

# The ilio-marsupialis muscle in the dasyurid marsupial *Sminthopsis douglasi*: form, function and fibre-type profiles in females with and without suckling young

P. A. Woolley<sup>1,\*</sup>, M. F. Patterson<sup>1</sup>, G. M. Stephenson<sup>2</sup> and D. G. Stephenson<sup>1</sup>

<sup>1</sup>Department of Zoology, La Trobe University, Bundoora, Victoria 3086, Australia and

<sup>2</sup>School of Life Sciences and Technology, Victoria University, Melbourne, Victoria 8001, Australia

\*Author for correspondence (e-mail: p.woolley@latrobe.edu.au)

All authors contributed equally to this study

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## Summary

The form, function and fibre-type profiles of the ilio-marsupialis muscles, branches of which insert on to the skin of the nipples and pouch, have been investigated in the small dasyurid marsupial *Sminthopsis douglasi*. Single fibres from the branches of muscles associated with unsuckled nipples in non-lactating females and with both unsuckled and suckled nipples at four stages during the 70-day suckling period were typed according to their sensitivity to the activators strontium (Sr<sup>2+</sup>) and calcium (Ca<sup>2+</sup>) into fast-twitch, slow-twitch and composite types. An unusual finding was the predominance of composite fibres in the resting state (unsuckled nipples). Changes in fibre-type composition were observed during the suckling period and these changes correlated with events in the development of the suckling young. Composite fibres declined during the suckling period and, at the stage when

the young can no longer be accommodated in the pouch but must still be carried by the mother while she is foraging, an increase in fast-twitch fibres that are associated with dynamic muscular activity was seen. Later in the suckling period, when the mammary tissue is greatly enlarged but the mother does not carry the young while out feeding, there was an increase in the proportion of slow-twitch (fatigue-resistant) fibres. The high proportion of fast-twitch fibres present late in the suckling period may be associated with vibratory movements that result in the young relinquishing the nipples.

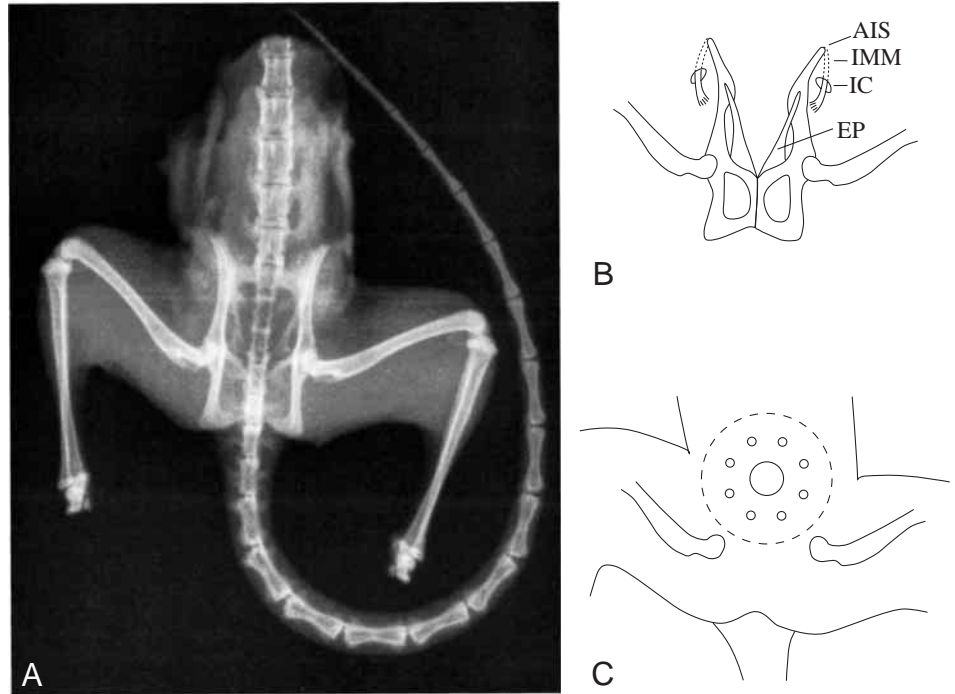
Key words: ilio-marsupialis muscle, anatomy, function, fibre-type composition, pouch, development of young, *Sminthopsis douglasi*, dasyurid marsupial.

