weight loss, and the underfur over the whole pelt remained heavily dyed up until this animal was euthanased at 21 months after dyeing.

No shedding lines or other evidence of wave patterns were observed in the coats of any live dyed animals, or surveyed pelts.

6.3.3 Follicle activity

Hair follicles were arranged in groups generally comprising a central primary follicle flanked by three to five compound follicles. In samples showing hair growth, individual follicles within the same compound follicle were invariably at different stages of the hair cycle, so that there appeared to be a mosaic pattern of activity.

The activity of individual samples at the dorso-lateral site as determined by skin histology is presented in figure 6.3. Highest activity occurred in pelts taken during summer, while activity was generally low or absent in those taken in autumn and winter. Follicle activity is therefore not completely out of phase ("mosaic") as there was a seasonal trend in hair growth.

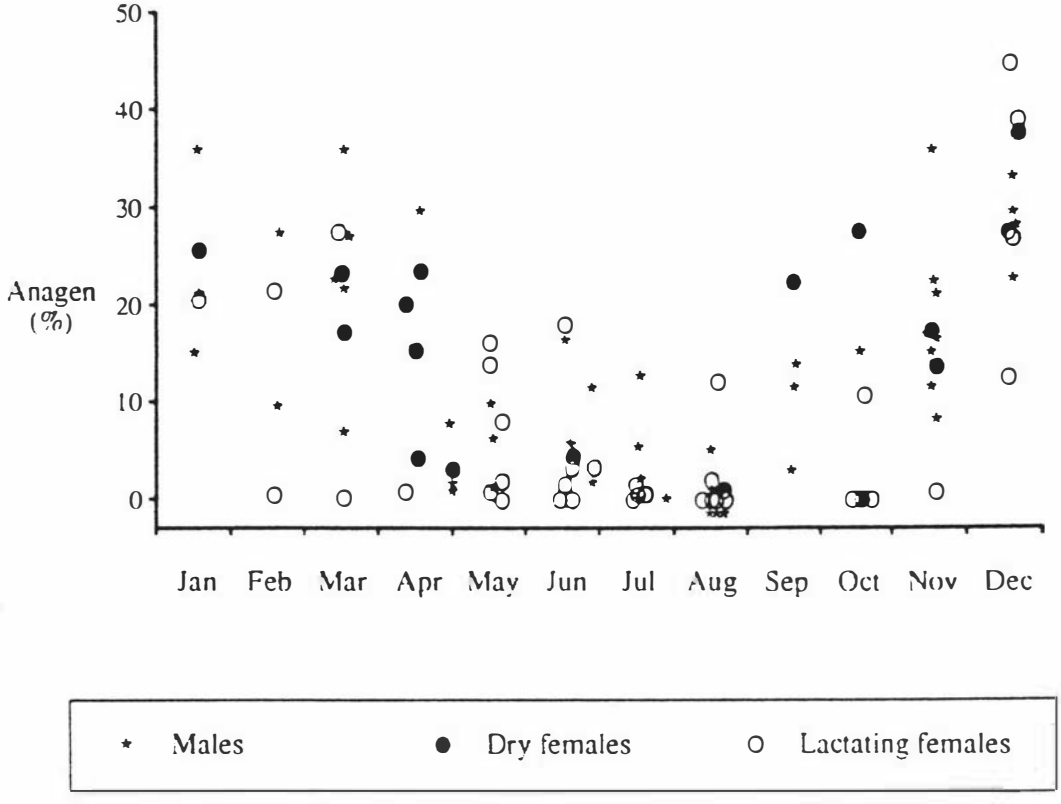

Figure 6.3 Follicle activity in wild possums. Percent of follicles in anagen at the mid dorso-lateral site of possums trapped each month for 25 months at Keebles Bush, Manawatu. The two years results are overlayed.

A suppressive effect of lactation on follicle activity is also indicated by figure 6.3. Means were 8.9 percent for lactating and 14.7 percent for non-lactating females, with S.E.D. of 0.40 ($P<0.10$). Note however, that the two highest percent activity values were found in lactating animals (during December).

Figure 6.4 shows the relationship between moult pattern and percentage of active follicles. Moult categories are arranged in order of activity level. Dark pelts generally had a high proportion of active follicles at the dorso-lateral site. However, in none of the skin samples - including those from the darkest pelts - were more than 45 percent of hair follicles in anagen.

6.3.4 Combining of moult categories

To simplify graphical presentation and analysis of some of the following results, moult categories depicted in figure 6.2 were lumped on the basis of follicle activity shown in figure 6.4 and overall pigment intensity. Four broader moult classes are listed in table 6.2 along with the corresponding follicle activity levels. Although both (1) "no moult" and (2) "light moult" have minimal follicle activity at the mid-side site, the two classes were distinguished since the latter represents patterns showing hair growth elsewhere on the pelt.

Table 6.2 Moult classes

Moult class	Moult pattern (figure 6.2)	Follicle activity (% anagen) Mean	Range
(1) no moult	A, B	0.8	0 - 6.9
(2) light moult	C, D, E	1.6	0 - 3.3
(3) medium moult	F, J	13.2	0 - 35.2
(4) dark moult	G, H, I	24.6	0.8 - 44.8

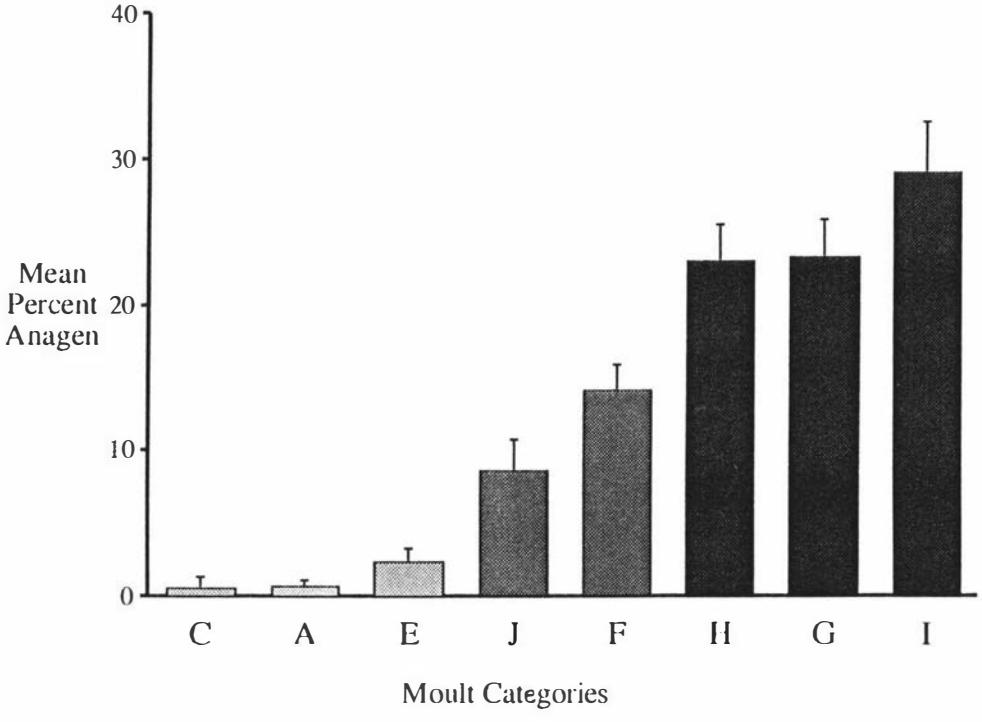

Figure 6.4 Follicle activity and moult category. Percent of follicles in anagen at the mid dorso-lateral site is shown for moult categories illustrated in figure 6.2. Error bars show the standard error of the mean. Skin samples were not obtained from examples of patterns "B" and "D".

For some analyses using binomial generalized linear models it was necessary to further combine moult categories, creating just two classes: no moult (patterns A and B) and moulting (patterns C - I). This allowed moult to be expressed as the proportion of pelts in the sample showing at least some pigmentation on the flesh side attributable to diffuse hair growth, and reduced the number of dimensions of the data set by one.

6.3.5 Age, sex and colour

Table 6.3 shows ratios of male:female, adult:juvenile, and fur colours occurring in each location. Sex ratios differed significantly from 50:50 in only Northland ($P<0.05$), and all regions combined ($P<0.01$). Each location was characterized by a predominant colour phenotype, either grey or brown (melanic). Both grey and brown possums showed age and sex related rufous colouration (Oldham *et al* 1985), older males being more red than younger females. The ratio of red:non-red graded pelts which occurred in each location is shown in table 6.3. This ratio is probably influenced by many factors, including age structure and genetic variation within possum populations.

Table 6.3 Sex, age and colour ratios (percent)

Location	Male/Female	Adult/Juvenile	Grey/Brown	Red/non-red
Northland	55:45	79:21	98:2	21:79
Urewera	52:48	76:24	1:99	29:71
Wairarapa	53:47	80:20	86:14	31:69
West Coast	52:48	72:28	4:96	39:61

Wairarapa was the only location in which comparison of moult in the two main colour phenotypes (grey and brown) of possums was possible. Amongst brown pelts, 55 percent showed some moult pigmentation, compared with 58 percent of grey pelts. The SED was 0.054, i.e. there was no significant difference in total proportion of moult between the two colours. A generalized linear model analysis with proportion showing moult (categories combined as in

section 6.3.4) as dependent variable and month, age, and colour as explanatory variables was conducted. All interactions involving month and colour were non-significant, indicating no difference between grey and brown possums in the timing of moult.

6.3.6 Seasonal and regional variation in moult

Moult patterns did not form a readily recognizable sequence through time, but a wide range of patterns occurred in adult possums of both sexes at almost all times of the year. As an example, the occurrence of each of the moult categories in each month in the West Coast location is shown in figure 6.5. Dark patterns tended to occur in summer; lighter patterns in winter and spring.

Similar trends were evident in the other locations. Figures 6.6 and 6.7 summarize seasonal moulting, showing combined moult classes for females and males respectively. Months from different years are combined in order to compare data of different locations. Log-linear analyses using both uncombined and combined moult categories gave highly significant moult x month x location interaction effects for both males ($P<0.001$) and females ($P<0.001$). These results indicate that the monthly occurrence of moult categories varies non-uniformly between regions in such a way that further analysis using log-linear models would not have been meaningful.

An analysis was conducted using generalized linear models with proportion of moult as a dependent variable (as described in section 6.3.4) and location, month, year, age and sex as explanatory variables. The age x month interaction was significant ($P<0.001$). That is, adults moult at different times to juveniles. Juveniles had generally higher proportions of moult than adults (88 percent of all juvenile pelts compared with 60 percent in adults). Almost all juvenile pelts sampled from October to May showed moult pigmentation. The low of 59 percent moult occurred in September. Adult moult was highest from November to January or February, with the low of 40 percent in August. Data from only three pelts (all with moult pigmentation) were available for the month of December.

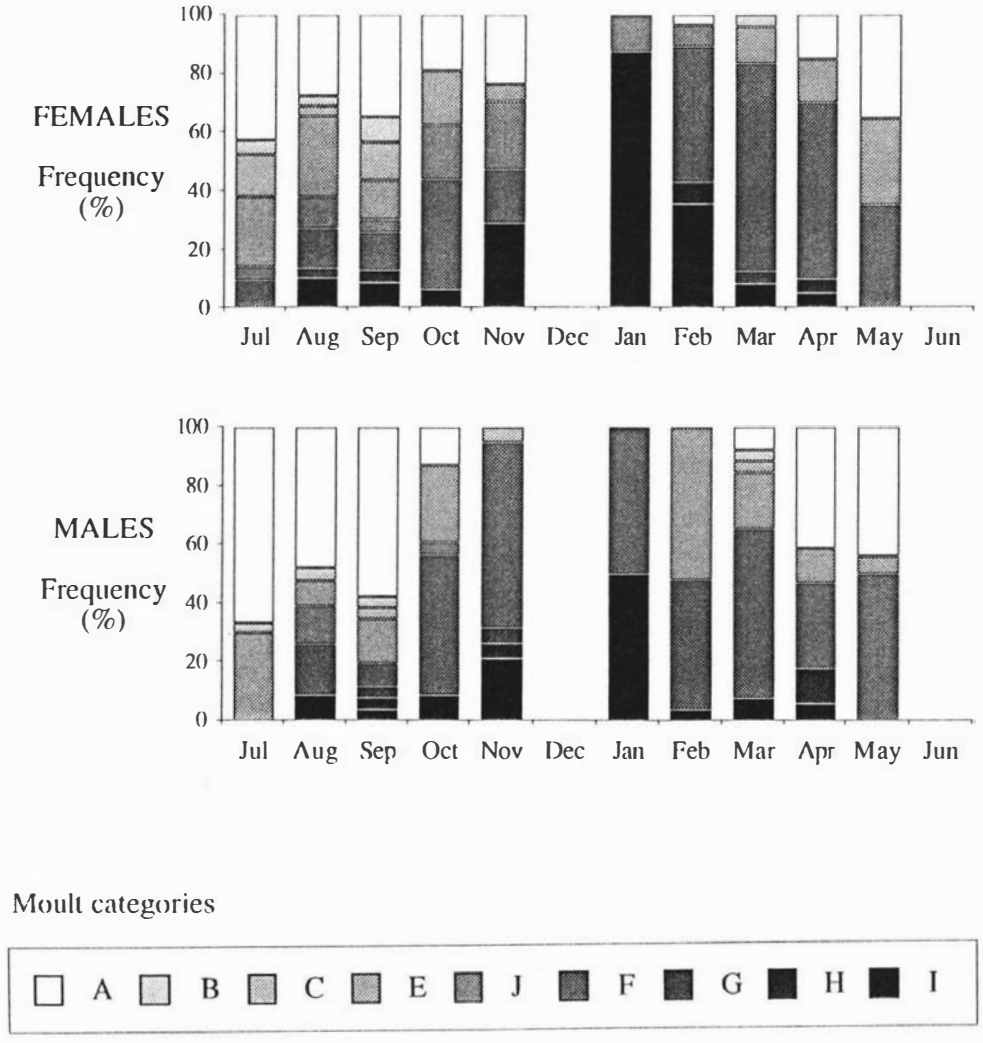

Figure 6.5 Frequency of moult patterns in West Coast. Seasonal change in frequency of moult pattern in possums of both age classes are shown, with females and males on separate graphs. Moult categories correspond to those