## Introduction

The mechanism of milk ejection in the lactating marsupial has, until relatively recently, been the subject of debate (Griffiths and Slater, 1988). Fundamental to the controversy has been the role played by the ilio-marsupialis muscle, which consists of skeletal muscle tissue originating on the pelvis and passing to the mammary area (see Fig. 1). Before it was known that the marsupial neonate was capable of sucking, it was postulated that voluntary contraction of the ilio-marsupialis muscle would compress the mammary glands and force milk through the nipples, hence the name *M. compressor mammae* was originally given to the *M. ilio-marsupialis* (see Griffiths and Slater, 1988, for discussion). The historical setting for this belief, and early observations on the ability of the young to suckle, have been documented by Hill and Hill (1955) and Enders (1966). Later workers (see Tyndale-Biscoe and Renfree, 1987) demonstrated that milk was not expressed from the glands by the contraction of skeletal muscle. It was suggested by Griffiths and Slater (1988) that the function of the ilio-marsupialis muscle was for mammary support and for retraction of the teats and attached young. This was considered by them to be especially important in 'pouchless' or 'pseudo-

pouched' marsupials, in which they found the muscles better developed than in species with a pouch. Contraction of these muscles would provide a mechanism for holding the exposed young close to the body of the mother, thus affording them some protection when the mother was active. Griffiths and Slater (1988) proposed the name 'retractor mammae' for the discrete branches of the ilio-marsupialis muscle that ran from the main body of the muscle up to the base of each nipple. They showed that the young of anaesthetised *Antechinus stuartii*, a 'pouchless' dasyurid marsupial, hung down from the pouch area but, as the anaesthetic wore off, they were pulled close to the mother's body. Similar observations were made on *Dasyuroides byrnei*, another 'pouchless' dasyurid, and on *Dasyurus viverrinus*, which Griffiths and Slater described as 'pseudo-pouched'. As classified by Woolley (1974), 'pouchless' dasyurid marsupials have a Type 1 pouch and 'pseudo-pouched' dasyurid marsupials have a Type 3 pouch. The six types of pouches found in marsupials are reviewed by Tyndale-Biscoe and Renfree (1987). The mammary area of the Type 1 pouch has no covering fold of skin and the nipples are exposed, whereas in a Type 3 pouch the mammary area is

Fig. 1. (A) X-ray of hindquarters of female *Sminthopsis douglasi*. (B) Diagram of pelvic region showing insertion of the ilio-marsupialis muscle (IMM) on the anterior iliac spine (AIS) and the passage of muscle through the inguinal canal (IC). The epipubic bones (EP) are embedded in the abdominal musculature. After passing through the inguinal canal, the ilio-marsupialis muscle divides into four branches, each passing to one of the four nipples on the same side. (C) Diagrammatic representation of the pouch. The broken line indicates the limit of the pouch area, and the solid line indicates the free edge of the fold of skin at the entrance to the pouch. The nipples, which lie under the pouch skin fold, are shown as small open circles.



covered by a circular fold of skin with a central opening and all the nipples are enclosed. Young attached to the nipples are exposed throughout lactation in species with a Type 1 pouch but are covered, at least in the early stages, in species with a Type 3 pouch.

In the present study, observations have been made on the ilio-marsupialis muscles of *Sminthopsis douglasi*, a dasyurid marsupial with a Type 3 pouch. There are eight nipples in the pouch of this species and this determines the maximum litter size because the young do not relinquish the nipple until approximately day 40 of the 70-day suckling period. In the event that the female does not have a full complement of young, the mammary glands associated with unsucked nipples do not enlarge and produce milk. For approximately 30 days, the young are covered by the pouch skin fold, after which they become progressively more exposed (Fig. 2). By 40 days of age, they hang out of the pouch but are usually held close to the body of the mother when she is moving around. From this age, they begin to be left in the nest while the mother is out feeding (P. A. Woolley, unpublished laboratory observations). *Sminthopsis douglasi* is nocturnal and insectivorous but little is known of its activity patterns in the wild. It would seem to be advantageous for the mother to be able to forage without the burden of large young. The mechanism by which the mother dislodges the young is not known, but young in captivity have been observed to relinquish the nipples following repeated rapid movements (vibration) of the abdomen (P. A. Woolley and M. Coleman, unpublished observations).