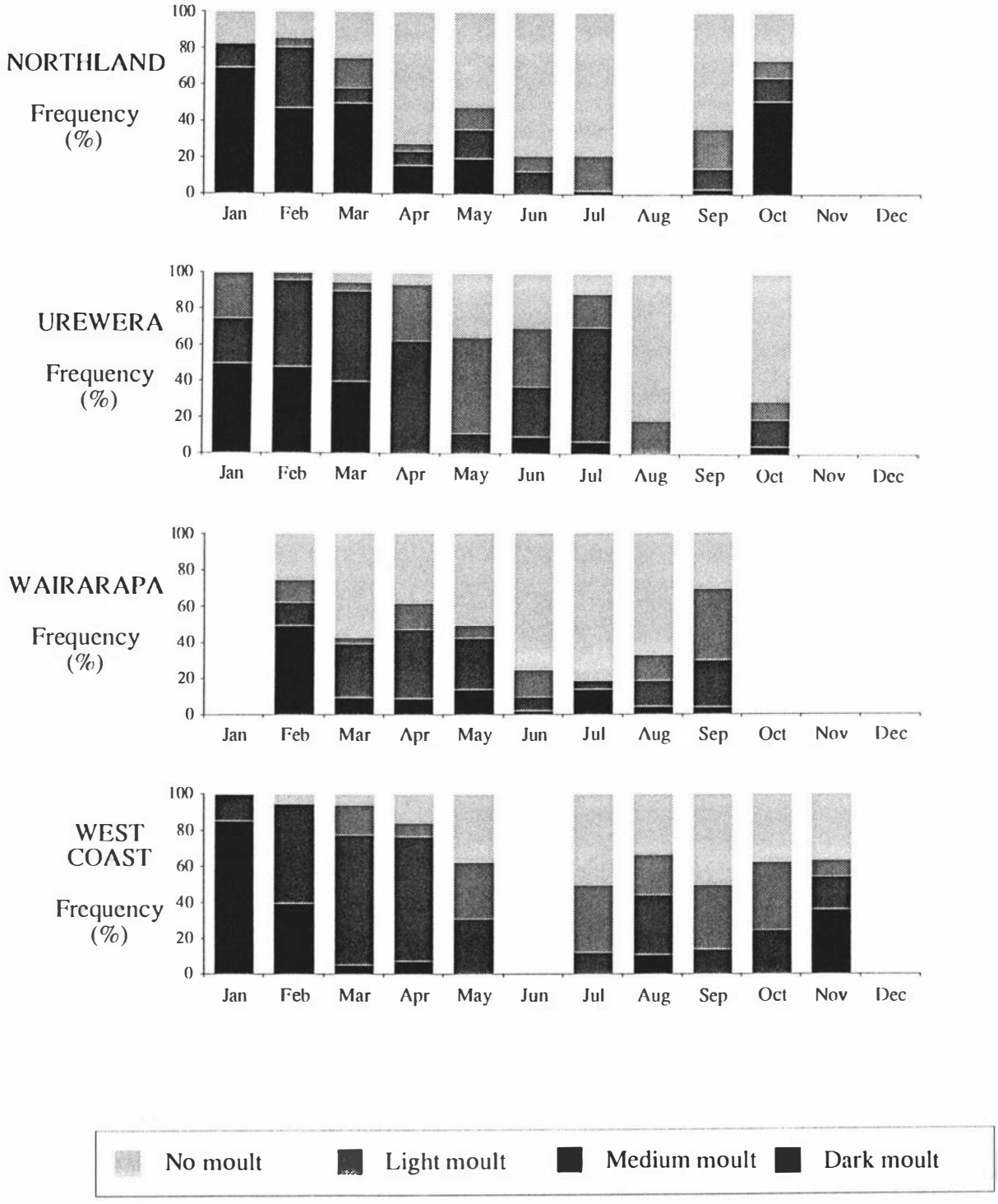

Figure 6.6 Frequency of moult patterns in four locations: females. Moult patterns of adult female possums are classed on a four point scale from heavily pigmented to no pigment (as in table 6.2). Months of different years are combined to allow comparison of moult in different locations

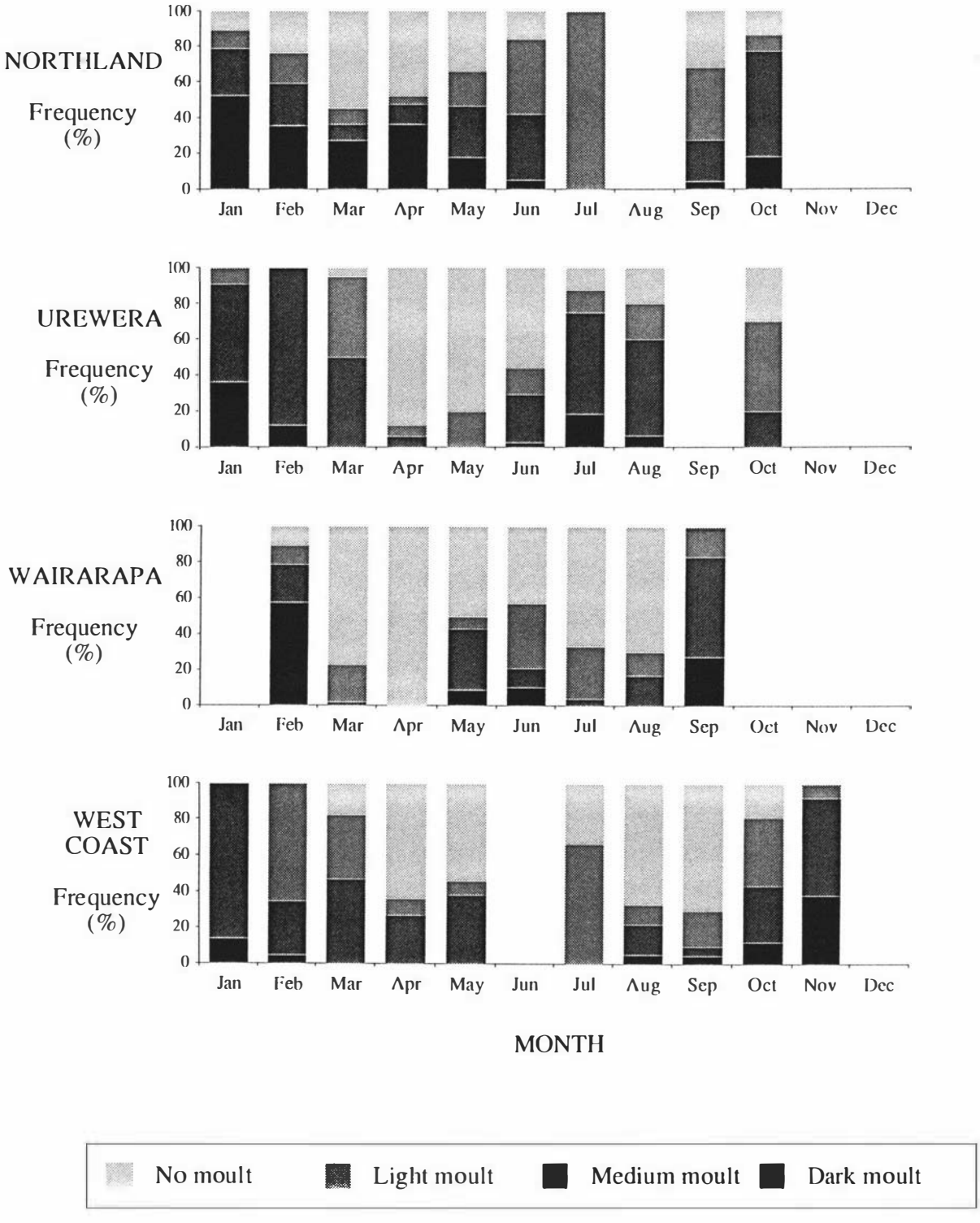

Figure 6.7 Frequency of moult patterns in four locations: males. Moult patterns of adult male possums are classed on a four point scale from heavily pigmented to no pigment (as in table 6.2). Months of different years are combined to allow comparison of moult in different locations

The same analysis showed month x sex ($P<0.001$), and location x sex ($P<0.001$) interactions, indicating sex differences in the proportions moulting in different locations and months. Separate analyses were therefore performed for each sex. Month x location ($P<0.01$) and month x year ($P<0.001$) interactions were significant for males, and month x location for females ($P<0.001$). Moulting could therefore have occurred at different times in different locations, but because of unbalanced data the effects of location, month and year can not be distinguished. Analysis of data from pelts of males demonstrated moulting in the two age classes occurred at different times ($P<0.001$), as described above. But for females, month x age was non-significant. Juvenile females showed higher proportion of moult than adult females, but there was greater similarity of seasonal variation between age groups than in males.

6.3.7 Effects of lactation

The proportion of lactating females sampled in each month indicated the time of breeding. During autumn, lactation increased from less than 40 percent of pelts to 70 percent or more, in all four locations. This increase occurred in April in Northland and Wairarapa, and May in Urewera and West Coast. In all locations except West Coast, the proportion lactating remained above 50 percent until at least October. Only in West Coast for the months of February and March were samples with no lactating females collected.

Moulting in lactating and non-lactating (dry) female possums from all locations is compared in figure 6.8. Both groups moulted in summer but dry females had generally darker moult in winter and spring. Using log-linear analysis, the moult x month x lactation effect was significant ($P=0.005$), indicating differences in the time of moulting in different regions of the two lactational status groups.

Generalized linear models were fitted using proportion of moult (section 6.3.4) as dependent variable and lactation, age, location, month, and year as explanatory variables. The month x lactation interaction indicated that the proportion of moult varied differently between lactating and dry states, in different months ($P<0.001$). Overall, 50 percent of lactating possums had moult pigmentation, compared with 84 percent of dry possums. Much of the variation contributing to a significant location x lactation effect ($P<0.001$) arose from the

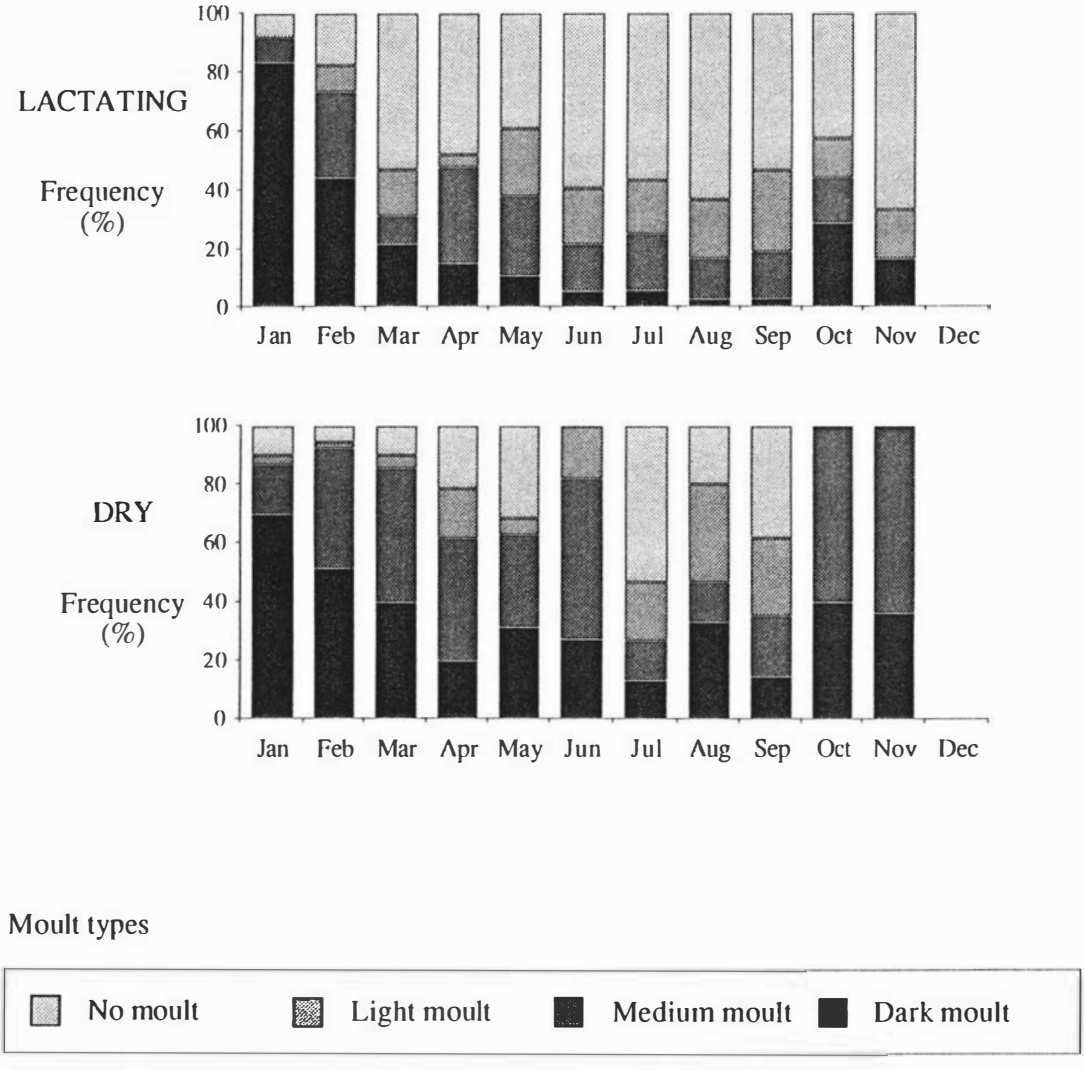

Figure 6.8 Moult patterns in pelts from female possums of differing physiological state.

differences between Urewera and the other locations. In Urewera, a higher proportion of all females exhibited moult, and dry females in particular (97 percent). The lactation x age interaction was non-significant, i.e. even though juveniles were generally dry and adults lactating (overall, 13 percent of juveniles lactating compared with 73 percent in adults), moult varied in a similar manner between lactating and dry possums within each age class.

6.3.8 Patchy fur growth

In addition to spontaneous seasonal hair growth occurring in the diffuse patterns described thus far, many possum pelts exhibited hair growth resulting from damage. Such patches can be seen on the flesh side of pelts as irregularly shaped, evenly coloured, clearly defined areas of melanin pigmentation also known as "ink spots". These are illustrated in plate 2. This form of growth associated pigmentation often appeared as a network of lines, particularly in the anterior region of the pelt. Corresponding to pigment patches was even length, growing fur, as shown in plates 3 and 4. Only 3.7 percent of possums pelts examined were completely devoid of such patches. In some individuals, (classed as having "extensive" patches) this form of hair growth was subjectively estimated to extend over at least half the area of the pelt.

The incidence of patchy fur growth amongst all possums is shown in figure 6.9. At all times of the year, most possums had some fur growing in discrete patches, but they were most prevalent in May (89 percent). Generalized linear models were fitted to the data with location, sex, age, month and year as main effects on the proportion of pelts with patches covering more than 5 cm^2.

The month x region interaction was significant ($P<0.001$), even though all locations showed the highest proportion of patchy growth in May (or June for Urewera) and lowest in October or September. West Coast differed from more northerly locations in having few pelts with patches in the spring months and all pelts with patches in January. This variation contributed to the result, but may have been due in part to the difficulty of scoring January pelts with much diffuse hair growth ("moult").

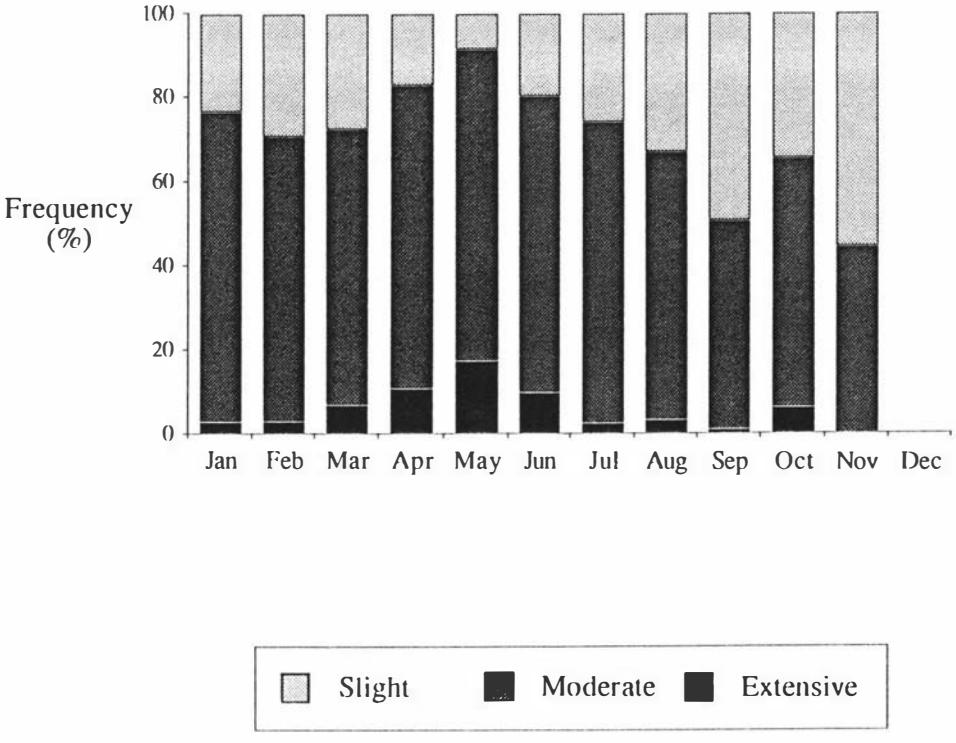

Figure 6.9 Seasonal occurrence of patches of fur regrowth in possum pelts.

Juveniles exhibited patches at different times from adults ($P<0.001$). Proportions with patches were generally lower in juveniles (67 percent over the whole year compared with 74 percent in adults). The highest values occurred in June for juveniles and in May for Adults. However, the month of lowest proportion with patches was October for juveniles (33 percent); a time when adults showed a minor peak (65 percent).

The proportion of pelts with patches varied differently between sexes over time ($P<0.01$). Both sexes showed greatest patchy growth in May (86 percent of pelts for females, and 91 percent for males). However, proportions of pelts with patches were higher in females, during the spring months. Patchiness also varied differently between sexes over different locations ($P<0.01$). The proportion of pelts with patches was similar for both sexes in Urewera and West Coast, but in Northland and Wairarapa fewer females had patches. Patches of regrowth occurred more frequently in adult males than in females or juvenile males ($P<0.05$).

Overall, the occurrence of discrete patches of regrowing fur summarized in figure 6.9 was influenced by many factors not shown by the graph. However, analyses showing many significant interactions involving month strongly suggest the existence of a seasonal pattern in the data.

6.3.9 Wounds

Small wounds were observed on some possum pelts. These were typically 2 to 4 cm long, "C" or hook shaped, red marks. They could be seen from both sides of the pelt, but were most visible on the flesh side. The incidence of such marks amongst possums of all locations, and both age and sex classes is shown in figure 6.10. A seasonal pattern is apparent in the three northernmost locations, with a main peak occurring about March or April. Generalized linear models were fitted, with proportion of pelts with wounds as dependent variable and age sex, location, month, and year as explanatory variables. The month x location interaction was highly significant ($P<0.001$). The proportion of pelts with wounds was generally high in Northland (31 percent of all pelts), and low in West Coast (4 percent of all pelts). A significant age x sex interaction ($P<0.01$) is attributable to similar proportions with wounds amongst adult and

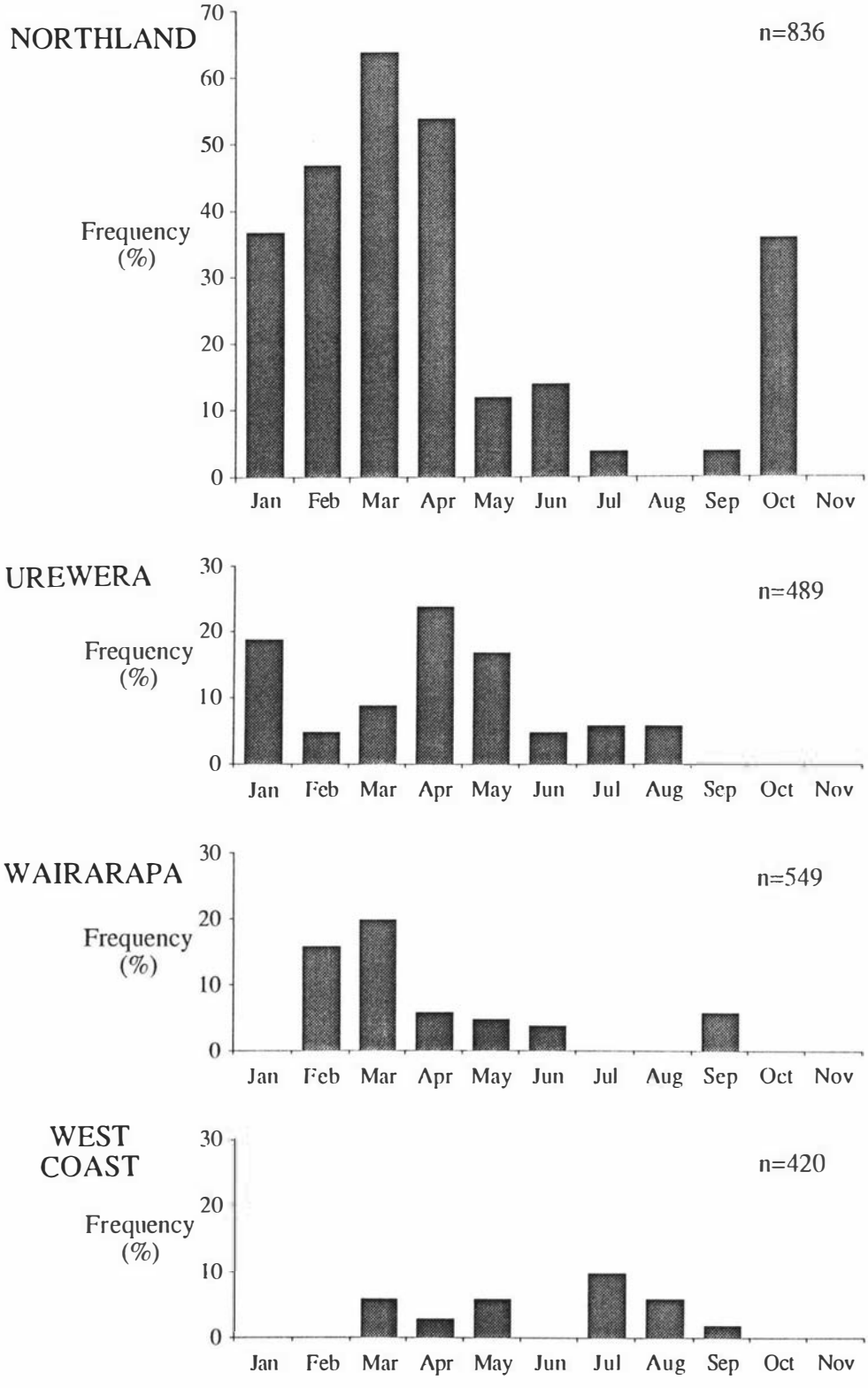

Figure 6.10 Seasonal occurrence of wounds in possum pelts.

juvenile males, while amongst females, proportions were higher in adults and lower in juveniles. The month x year interaction ($P<0.001$) showed some significant variation due to seasonal, annual or regional effects, but because of unbalanced data, this could not be resolved. Month x sex, month x age, location x sex, and location x age interactions were all non-significant, indicating that the proportion of pelts with wounds varied consistently in different times or places, amongst age groups or sexes.

6.3.10 Fur grading

Table 6.4 summarizes the relative frequency of grades assigned to survey pelts by a professional fur-grader prior to auction. "Summer" pelts are those collected from February to April, while "winter" pelts are collected from June to September. (Note that tabulated values are percentages, and therefore zeros do not necessarily denote complete absence of pelts.)

Table 6.4 Relationship of grading to location and season. Grades are given by size and quality (e.g. A pelt graded "large 3" is a larger but poorer quality pelt than one graded "medium 1"). Values are percent of pelts from each location/season.

Location/	Large			Medium/large			Medium		Small		
season	1	2	3	1	2	3	1	2/3	1/2/3	4th	Inferior
Northland											
summer	0	1	4	0	2	32	0	25	16	19	0
winter	1	1	1	2	10	36	1	18	10	18	2
Urewera											
summer	8	22	14	0	2	18	0	12	6	18	0
winter	13	22	20	0	7	16	0	5	2	15	1
Wairarapa											
summer	3	10	18	2	16	27	0	15	5	6	0
winter	3	10	10	6	20	28	0	14	2	7	0
West Coast											
summer	2	14	26	1	5	24	0	10	5	14	1
winter	0	7	7	1	9	30	0	15	4	26	2

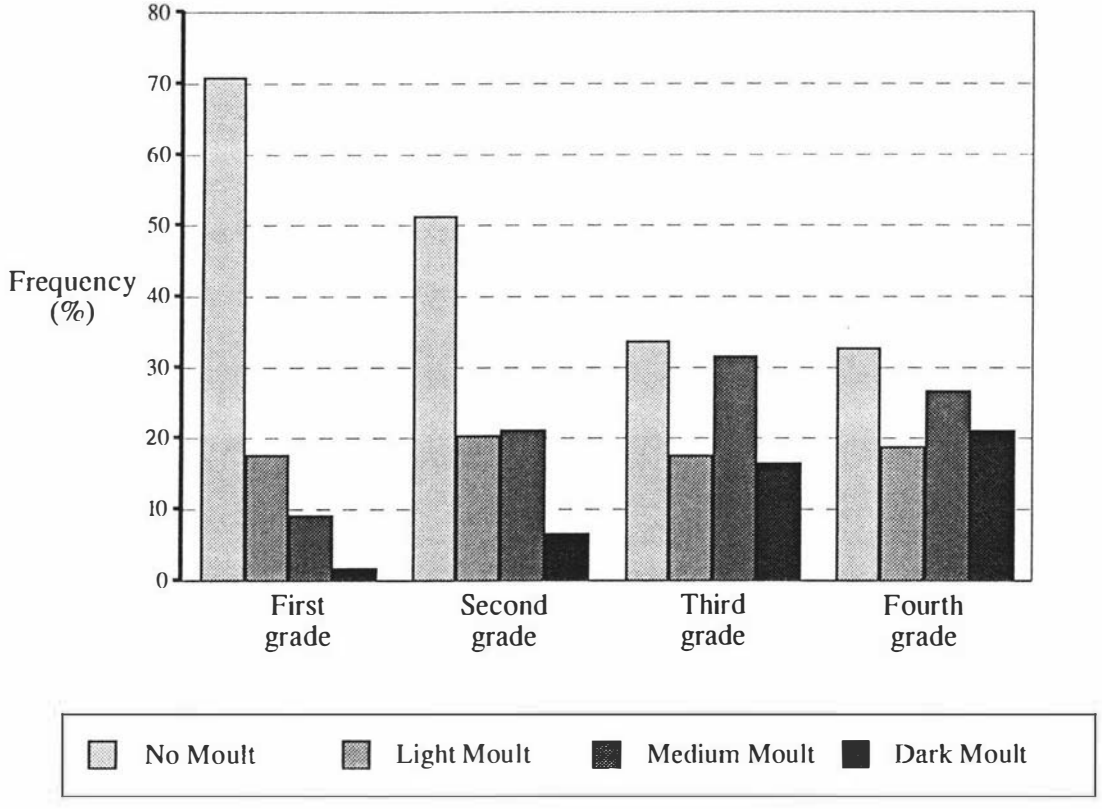

Figure 6.11 Moult categories and grade. Moult patterns are classed on a four point scale from heavily pigmented to no pigment (as in table 6.2). Grades are those assigned prior to sale of the pelts.

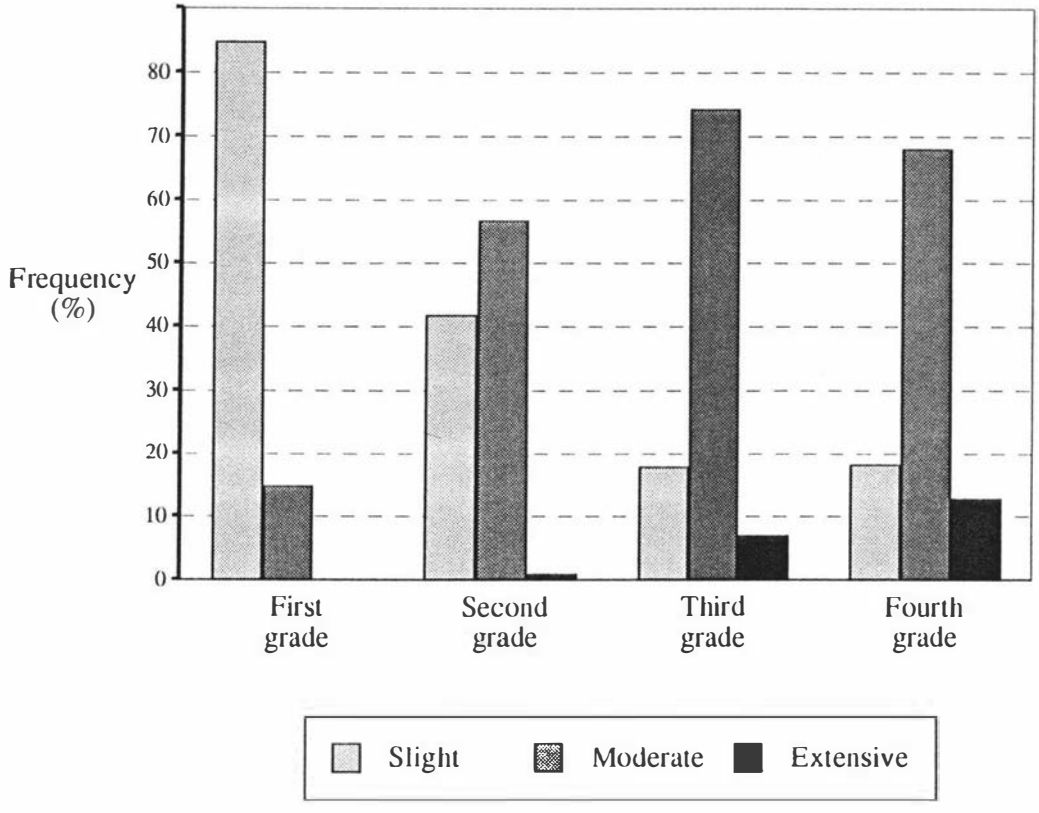

Figure 6.12 Regrowth patches and grade. Growth patches are scored on a four point scale (table 6.1). Grades are those assigned prior to sale of the pelts.

Statistical analysis involving grade was hampered because quality grades of smaller pelts are lumped together, as are sizes of fourth grade pelts (see table 6.4). Log-linear analysis to test location and season effects was inconclusive, giving a significant three term (grade x location x season) interaction ($P<0.001$). A generalized linear model was fitted treating medium 2/3 grade as an average 2.5, and small grade as an average 2.0. No significant difference between summer and winter pelts was evident using both age classes, but when juveniles were excluded (removing many small and medium sized pelts) the result showed pelts obtained in the winter trapping season were graded higher than those obtained in summer ($P<0.05$). However, these results should be treated with caution, because high grades were less frequent than lower grades amongst larger pelts, suggesting that the average values estimated for smaller sizes were not valid.

The relationship between grade quality (irrespective of size) and moult category is illustrated in figure 6.11, and grade quality and patchy growth in figure 6.12. Small and medium sizes were excluded in order to show all four grades. Log-linear analysis using grade, patches, and moult (four categories as in section 6.3.4) showed all two term interactions were highly significant ($P<0.001$). That is, pelts with extensive patches tended to have slight moult pigmentation. This relationship was reflected in the analysis of the timing of the two forms of growth, reported above. Also, higher grade pelts tended to have less, and lower grade pelts more hair growth of both kinds, as is apparent from the graphs. Nevertheless, grade was not readily predicted from pelts with slight moult. Many pelts with slight moult ("prime") were downgraded due to patches, or other defects such as worn and weak fur, bullet holes, stained fur, and "windows" (areas of plucked fur).

6.4 Discussion

This study provides the first description of moult patterns in a marsupial, and shows attributes not commonly seen in eutherian mammals. In brushtail possums, spontaneous (seasonal) hair growth is diffuse and highly variable in timing. There is a poorly defined summer growth season, together with much regrowth of fur damage in patches. These features should be interpreted not

only in the light of existing views of seasonal adaptive coat changes in mammals (Ling 1970, Johnson 1977b, 1984) but also taking into account developmental and histological studies of hair follicle activity in possums (chapters four and five, Lyne 1970a).

6.4.1 Topographic pattern

The comparatively uniform skin pigmentation patterns together with gradual, even replacement of colour in dyed possums indicate that hair growth is diffuse, and does not progress in wave-like fashion as in many mammals (Ling 1970, Johnson 1972). Dyeing of domestic rabbits in a manner similar to that described in section 6.2.2, provided a graphic illustration of the contrasting topographic moult patterns occurring in the possum and a eutherian of similar size (appendix II, plates 17 and 18).

However, patterns superficially similar to those reported here for possums have been observed amongst eutherian mammals. How then, do the moult patterns in possums compare with these examples of diffuse moulting?

In so called "diffuse" moults of some other species, e.g. muskrats (Linde 1963) and water rats (Olsen 1980), follicle activity occurs in irregular patches or islands within the skin. Diffuse growth in possums is a fine mosaic with adjacent follicles at different stages of growth. More commonly though, diffuse moulting in eutherian mammals alternates seasonally with wave moulting, or occurs as juvenile or old age moults in species otherwise exhibiting seasonal wave moulting (e.g. Kryltzov 1964, Morejohn and Howard 1956, Ling 1970, Olsen 1980). In the case of the possum, and other marsupials, non diffuse patterns have not been observed.

More significantly, all hair growth patterns (both "wave" and "non-wave") have long been interpreted in terms of coordination between hair follicle cycles occurring in a definitive number of mature follicles (e.g. Chase and Eaton 1959, Johnson 1977b). In non-wave patterns, cycles of follicle activity may be either fully synchronized,or conversely, out of phase to give mosaic or diffuse patterns. For example, Baker (1974) reported that dyed cats regrew hair uniformly throughout their pelage, and that follicles showed a local mosaic pattern of activity. In the guinea-pig also, adjacent follicles appear out of phase (Dawson 1930, Bosse 1965). However, in both cases a degree of synchronization of follicle activity has been shown (Jackson and Ebling 1972, Ryder 1976). Hair

growth or "moult" in the possum differs fundamentally from these other types of diffuse moulting, even though the resulting pattern of follicle activity may be similarly uniform over the skin surface (non-wave pattern). The fibre growth manifested as pigmentation patterns on pelts represents not only regenerative hair cycles, but also *de novo* formation of hair follicles by branching within compound follicles (chapter five).

6.4.2 Timing and variability of diffuse fur growth

The analysis of moult over time was not simple, with interactions of several factors contributing to variation. However, there was strong evidence of real seasonal effects. Within each population sampled, a higher proportion of darkly pigmented skins were observed in summer than at other times of the year, and there was evidence of a subsidiary peak in winter for males in all locations and females in Urewera (figures 6.6 and 6.7). Although samples of pelts were missing for the month of December, it can be assumed that most pelts had comparatively high levels of growth at this time since trappers were avoiding dark, reputedly poor quality, pelts (B. Mercer, C Ritchie pers. comm.). Skin samples from possums taken from Keebles Bush also showed highest activity at this time (figure 6.3).

A wide range of patterns occurred over much of the year (figures 6.5 to 6.7), indicating that the timing of hair growth was highly variable between individual possums, as was also observed in captive possums (section 5.3.3). Seasons of "moult" (hair growth) and "prime" (completed fur growth and quiescent follicles) are thus less well defined than in many northern hemisphere eutherian fur bearers (e.g. Flux 1970, Hewson and Watson 1979, Maurel *et al* 1986), which exhibit marked seasonal changes in pelage density and, in some cases, colour. The timing of moult in some other mammals from less extreme climes may be as variable as in possums, but detail of variability is lacking in many accounts. In the Australian water rat (*Hydromys chrysogaster*) moulting individuals were found in all months of the year (Olsen 1980). Nutria (*Myocastor coypus*), a native of South America, are also reported to moult throughout the year (Pastirnac and Gruia 1987).

The timing and variability of moult in possums suggests that hair growth is not cued primarily by photoperiod as shown in many mammals (Ebling and Hale 1970) but probably also involves other factors, such as body growth, reproductive status and nutrition.

A high proportion of juveniles showed hair growth until the month of June. Given an April-May peak in births, many of these animals would be approximately 14 months of age at this time. These events could be interpreted as a "juvenile" or "subadult" moult, such as seen in many mammals. However, more detailed examination of this hair growth over time in captive juveniles (section 4.11) demonstrated that it is not comparable with cases in which a separate juvenile coat is grown and completely replaced with adult pelage (e.g. Kryltzov 1964, appendix II). Central primary follicles may be involved in replacement cycles, but much of the hair growth represented by pigmentation in juvenile possum pelts is part of the process of follicle formation which continues into adult life. It must also be remembered that assessment of age was subjective, and that in spring each year a new cohort of young become independent and juveniles of the previous year join the adult class. Variation in moult associated with age at this time could therefore be an artifact.

Lactating females were present in all monthly samples (except from West Coast) and increased markedly in autumn, indicating that while the main birth peak occurred about this time, some births were distributed over most of the year (in the three northernmost locations at least). Earlier studies of breeding have also shown that births peak in autumn, but depending on location can occur at almost any time of the year (e.g. Bell 1981, Kerle 1984). Hair growth manifested as skin pigmentation of surveyed pelts similarly occurred throughout the year, with a nadir in the proportion of pigmented pelts at about the time of breeding (for males) or later in winter (for females). Non-lactating females tended to have darker pelts than lactating females. From this survey data it is not possible to determine cause and effect, however endocrine control mechanisms, such as those proposed by Khateeb and Johnson (1971c) and Johnson (1981) could be involved in the association between reproduction and hair growth.

Kerle (1984) related time of birth of young possums to plant growth index, and suggested that nutrition is the most important proximal factor controlling seasonal distribution of births. Moulting may likewise be influenced by nutrition, either through similar physiological pathways, or by other effects of nutrition on metabolism and growth. Such an effect would explain the variation in moult between individuals, and between locations, if food availability and quality varied both within and between locations. This contention is supported

by anecdotal evidence from trappers. Experienced trappers move trap sets between sites of differing vegetation through the season to obtain optimum proportions of pelts in "prime" or no moult condition (V. Comer pers comm.). The trapper from Urewera further supported this claim with small samples of pelts taken on farmland at about the same latitude as the samples from beech/podocarp forest. The 22 farmland pelts, taken in the months of January and June to September, were generally exhibited more hair growth than those from forested areas at the same times.

6.4.3 Patchy hair growth

In addition to diffuse seasonal hair growth much hair growth observed in possum pelts occurs in discrete patches. Characteristically shaped wounds were also present, which are interpreted by trappers and fur graders as claw marks. Such wounds induce hair growth patches of the same shape, but the majority of growth patches were not attributable to wounds and presented no corresponding damage to the skin itself.

Both patchy fur growth and wounding had a seasonal distribution which coincides with breeding activity. The incidence of patchy growth increased from March to a maximum in May (figure 6.9), while the incidence of wounds was highest in March or April (figure 6.10). The incidence of lactation indicated that birth peaks occurred in April or May, as generally reported in previous studies (e.g. Crawley 1973, Kean 1975, Bell 1981). Since the gestation period is about 16 to 18 days, the appearance of new patchy hair growth coincides with the approximate time of mating activity. It seems probable therefore that patchy hair growth results from the raking of skin and fur during intraspecific contacts associated with mating, territoriality or other social interactions. The wounds too, are testimony to physical conflict.

Fur growth by this means occurs at times when seasonal growth is at a minimum (compare figures 6.5, 6.6 and 6.9). Hair replacement by the regrowth of plucked patches may thus be an important annual event in possums. Although fur damage has been noted in similar studies of other species (Linde 1963, Stodart 1965, Olsen 1980), the ease with which possum fur can be plucked is a peculiarity not noted in other mammals. The possum therefore appears predisposed to loss and subsequent regrowth of large amounts of fur by "accidental" epilation. Furthermore, patchy growth probably accounts for a very

high proportion of annual hair production in many individuals, given the low levels of follicle activity (usually less than 30 percent) associated with spontaneous growth.

6.4.4 Fur growth and pelt grading

Despite confounding effects such as differing pelt presentation and many types of faults, there was a clear relationship between grading and the two forms of hair growth. This is not surprising when it is considered that skin pigmentation is a major criterion used in grading. The issue of greater importance is the correlation between pigment in growing hair follicles and fur quality.

The pelts of most fur bearers are presented fur side out and graded for sale in a multi-step process (Joergensen 1985). However, the majority of possum pelts are graded in New Zealand with at most a cursory inspection of the fibres. Possum is one of the few remaining furs graded by assessment of pigmentation of the flesh-side (R. Grimes pers. comm.). The suitability of this continuing practice has been called into question by Dellow *et al* (1985), who had the opportunity to compare grading of pelts before and after they were dressed. They concluded on the basis of a small sample of pelts that the flesh side colour of dried pelts was not a good predictor of the grades assigned after tanning, and by implication, their true quality and usefulness in garment manufacture.

There is an historical explanation for flesh side grading of possum pelts. Moult patterns were used for grading rabbits prior to their decommercialization in 1957, and until recently, many of the small number of possum fur graders in New Zealand had earlier experience with rabbit fur. The principle of using moult patterns was therefore incorporated into the present possum grading system, and appeared to provide a quick and simple to use criterion of fur quality. It may be that there is a correlation between pigmentation and poor fur quality due to worn or growing fibres, especially guard hairs. However, this relationship has not been objectively determined.

It is acknowledged by some that moult patterns in possums do not always correlate with fur quality (D. Campbell, pers. comm.). This is borne out by the gradings of raw pelts observed during this study. In many pelts, the flesh side pigment was absent, but fur was of poor quality, being weak, worn and thin. Some instances of highly graded pelts with dark pigmentation were also noted

(figure 6.11). However, the very darkest pelts generally had poor fur. It was similarly found that lack of pigment does not always correspond with prime condition in muskrats (Linde 1963).

The comments of Dellow *et al* (1985) may therefore be valid with regard to diffuse pigmentation and seasonal hair growth. On the other hand, dark patches in pelts resulting from regrowth of plucked fur ("ink spots"), always correspond to patches of growing fur which devalues the pelt (see plates 3 and 4). If the pelt is used for furrier work, such defects must be painstakingly repaired. In cases where the area of regrowing fur is small, narrow in shape, or the fibre almost fully regrown, the fault may be less noticeable and especially difficult to detect in dressed pelts from which flesh side pigmentation is removed. Nevertheless, the defect is still present in the fur. Grading by examination of the flesh side of the pelt for "ink spots" therefore serves the purpose of detecting common, serious faults peculiar to possum pelts which are later obscured by dressing.

A larger objective study examining the relationships between skin pigmentation due to the two forms of hair growth, grading before and after tanning, and pelt quality as determined by manufacturers would be useful to direct improvements in the grading system presently used. This might not be warranted for the comparatively low value and small volumes of wild pelts used for furrier work, but would be important should farmed pelts of high fur quality be marketed in larger quantities.

In summary, skin samples from wild possums captured in the Manawatu district showed that the darkness of flesh side pigmentation is correlated with follicle activity. As with the captive study (chapter five) less than half of the follicles were active at one time. Regular sampling of pelts from four widely separated and environmentally diverse locations showed that maximum fur growth (manifested as pigment patterns) occurred most frequently over summer, but also as reported in chapter five, high individual variation in the timing of hair growth was observed in all locations. It is therefore reasonable to conclude that the unusual findings of investigations using small numbers of captive possums are broadly representative of hair growth and replacement occurring in wild possum populations in New Zealand.

Chapter 7
SUMMARY AND GENERAL DISCUSSION

7.1 Summary of seasonal hair growth patterns in possums

Current concepts of hair follicle development and patterns of follicle activity are founded on studies of eutherian mammals, mostly laboratory rodents, and northern hemisphere ungulates, carnivores and lagomorphs. In this study, seasonal hair growth and replacement in a marsupial - the brushtail possum - was found to differ fundamentally in several respects from that described in eutherians. Replacement of fibres does not occur on an annual, biannual or continuous basis as reported in many species (Ling 1970). Nor can hair growth in possums be classified simply by the pattern of synchronization of follicle cycles in the manner proposed by Ebling (1965a) and Johnson (1972). Rather, the processes of follicle development and cyclic replacement are much less distinct in possums. Both processes, together with regrowth of damaged patches, are important in the total hair growth of sexually mature members of the species.

This combination of mechanisms is best explained by beginning with the development of hair follicles and pelage. The sequence of development of follicle types and groups, briefly examined in this study, conformed to that described by Lyne (1970a). The earlier formed primary central follicles produce monotrichs and guard hairs in the adult (section 4.6). Lateral bundles (compound follicles) develop from lateral primary and secondary follicles and grow mostly underfur. In two female possums, (section 4.11) full length fur was grown by about 30 weeks of age, marking the end of a growth cycle in primary central follicles. Development of compound follicles was most rapid up until about 40 weeks, but derived follicles continued to form in adult life. Increases in follicle group size were similarly observed in other adult captive possums (section 5.3.4). This type of neogenesis is unusual. Previous studies of other species have lead to the conclusion that follicle initiation generally ceases in late embryonic or early post-natal life, and moulting of adult pelage in most species involves a definitive complement of follicles (Billingham 1958, Mann 1962, Lyne and Brook 1964). Lyne (1957a) recognized the same phenomenon in

another marsupial, the bandicoot, but it has not been described in other mammals.

The discovery of follicle neogenesis in possums suggested that much of the total fibre growth observed in adult possum skin is not attributable to replacement of old hairs in existing follicles but fibre growth in newly formed derived follicles. Furthermore, it was found that total levels of seasonal hair follicle activity were very low in comparison with those previously reported in other mammals (section 5.3.3). Follicle activity at the mid dorso-lateral site was not observed to exceed 40 percent anagen in captive possums, or 45 percent in the wild population (section 6.3.3). It was calculated that amongst six possums (eight possum-years), no more than 48 percent of the initial follicle/fibre population was replaced over one year, with an average (± S.E.) of 12.8 ± 1.0 percent.

Hence, there are two types of seasonal hair growth in possums, which tend to be associated with different follicle types. Normal replacement cycles occur in central primary follicles, renewing guard hairs, whilst branching to form new derived follicles results in the growth of underfur. The proportion of total fur growth attributable to replacement cycles ranged from 0 to 100 percent (section 5.3.7). In older animals with proportionally lower weight gains, the proportion of replacement was higher, indicating a body growth related change in partitioning of the two types of hair production (as discussed in section 5.4.1). Both processes occur in a fine mosaic throughout the skin, producing a diffuse topographic pattern. This was evident in the growth related pigmentation patterns observed in possum pelts (section 6.3.1), and demonstrated by dyeing the fur of live possums (section 6.3.2).

Given the low amount of growth and replacement, pelage changes might be expected to be gradual in possums. This conclusion was supported by measurements of coat length (section 5.3.5) and fibre diameter (section 4.7), and subjective observation of coat structure. The gradual nature of fibre replacement was demonstrated clearly by the many dyed fibres which were retained in the coat for at least 21 months (section 6.3.2).

The times of diffuse growth varied greatly between individuals in the captive study (section 5.3.3) and similar large individual variation in fur growth was evident in surveyed pelts (section 6.3.5), and skin samples from wild possums (section 6.3.3). Generally, maximum hair growth occurred over the

late spring-summer period, but some hair growth occurred in some possums throughout the year. Males and females differed significantly in their seasonal patterns of growth. Higher proportions of juveniles than adults showed hair growth manifested by pelt pigmentation. Lactating females showed less growth than non-lactating females (section 6.3.7). Thus some of the high variability is associated with differing physiological and metabolic states, as discussed below.

Within a population, no differences were found between fur colour phenotypes (grey and brown) in either total proportion to pelts showing diffuse hair growth, timing of hair growth, (section 6.3.6), or length of fur (section 4.5). The most parsimonious interpretation is that there is a simple genetic basis for colour, as in other mammals (Searle 1968, Silvers 1979), even though there are between population differences in gene frequencies for this character and others, related to ancestry (Triggs 1987).

In addition to the two forms of diffuse growth, possums commonly grow fur by a third means. Patches of fur growth result from physical damage, incurred especially at the time of breeding. Fur loss and regrowth in this manner is another unusual feature of possum pelage. When fibres are plucked, some cells of the external root sheath are removed with the club end (section 4.4). However, the dermal papilla and epithelium which are necessary for regeneration (Oliver and Jahoda 1989) remain in the skin. Loss of fur in this manner is a regulated response, since the force required to pluck fibres can be reversibly manipulated by stress or pharmacological means (section 4.4). An unusually large number of mast cells were found in close association with hair follicles (section 4.4). It is possible that this cell type has a role in the regulation mechanism. Mast cells are known to synthesize and release many compounds having marked effects on skin physiology (Junqueira and Carneiro 1980). Mast cells and their products have also been associated with spontaneous hair growth cycles (Moretti *et al* 1969). Further histological investigation to determine conditions corresponding to mast cell degranulation could help to explain this phenomenon.

Coat replacement by regrowth of plucked patches certainly exceeded that occurring by diffuse growth in some of the possums observed in the course of the pelt survey. Furthermore, the two forms of growth predominated at different times of the year. Diffuse seasonal growth was generally initiated in spring and involved little replacement, whereas loss and renewal of fur in patches attained a

peak in autumn (section 6.3.8). It is therefore tempting to speculate that the two forms of growth are in some way adaptively interrelated. Patchy renewal of fur might be a means of complementing limited seasonal replacement, or alternatively, lower seasonal replacement could be a response to patchy loss of fur which has arisen as a result of some other selection pressure. There is a popular notion that the ease with which possums may be plucked is an adaptation to predation (Moresby 1984). This could be termed "the lizard's tail hypothesis", in reference to the analogy often made by possum trappers. However such a proposition is difficult to test. It should be borne in mind that possums are nocturnal and arboreal, and are therefore less exposed to predation than many other mammals which have not evolved such an adaptation.

How general is this set of hair growth characteristics amongst marsupials? From the limited available evidence, it would appear that the diffuse pattern is a characteristic of marsupials (section 4.2). Moult patterns in tammar wallabies (*Macropus eugenii*) are reputed to be similar to those of possums (N. Masters, D. Campbell pers. comm.). Neogenesis possibly occurs in other marsupials (section 5.4.1), however, in view of the seasonal coat change related to thermoregulation reported in kangaroos (Dawson and Brown 1970), it seems that the capacity for annual replacement of at least some of the coat exists in macropods. Patches of fur growth were noted in other possum species (*Trichosurus caninus* and *Pseudocheirus perigrinus*) (section 4.2), but they are not conspicuous in wallaby pelts (N. Masters pers. comm.), and readily plucked fur has not been reported in other marsupials.

Several features of hair growth in the brushtail possum make this species a potentially useful model for understanding the general mechanisms of follicle initiation and shedding, which are of both economic and theoretical significance (Nagorcka and Mooney 1985, 1989). The entire sequence of follicle development in possums occurs in post-natal life (Lyne 1970a), making the process much more accessible to experimentation than in other commercial fibre producing species. An insight into cellular interactions which underlie continued follicle initiation in adult possums could contribute to an understanding of follicle density in other species. This is of interest since follicle density and group composition is relevant to both wool production in sheep (Ryder and Stephenson 1968) and pelt quality in fur bearers (Keiji *et al* 1988).

7.2 Control of hair growth in relation to reproductive physiology in possums

Hair replacement is important to survival in many mammals. Accurately timed and reliable modification of pelage colour, insulation or reflective properties may be required to meet seasonal environmental changes. Reproductive strategies too may depend on an animal's ability to measure some environmental cue - photoperiod in particular - in order to optimise survival of offspring by timing birth and associated nutritional demand to the most favourable time of year. Such strategies have been recognised in marsupials, including brushtail possums (Tyndale-Biscoe 1980, Tyndale-Biscoe and Renfree 1987). Thus, a parallel relationship between breeding and moulting is evident in many cases (Ebling and Hale 1970). Both aspects of biology have been shown to depend on the mechanism involving pineal secretion of melatonin for transduction of the photic message into a hormonal one (e.g. Hoffmann 1981, Lincoln and Ebling 1985), and many hormones influence both reproduction and hair growth (Mohn 1958, Johnson 1981). How then do the patterns of hair growth described in possums relate to other aspects of physiology, breeding in particular?

In the present study, hair growth in possums was seasonal, but highly variable amongst animals exposed to identical conditions of photoperiod and temperature. Possum pelts trapped in the same location also showed marked variability in the proportion with (hair growth related) pigmentation. Similarly, a seasonal pattern in breeding with a peak in births about the time of the autumn equinox, and a high degree of variability, was evident in this study, as in many others (e.g. Gilmore 1969, Crawley 1973, Kean 1975, Bell 1981). Male possums are probably reproductively capable throughout the year (Gilmore 1969), and females are seasonally polyoestrous, although some may breed at almost any time (Pilton and Sharman 1962). Breeding thus occurs widely throughout the year. The pattern of births is reported to vary between years (Bell 1981) and between locations (Kerle 1984), and it has been argued that both nutrition and photoperiod are major proximal cues for onset of breeding.

The question of the role of photoperiod and associated hormonal pathways in reproduction of possums was addressed by Gemmell (1987a). He showed that exogenous melatonin could induce out of season breeding. Three out of

five melatonin treated possums showed an advance in the prenatal rise of progesterone levels and date of birth. Gemmell suggested that in possums, as in other short day breeders, exogenous melatonin overrides the short day response. Gemmell (1987b) also demonstrated altered time of breeding using melatonin in a marsupial long day breeder - the bandicoot. The large individual variation in the timing of hair growth in possums shows that photoperiod is by no means the only regulating factor, but from its limited role in reproduction, melatonin might also be expected to influence the timing of fur growth, as clearly demonstrated in other species (e.g. Allain and Rougeot 1980). The effects of melatonin on fur growth in possums have been explored, although no published information is available. In an experiment in which melatonin implanted and control possums were matched for sex, colour and age, no measurable treatment effects were found on pelt quality and skin pigmentation (follicle activity) (A. Pearson, unpubl. data). Melatonin fed to possums also showed no marked effect on pelt quality (A. Keber pers. comm.). However, dose rate, release profile, and particularly previous exposure to light are all likely to affect the outcome of such experiments (e.g. Hoffmann 1981, Allain *et al* 1981). Further efforts to adequately test the role of photoperiod and melatonin would require many trials with large numbers of animals, especially given the large natural individual variation in fur growth.

It has been suggested that melatonin exerts its influence on hair growth indirectly via other hormones, prolactin in particular (Lincoln and Ebling 1985, Martinet *et al* 1984, Duncan and Goldman 1984b, Rose *et al* 1987). High prolactin levels generally coincide with suppressed hair growth. Hinds and Janssens (1986) showed that plasma prolactin concentrations were initially low in lactating female possums, but became elevated at about 120 days until about 180 days after birth. Non-lactating possums had no consistent pattern, but had generally lower prolactin levels. The lower hair follicle activity and lighter moult patterns found in lactating possums (section 6.3.7) could be associated with the effects of prolactin, as shown in other species. However, caution must be exercised when extrapolating from strongly photosensitive species such as mink to the apparently weakly seasonal possum.

Fluctuations in androgen levels of possums measured by Curlewis and Stone (1985) were not related to breeding season. But Gemmell *et al* (1986) found that testosterone concentration increased prior to the start of the breeding

season with a peak in March, then declined to a nadir in September. Body weights showed similar seasonal variation, even though animals were kept on a constant diet. These changes were correlated with day length. Although this pattern of secretion mirrors the general trend in hair growth observed in possums, there are no grounds to attribute a direct relationship. Various steroids have been reported to have a range of modulatory effects on hair growth in some species (Mohn 1958, Khateeb and Johnson 1971c), but effects of gonadal steroids have been discounted in others (Rust *et al* 1965). Therefore, while it is possible that they could be involved in the slightly different proportions of males and females moulting at different times, gonadal steriods are probably not responsible for the overall pattern. The pattern and variation in hair growth described in the present study would suggest that treatment effects may be difficult to measure, and modes of action difficult to interpret in any investigation of endocrine control of hair growth in possums.

Nutrition has been identified as an important factor in breeding of possums (Tyndale-Biscoe 1980), as shown by the associations between body condition and reproductive success (Bell 1981) and between breeding times and plant growth index (Kerle 1984). The anecdotal evidence that moult timing and intensity differs in habitats of differing vegetation suggests that hair growth is likewise responsive to nutrition. The notable decline in follicle activity during periods of ill-health and weight loss also supports the view that nutrition, condition and stress have a comparatively large impact on hair growth in this species. Efforts were made in this study to standardize diet and cage conditions, but response to capture and long term confinement were highly variable. Special attention should therefore be given to animal health, diet, feed intake and homogeneity of stock in any future research.

When reviewing regulation of reproduction in possums, Tyndale-Biscoe (1984) pointed out that firm experimental evidence is lacking and that the mechanism by which external cues are translated into physiological responses is poorly understood. Even less well known are the interactions between environmental factors such as daylength and nutrition, and physiological processes such as growth, reproduction and hormone secretion which might operate to control hair growth. The present study gives evidence that these interactions are likely to be complex in possums.

7.3 Implications for commercial possum fur production

Management of wild possums

The exploitation of wild possums for their fur in New Zealand was for many years disorganized and information on seasonal changes in possum fur has been largely in the form of folklore. With the advent of contract hunting for pest control (Morgan and Warburton 1987) and the consideration of sustained yield harvesting (Clout and Barlow 1982), some basic knowledge about the nature and timing of fur growth provided by this study might be of relevance to commercial exploitation of possums.

First, some findings present a challenge to certain beliefs about fur growth in possums held by those in the fur industry. The comparatively low levels of follicle activity, continued formation of new follicles in the skin of adult possums, and resulting low annual replacement of fur described in this study (section 5.3.7) lead to the conclusion that seasonal changes in coat structure are minimal. This is supported by measurements of fibre length (section 5.3.5) and diameter (section 4.7) showing little seasonal variation. Some changes in pelt quality could occur when fur is growing due to uneven fibre length and replacement of guard hair in central primary follicles (section 5.3.3). However, there is no support for the popular view that possums undergo seasonal changes to give a denser coat in winter, and a lighter coat in summer. If this were so, diffuse hair growth would occur in autumn. In fact the converse was found. Both captive possums and surveyed pelts showed spring-summer growth, indicating that autumn would be a time of optimum pelt quality were it not for the occurrence of patchy hair growth at this time. There is therefore a need for a reconsideration of some of the terms and concepts used in the possum fur industry. In particular, notions of "winter" and "summer" furs, the terms "moult" and "prime" (discussed in section 5.4.), and aspects of the grading of possum fur (discussed in section 6.4.4).

Second, the description of timing and variability of two forms of hair growth indicate suitable times for harvesting possum furs and, in general, the knowledge and practices of trappers were verified. Diffuse growth reaches a maximum between October and January. Patches, or "ink spots", are most frequent from April to July. This therefore leaves two periods in the trapping calender when pelts are more likely to be of optimum quality, i.e. February to

March/April and July/August to September. These times approximate those recognised by experienced trappers (e.g. Comer 1984, Marshall 1984), although many work throughout the year except mid summer. Given the observed differences in times of moult and fur damage in subgroups within possum populations, further refinement of hunting strategies would be possible if selective trapping methods were available, eg. taking higher proportions of different sex and age classes at different times. Return on trapping effort might also be enhanced by investigation of the relative importance of the two forms of hair growth according to manufacturers' quality criteria. However, such information might have limited application in the industry because of sociological factors.

Anecdotal accounts of large annual and local variation in possum pelt quality were supported by the variation in hair growth shown within and between locations sampled in this study. Just as with the breeding of this species (Bell 1981), ecological interactions influencing pelt quality are likely to be complex. Not only the environmental factors controlling the timing of seasonal growth, but also characteristics of the possum population such as density and age structure probably affect the average type and quality of pelts harvested. For this reason, local knowledge based on handling of animals is more likely to be of use in a given situation than general principles derived from this study.

Farming of possums for fur

The biological principles relating to the trapping of wild possums also apply to the farming of possums for their fur. "Fur finishing" has been regarded as a step toward true farming, and is an attempt to make use of the special features of hair growth in this species. Wild caught possums are held in cages in order to improve their pelt value. The effectiveness of fur finishing therefore lies not so much in harvesting of pelts at their seasonal prime, but in isolating the animal while patches of damaged fur regrow. Since "priming" in possums is not the result of complete coat change, and there is no clearly defined, synchronized period of prime, animals may be collected over much of the year. However, the greatest advantage would be gained by live capture between March and May, allowing any damaged fur to regrow and preventing blemishes when they appear on many wild skins. In this study (section 5.3.7) the mean

time (± S.E.) required for half of the follicles to regenerate, grow new fibre, and reach telogen following plucking was estimated to be 82 ± 2 days. Guard hairs alone took longer to grow than all fibre types together. Average growth time of longest guard hairs was 99 ± 4 days. Optimal holding time allowing for complete regrowth is therefore likely to be in the order of 100 days. This is in accord with the period suggested by Moresby (1984). Neither median duration of growth nor guard hair growth times were significantly influenced by temperature. There is therefore no evidence that ambient temperature would affect holding time (in so far as animal health is not affected).

Fur finishing has not yet proved to be viable, as an independent, long-term operation, even when attempted on a large scale with road access to large areas of exotic forest. Difficulties with adjustment of large numbers of wild animals to captivity was a major factor in failure of the model venture in the central North Island (A. Pearson *et al* unpubl. report). While fur finishing might not provide an economic bridge to true farming as once hoped, it has provided experience with animal husbandry which would be required for the selection of potential breeding stock with appropriate temperament and fur qualities.

Trials with farming of domesticated possums independently of wild stocks should be designed with an appreciation of the mechanism of hair growth in possums, and the ways in which it differs from other intensively farmed species. The age at pelting is likely to be an important parameter influencing the economic feasibility of possum farming. The brief study of maturation of pelage (section 4.6) suggests wild possums coats reach adult length at about 30 weeks of age, but there is a rapid increase in the number of derived follicles between 30 and 40 weeks. The end of this growth period might represent a suitable harvesting time, since many fibres are newly grown and follicle activity is at a nadir. However, further work relating these changes to body weight, fur density and fibre strength would be required to more precisely define fur maturation and pelting times.

The gradual, diffuse nature of hair growth in possums contrasts with the rapid, synchronized and complete priming of pelage in other species farmed for their fur (e.g. fitch, mink, rabbits). The management system for farmed possums might therefore also be very different from that used for other fur bearers. Pelting times could be much more flexible, and fitted to a variety of breeding programmes. Possum farming might also be integrated with other forms of

intensive agriculture, fitch farming in particular. In this situation the same cages could be used for both species at different times of the year (R. MacGibbon pers. comm.)

Artificial control of hair growth would seem to be an especially valuable management tool, given the unpredictable occurrence of hair growth in possums. However, as discussed above, the control mechanisms appear complex and some initial trials with melatonin were not promising. Hormonal manipulation is therefore unlikely to be possible in the near future. A more effective means of regulating desirable hair growth might be to focus effort on improved animal husbandry, so as to ensure healthy, growing animals, which are well adjusted to life in captivity. Once management techniques have been perfected and a more uniform domesticated stock has been bred, experiments with hormonal manipulation would be more informative.

True farming of possums would allow genetic improvement of stock. Selection for desirable traits could create a distinct quality product, readily distinguished from wild fur, and commanding a premium price. Models for such developments exist within the fur industry. Much effort has gone into breeding and marketing consistent colour varieties of mink and fox (Nef *et al* 1988). Some traits that show variability and could be selected for in possums are: increased pelt size, evenness of fur length over the whole pelt, unusual and desirable colour varieties (e.g. brown, "blue"-grey, golden), and reduced redness of the anterior region (due to skin gland secretions).

Such developments are not favoured at present as the commercial conditions responsible for the initial interest in possum farming no longer exist. At the time of writing, fur prices are depressed, and the wider economic climate does not favour high risk investment. However, these conditions are typically changeable. The feasibility of possum farming ultimately depends on increasing the reproductive rate of the species, and establishing a premium market (Chai 1984). Should conditions favour further development of the use of possums for their fur, a necessary basic understanding of hair growth patterns in the species is available.

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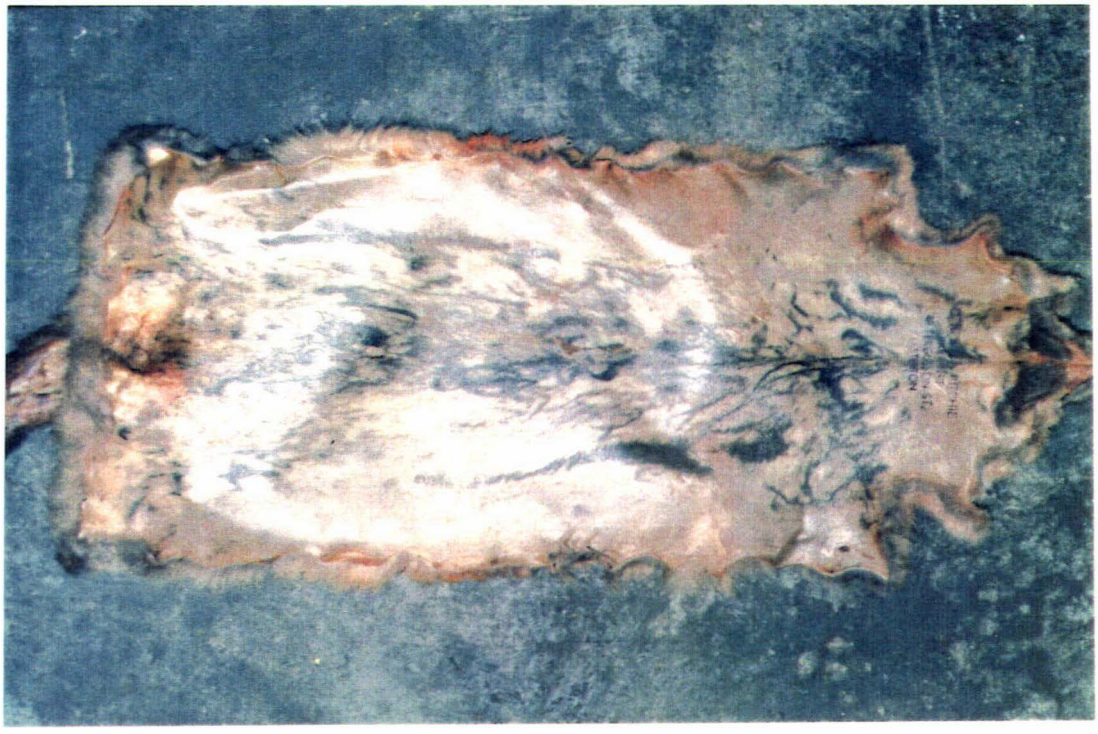
Plate 8 Transverse section of hair follicles in a 146 day old possum. All original follicles have developed. H&E x156.

Plate 9 Transverse section of hair follicles in a 209 day old possum. Derived follicles have developed by branching from original lateral primary and secondary follicles to form lateral bundles. Guard hairs growing from central primary follicles have reached full length. H&E x156.

Plate 10 Transverse section of hair follicles in a 480 day old possum (same individual as plates 7 and 8). Development of derived follicles within bundles has continued in adult life. H&E x156.

Plate 1 Typical melanin pigmentation on the flesh side of a possum pelt, associated with diffuse fur growth.

Plate 2 Typical "ink spots", or melanin pigmentation the flesh side of a possum pelt, associated with discrete patches of fur growth.



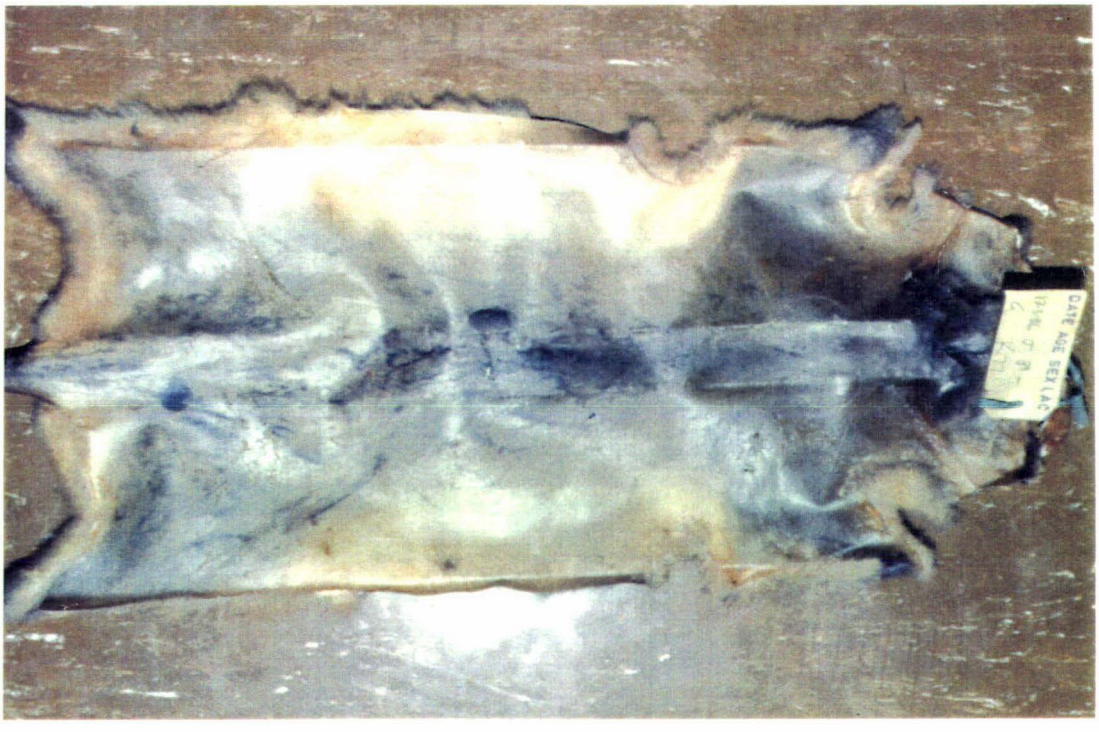

DATE AGE SEX LAC

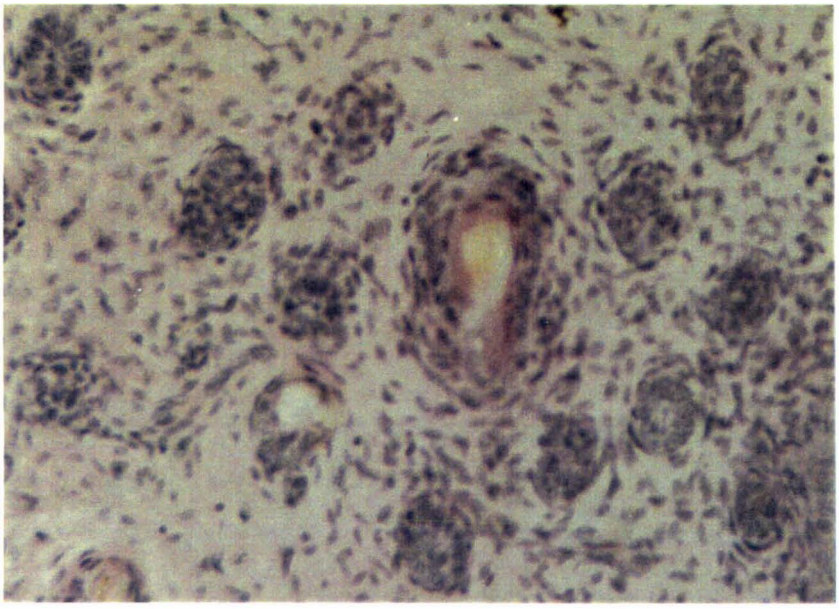
Plate 3 Pigment patch, or "ink spot", corresponding to growing fur shown in plate 4

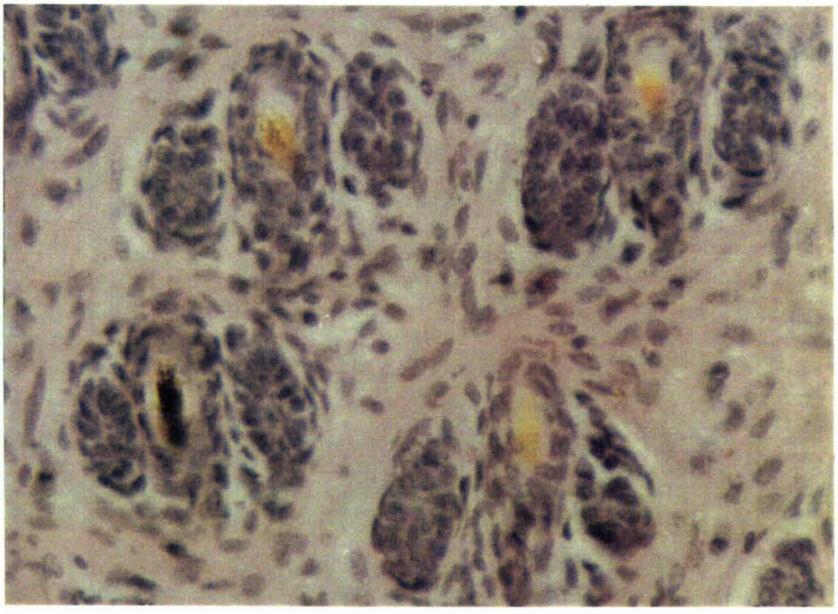
Plate 4 Patch of growing fur corresponding to the "ink spot" shown in plate 3.

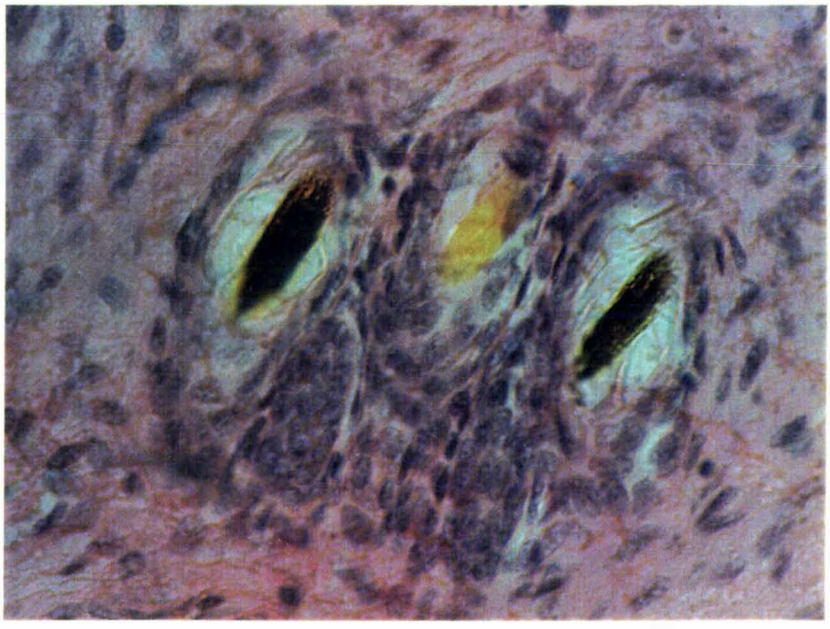
Plate 5 Transverse section of hair follicles in a 61 day old possum. Follicles with fibres are the first generation primary central follicles. The remainder are second generation primary central follicle primordia. H&E x425.

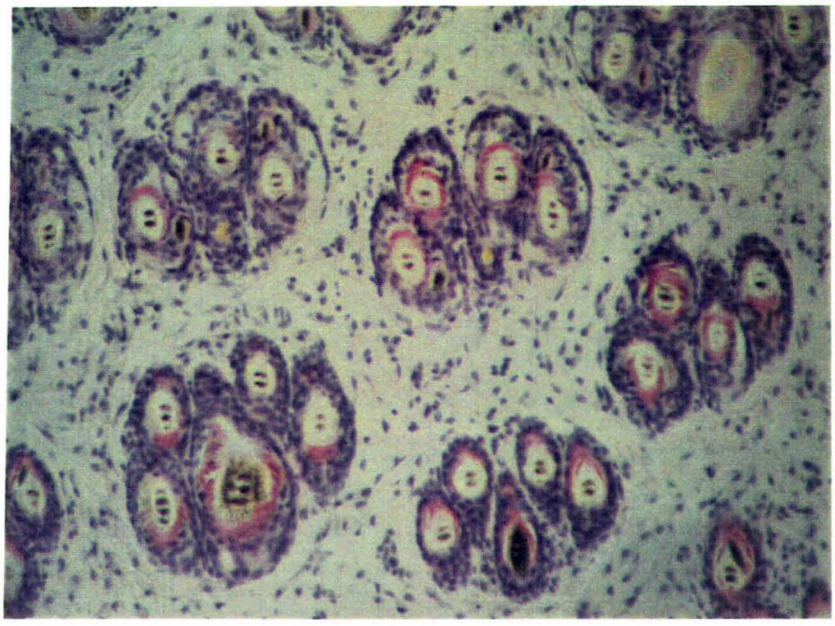
Plate 6 Transverse section of hair follicles in an 84 day old possum. Follicle development is at the primary lateral primordia stage. H&E x425.

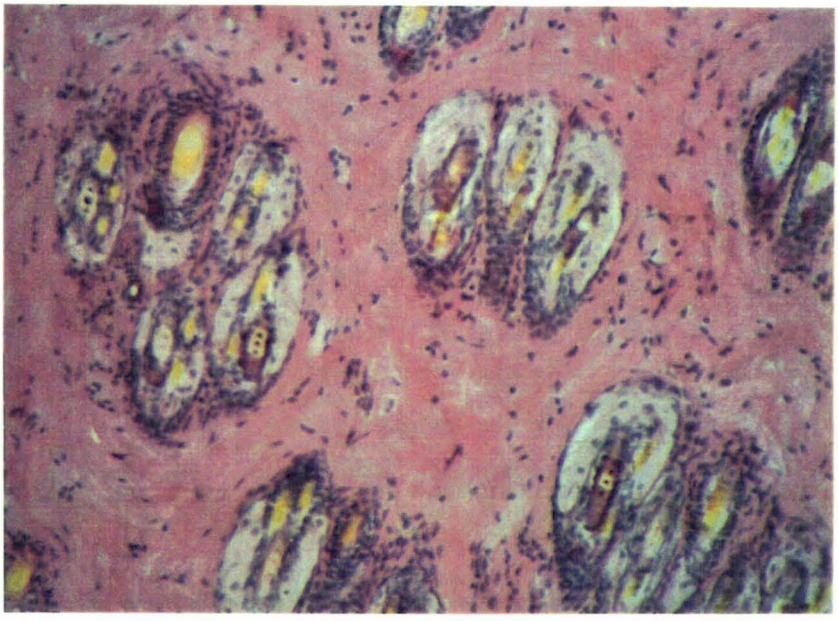
Plate 7 Transverse section of hair follicles in a 114 day old possum. Follicle development is at the secondary original primordia stage. H&E x630.











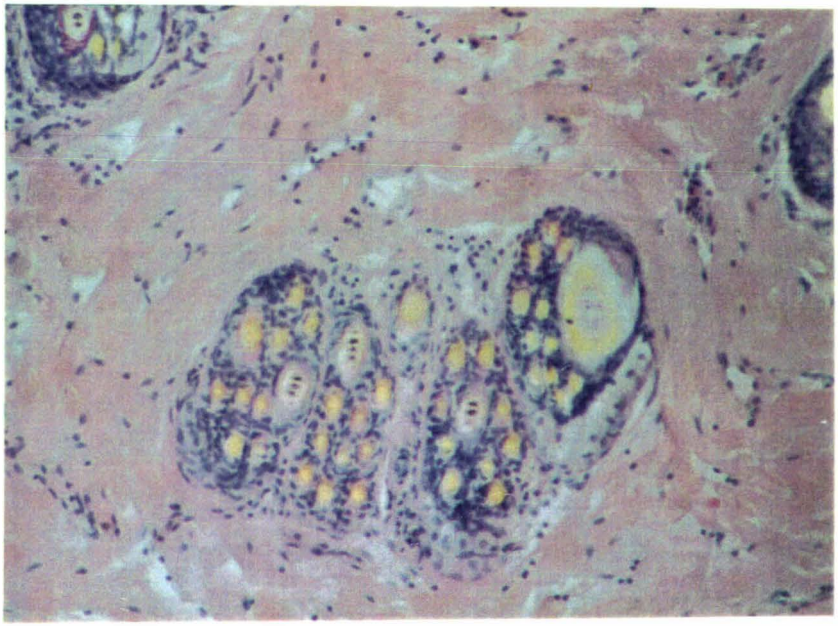
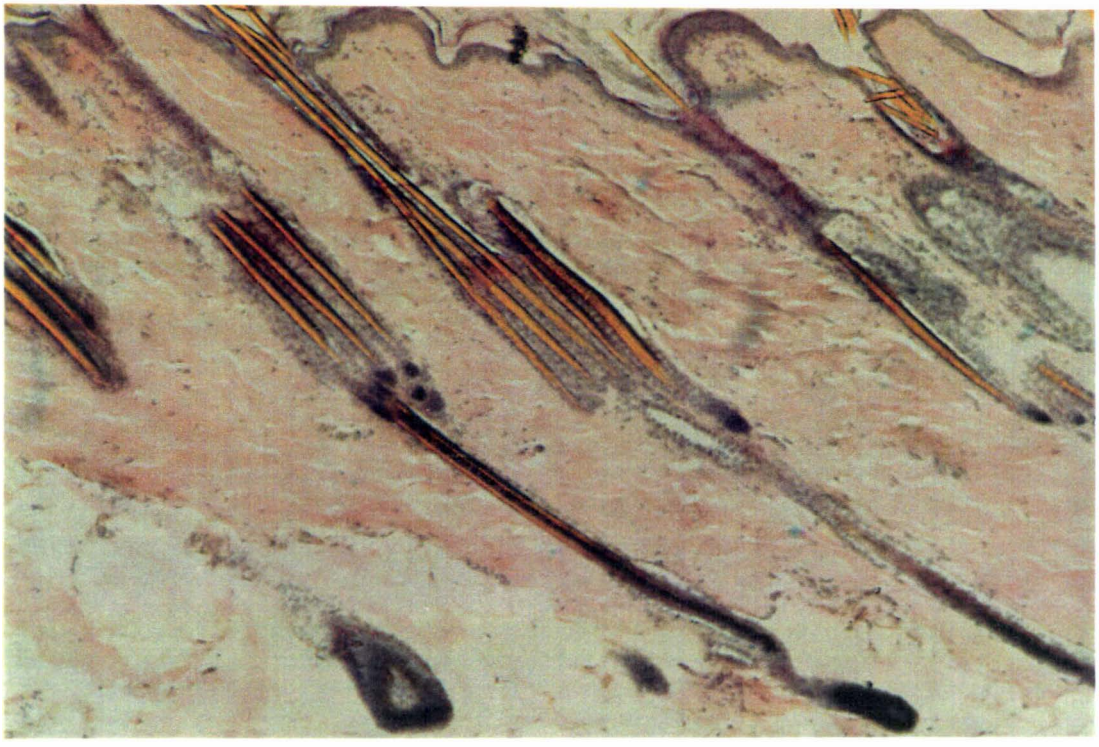


Plate 11 Longitudinal section of hair follicles. Note: fibres of bundle follicles sharing a common hair canal, shorter telogen follicles containing fibre club ends, longer anagen follicles extending down into subdermal adipose layer and containing medullated fibre. H&E x150.

Plate 12 Tips of possum fur fibres. From top to bottom: monotrich, guard hair, intermediate, underfur. x16



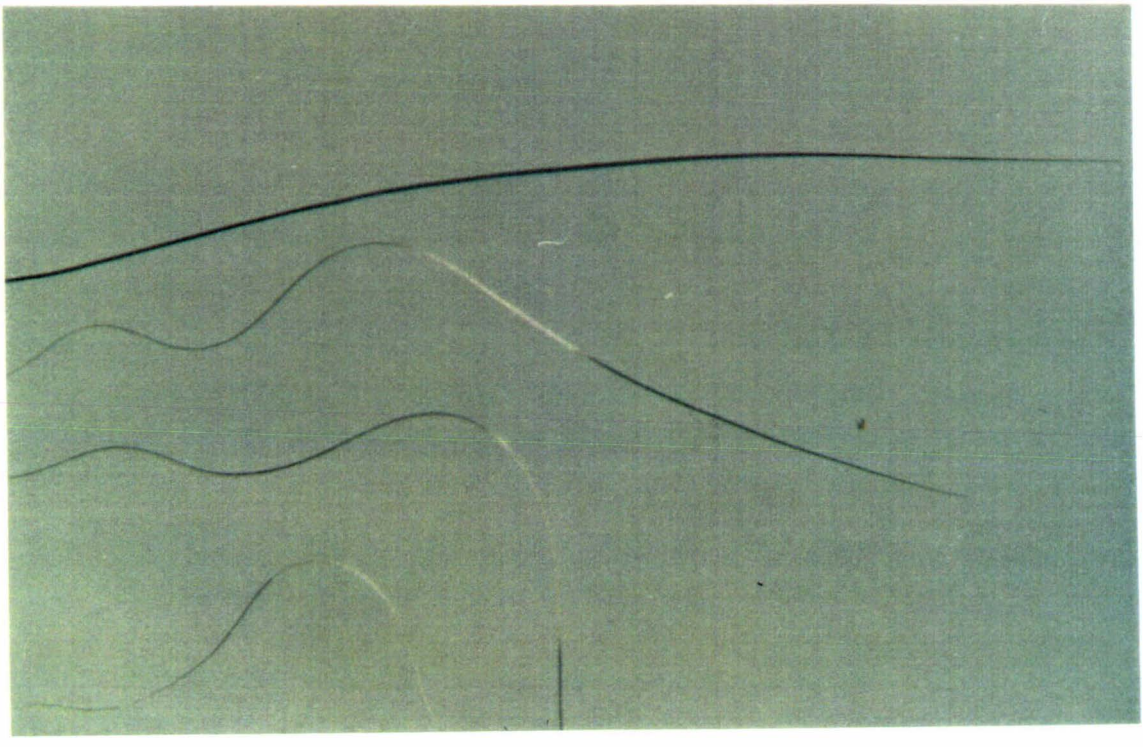


Plate 13 Mast cells associated with hair follicles. Cytoplasmic granules within mast cells are stained red with safranin. (Nuclei black, keratin yellow, collagen blue, outer root sheath green.) Sacpic x1560.

Plate 14 A fine network of elastic fibres surrounding hair follicle groups. (Elastin black, collagen orange, nuclei black, keratin yellow.) Verhoeff's elastin stain x380.

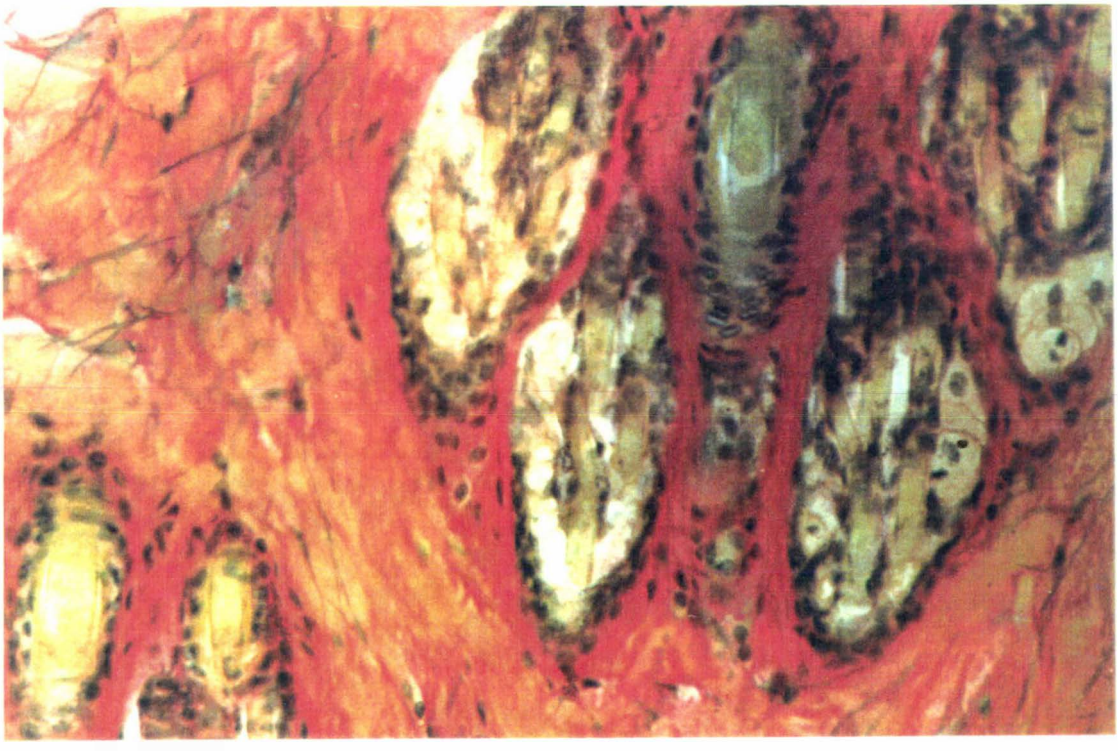
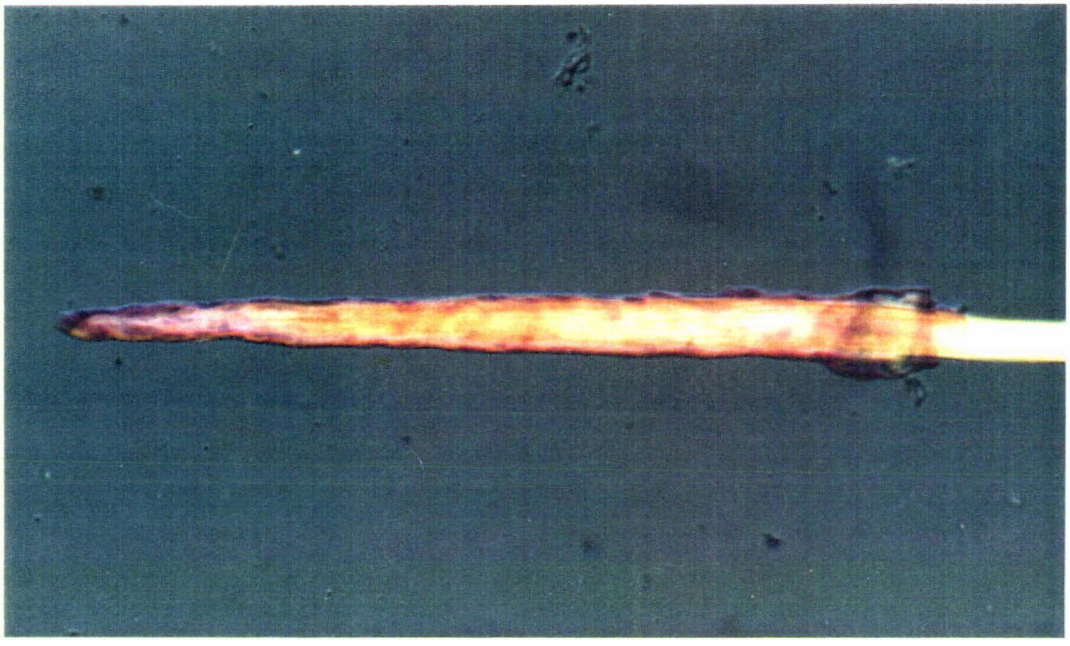


Plate 15 Club end of plucked fibre. Note layer of epithelial cells. Stained with haematoxylin, eosin and picric acid, x97

Plate 16 Oblique section of hair follicles in epilated possum skin. H&E x156.



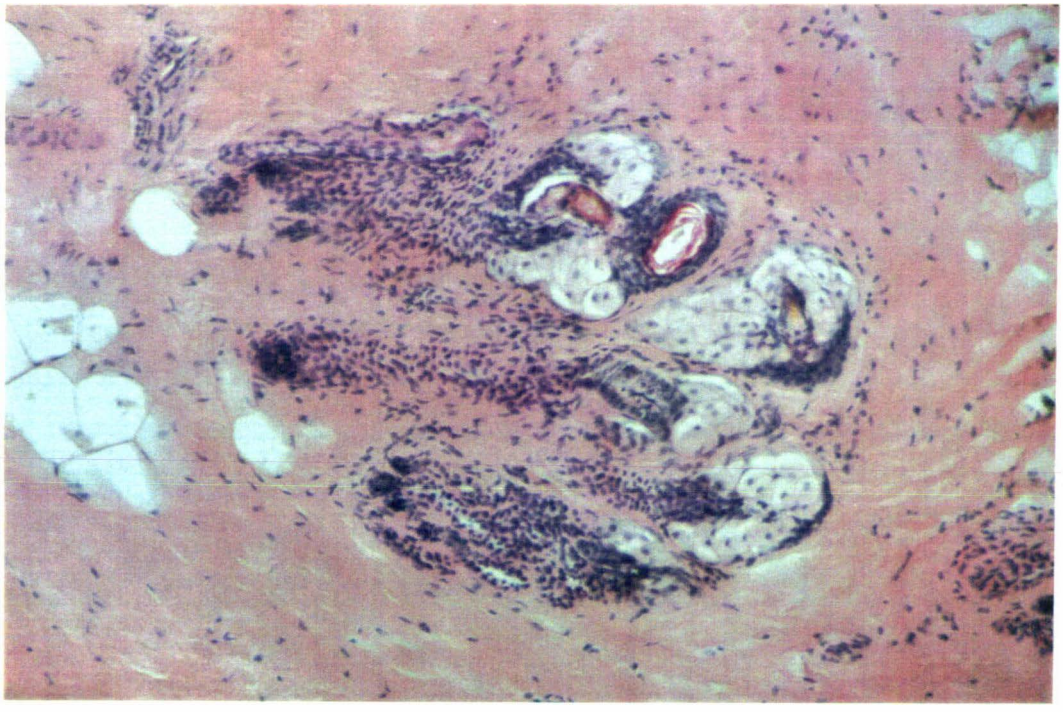


Plate 17 Male possum 223 days after dying of the coat. A diffuse pattern of replacement was evident. Fur on the mid side is brushed apart to show dyed fibres retained in the coat.

Plate 18 Male New Zealand White rabbit 71 days after dying of the coat. A wave pattern was demonstrated, in which a ventrad wave of fibre replacement progressed down the back to meet a dorsad wave. This was the first adult (seasonal) moult.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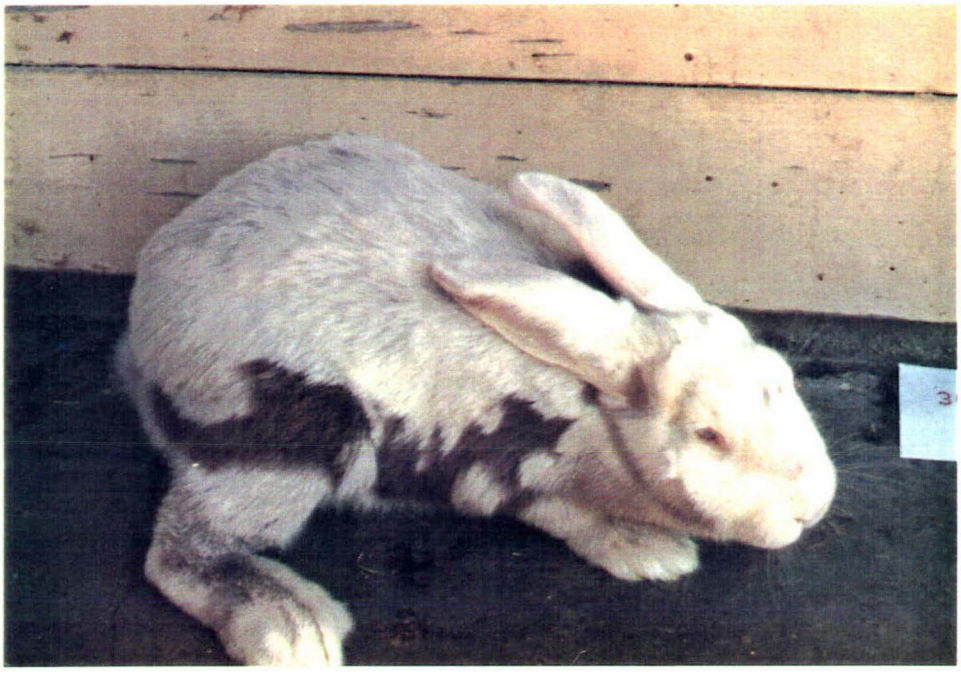


Appendix I
A BASIC COMPUTER PROGRAM FOR PROCESSING FOLLICLE SCORE DATA

```
LIST
10 CLEAR 1000
20 PRINT CHR$(12)
30 PRINT "*************************************************************************"
40 PRINT "*****************     HAIR FOLLICLE ACTIVITY     ******************"
50 PRINT "*************************************************************************"
60 PRINT
70 ' This program calculates percentage activity in primary central, lateral,
80 ' bundle, and total hair follicles from scores obtained by microscopy.
90 ' A.J.Nixon,  12-9-85
100 '
110 DIM CO(50), CA(50), LO(50), LA(50), NB(50)
120 OPEN "A:\apossum\A11.DAT" FOR INPUT AS #1
130 OPEN "A:\apossum\a11.mod" FOR OUTPUT AS #2
140 '***********************   MAIN PROGRAM   ******************************
150 INPUT #1,PS$
160 GOSUB 230 'INPUT
170 GOSUB 340 'CALCULATE
180 GOSUB 620 'OUTPUT
190 INPUT #1,PS$
200 IF PS$<>"EOF" THEN GOTO 160
210 PRINT "FINISHED"
220 END
230 '***********************    INPUT     ************************************
235 'INITIALISE ALL VARS
240 FOR I=1 TO 30
250      NB(I)=0: CA(I)=0: CO(I)=0: LA(I)=0: LO(I)=0
260 NEXT I
270 CS=0: LS=0: CT=0: LT=0: TN=0: NG=0
280 SP=0: SQ=0: SR=0: SS=0: SB=0: SD=0: SE=0: SF=0
290 INPUT #1,DD$,SM,N
300 FOR I=1 TO N
310     INPUT #1,NB(I),CA(I),CO(I),LA(I),LO(I)
320     NEXT I
330 RETURN
340 '************************** CALCULATE  ******************************
350 FOR I=1 TO N
360     TN=TN+NB(I)
370     CS=CS+CO(I)
380     LS=LS+LO(I)
390     CT=CT+CA(I)
400     LT=LT+LA(I)
410 NEXT I
420 G=CS+CT
430 H=LS+LT
440 F=G+H
450 GS=F/N
460 BS=H/TN
470 CX=CT*100/G
480 LX=LT*100/H
490 TX=((CT+LT)*100)/F
500 FOR I=1 TO N
510     SP=CA(I)+CO(I)+LA(I)+LO(I)
530     SQ=SQ+(SP-GS)^2
540     SR=SR+(((LA(I)+LO(I))/NB(I))-BS)^2
545     SS=SS+(((CA(I)+LA(I))/SP*100)-TX)^2
550 NEXT I
560 SD=(SS/(N-1))^.5
570 SG=(SQ/(N-1))^.5
580 SB=(SR/(N-1))^.5
590 SE=SD/(N)^.5
600 SF=SB/(N)^.5
610 RETURN
```

```
620 '************************** OUTPUT **********************************
630 PRINT "******  HAIR FOLLICLE ACTIVITY RESULTS  ******  ";
640 PRINT DATE$;:PRINT "  ******"
650 PRINT "POSSUM:"PS$ "       MOULT:"SM "       DATE:"DD$
660 PRINT:PRINT
670 PRINT "  TOTAL FOLLICLES SCORED      ="F
680 PRINT "  NUMBER OF GROUPS SCORED     ="N
690 PRINT USING "  AVE FOLLICLES / GROUP       = ##.##";GS
700 PRINT USING "  STD DVN OF GROUP SIZE       = ##.##";SG
710 PRINT
720 PRINT USING "  AVE CENTRAL PRIMARY / GROUP = ##.##";G/N
730 PRINT USING "  AVE LATERAL BUNDLE / GROUP  = ##.##";TN/N
740 PRINT USING "  AVE LAT FOLLICLES / GROUP   = ##.##";H/N
750 PRINT
760 PRINT USING "  AVE FOLLICLES / LAT BUNDLE  = ##.##";BS
770 PRINT USING "  STD DVN OF BUNDLE SIZE      = ##.###";SB
780 PRINT USING "  STD ERROR OF BUNDLE SIZE    = ##.###";SF
790 PRINT
800 PRINT USING "  CENTRAL PRIMARY ACTIVITY    = ###.## %";CX
810 PRINT USING "  LATERAL BUNDLE ACTIVITY     = ###.## %";LX
820 PRINT USING "  TOTAL  ACTIVITY             = ###.## %";TX
830 PRINT USING "  STD DVN OF ACTIVITY         = ##.###";SD
840 PRINT USING "  STD ERROR MEAN TOT ACTIVITY = ##.###";SE
850 PRINT:PRINT
860 PRINT #2, PS$;SM;DD$;
865 PRINT #2, USING "### ##";F;N;
870 PRINT #2, USING "###.#";CX;LX;TX;
880 PRINT #2, USING " ##.##";SD;SE;
890 PRINT #2, USING " ##.# ##.## ##.# ##.## ##.##";GS;SG;BS;SB;SF
900 RETURN
```

Appendix II
HAIR GROWTH PATTERNS IN YOUNG NEW ZEALAND WHITE RABBITS

Introduction

Moult patterns have been demonstrated by dyeing of the whole coat in mice (Borum 1954) and cats (Baker 1974). Whiteley and Ghadially (1954) used the same method to show the progression of moult in adult rabbits. Adult rabbits generally exhibit biannual wave-type hair replacement whereas the pattern in juveniles is reported to be more diffuse (Robinson 1958, Stodart 1965). In order to provide a record of early hair growth and replacement, some young New Zealand White rabbits were dyed to show the patterns of hair growth as dyed fur was replaced with new white fur. Some indication as to the state of the coat at different ages thus obtained might be useful in the management of this commercial meat breed in which the fur is a by-product.

Methods

Eight kittens from a single litter, born in mid September were dyed using "durafur black". They were first washed in a warm detergent solution, then bathed in the dye prepared as described by Chapman and Wheeler (1963). They were wiped dry and kept warm under a heat lamp or fan heater for several hours.

Four kittens were dyed at three weeks of age (weight 400-450 g). At nine weeks of age, after the first new hairs appeared, one animal was re-dyed and another dyed for the first time. Three rabbits were dyed again at 22 weeks of age. A tranquilizing dose (0.5-1 ml/kg) of "saffan" (alphaxalone 0.9% w/v, alphadelone acetate 0.3% w/v) was administered for all except the initial dyeing.

The entire litter was left with the doe until eight weeks of age, then kept in groups of two or three until 18 weeks when the numbers were reduced to three (one male and two females). The wire cages were enclosed within a three-walled, naturally-lit shed. Commercial rabbit pellets and hay were fed until 18 weeks when pellets were rationed.

The animals were photographed at approximately monthly intervals, or whenever new features of hair growth became apparent.

Results

The appendix figure portrays the sequence of changes in appearance of dyed rabbits from the first growth of new fibres to the start of the second adult moult at about eight months of age. Original (dyed) fur is represented by unshaded areas. The first new fibres came through the coat of the kittens at age eight weeks. Growth started on the side of the shoulder and underside (figure a) followed by the side of the head and hind regions (figure b). The last parts of the coat to grow new hairs were the neck and mid-lateral region (figure c). By about 15 weeks newly grown fibre covered the original coat which was retained in its dyed state as the underfur. At this stage the young rabbits had lost their fluffy appearance and acquired a coat with adult characteristics.

In three of the five dyed animals, all fibre types were replaced in a wave which began at about 17 weeks. This wave started mid-dorsally and progressed to meet the new belly fur as shown in figures d-f. A second wave had started in the male before the replacement was complete in patches on the neck and flanks (figure g). Dyed fibre was still evident in the coat after the first seasonal moult in the two females and the second moult of the male. At one year of age patches of dyed fur were still evident in the two surviving adult animals (one of each sex).

There was much variation between individuals in the timing of events, such that at 17 weeks (weight range: 2.8 - 3.5 kg) the moult pattern ranged from that illustrated in figure c to figure e. (Both the animal that was dyed twice and the one not dyed with the others at four weeks were in the middle of this range, giving no evidence that dyeing affected the timing of moult.) Two of the five dyed rabbits did not undergo the seasonal moult before they were culled at 18 weeks. The patterns were variable also. Some appeared as a smooth front passing down the back, while others were patchy. The figure must therefore be regarded as an average representation of moult observed in the young New Zealand White rabbits.

Discussion

There were two main features of hair growth in the first eight months: the growth of overhairs through the juvenile coat, and later, a seasonal moult with a wave proceeding from back to belly. A separate wave of moult on the belly was also observed as part the seasonal moult pattern, like that reported by Whiteley and Ghadially (1954).

The so-called juvenile moult does not involve the bulk of the underfur which is still present until the first seasonal moult at about 16-18 weeks. Juvenile moult may be regarded as a developmental process by which guard hairs of the adult coat are acquired. This process of hair growth in juveniles shows similarity to some of the descriptions reviewed by Robinson (1958), particularly the report of growth of stouter hair types in a silvered breed.

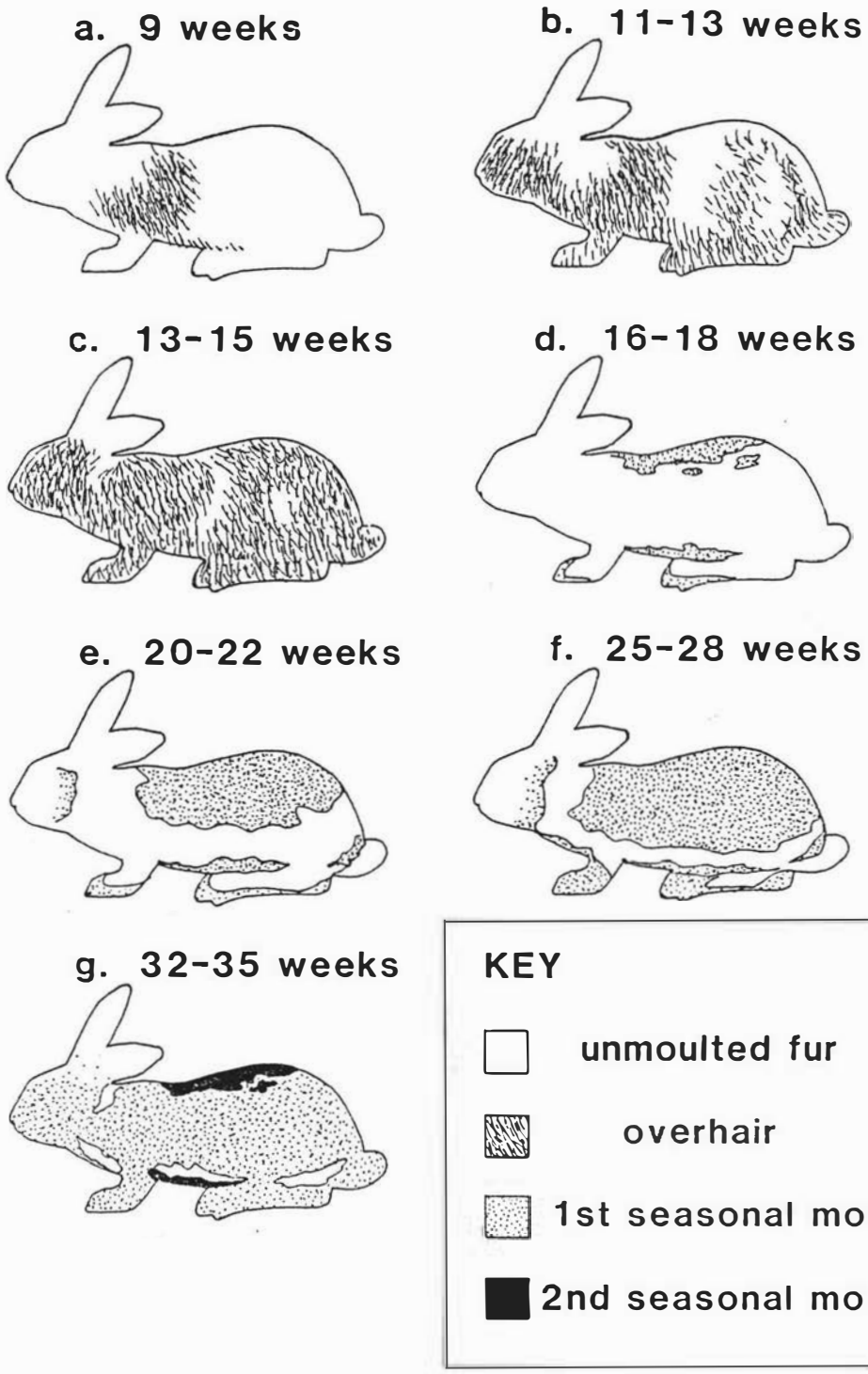
Wave patterns moulting observed in maturing rabbits in this study were described by Robinson as biannual, or sometimes annual events. Stodart (1965) found a spring-summer "dorsad" as well as an autumn-winter "ventrad" moult in Australian wild rabbits. In both types of seasonal moult, growth waves commence at dorsal and ventral centres spreading to meet in a lateral band (as in figure f). It may be that some of the variation observed in this study is due to these two forms of moult. Stodart also found marked differences in timing of moult between climatic regions which might suggest that environmental as well as genetic factors could account for differences that might exist between wild rabbits and various domestic breeds.

It is possible that some of the variation between individuals encountered in this study may be due to sexual differences. Such an effect is more likely as sexual maturity is approached, and there was a marked difference between the male and the two females at 30 weeks. Stodart noted greater irregularity in the moulting of wild female rabbits. Seasonality is another factor which is likely to affect variation in moult timing. Three of the five rabbits underwent a seasonal moult in mid summer, which could be asynchronous with normal adult moulting.

The results of dyeing suggest that the best time to harvest New Zealand White rabbits with optimum pelt quality would be about 15 weeks of age. At this stage they have acquired their adult coat, but have not yet entered their first seasonal moult (which gives inconsistencies to the pelt). Killing on the basis of

age at some later stage, say between moults at about 25 weeks, would be unreliable because of individual variation and overlap in successive moults.

Wave progression shown in rabbits is a common characteristic of eutherian moulting, and contrasts markedly with the diffuse growth pattern shown for possums (section 6.3). Furthermore, no marked juvenile or sub-adult moults occur in possums. After initial growth of very short fibres (Lyne 1970) possums develop an adult type pelage as pouched or back young (section 4.6), whereas juvenile moult patterns in many small to medium sized eutherian species, including rabbits, differ from adult seasonal patterns. Further investigation of juvenile moults of rabbits may be warranted to determine the activity cycles and fibre production of each follicle type required to achieve mature coat structure.



Appendix figure: Hair growth patterns in young New Zealand White rabbits.
Shaded areas indicate topographic progression of successive waves.