From this brief description of events in the development of the suckling young, it seems likely that the demands placed on the ilio-marsupialis muscle may change over the suckling

period. As mammalian skeletal muscles in general display plasticity (Pette and Staron, 1997, 2001; Pette, 1998), changing demands on the ilio-marsupialis muscle would be expected to induce changes in the fibre-type composition. Therefore, bearing in mind that dynamic muscular activity is usually associated with fast-twitch muscle fibres, and postural-type activity is usually associated with slow-twitch, fatigue-resistant muscle fibres (Jones and Round, 1990), it was of interest to investigate whether or not changes occurred in the type of muscle fibres present in the ilio-marsupialis muscles of *Sminthopsis douglasi* in the course of the development of suckling young. Identification of such changes might also help to better understand the function of this muscle.

## Materials and methods

### Animals

Ten females bred in captivity were obtained from a colony of *Sminthopsis douglasi* Archer 1979 kept at La Trobe University (Woolley, 1995). Eight of the females were suckling young. Young less than 40 days of age were removed from the nipples and formed part of a separate study on growth and development, while those older than 40 days were fostered to other mothers. The adult females were killed by gaseous anaesthetic overdose, and a midline incision was made through the pouch and abdominal skin to expose the mammary tissue and ilio-marsupialis muscles. Branches of the muscles supplying particular nipples were identified, and 3–5 mm lengths were removed, blotted dry on filter paper and then placed under paraffin oil on a base of Sylgard 184 (Dow Chemicals, Midland, MI, USA). Some of the lactating females did not have a full complement of young and so muscles



Fig. 2. A wild-caught *Sminthopsis douglasi* with eight young estimated to be 35–40 days old. Before handling, the young were partially covered by the pouch skin fold, part of which is indicated by the arrow below the thumb of the handler.

associated with both suckled and unsuckled nipples were obtained from most of these individuals. One half of the pouch area (skin, mammary glands and muscles) from each of two individuals was fixed in Bouin's solution (glacial acetic acid, 40% formaldehyde, saturated aqueous picric acid, 1:5:15), and serial 10  $\mu\text{m}$  paraffin sections were prepared. Alternate slides were stained with Haematoxylin and Eosin, and Haematoxylin, Xylidene Red and light green SF (Masson's trichrome method). Carcasses were preserved for later dissection to establish the point of insertion of the ilio-marsupialis muscles on the pelvis.

#### Fibre typing

It has been shown that muscle fibres from eutherian mammals (Fink et al., 1990; Lynch et al., 1995) and dasyurid marsupials (Wilson and Stephenson, 1990) can be classified according to their sensitivity to the activators strontium ( $\text{Sr}^{2+}$ ) and calcium ( $\text{Ca}^{2+}$ ) into three groups: fast-twitch, slow-twitch

and composite. Compared with slow-twitch fibres, fast-twitch fibres display a greatly reduced sensitivity to activation by  $\text{Sr}^{2+}$  ions and a slightly decreased sensitivity to activation by  $\text{Ca}^{2+}$  ions. Composite fibres display high  $\text{Sr}^{2+}$  sensitivity at lower levels of activation (similar to slow-twitch fibres) but low  $\text{Sr}^{2+}$  sensitivity at higher levels of activation (similar to fast-twitch fibres). This physiological method of fibre classification has been validated previously by an electrophoretic method for the determination of the myosin heavy chains in the same fibres for which activation by  $\text{Sr}^{2+}$  and  $\text{Ca}^{2+}$  was measured (Bortolotto et al., 2000).

Between two and nine fibres per animal were examined. Single fibres were randomly isolated from freshly dissected branches of the ilio-marsupialis muscle associated with a particular nipple, and their  $\text{Sr}^{2+}$  and  $\text{Ca}^{2+}$  activation characteristics were determined after brief treatment with Triton X-100, a non-ionic detergent, to remove the membrane barriers and enable access to the contractile apparatus (Bortolotto et al., 2000). The fibres were then activated in  $\text{Sr}^{2+}$ - and  $\text{Ca}^{2+}$ -buffered solutions and classified as fast, slow or composite, and the proportions of the different types of fibres were calculated. This method has been shown previously to produce a fibre-type profile that is very close to that obtained from the sampling of the entire muscle (Bortolotto et al., 2000).

Samples of the ilio-marsupialis muscle associated with individual nipples were taken from non-lactating and lactating females and grouped as follows:

Group 1 – nipple never suckled (two non-parous individuals),

Group 2 – nipple being continuously suckled (three individuals with young of 35 days old, 36 days old and 39 days old, respectively),

Group 3 – suckling intermittent (three individuals with young of 51 days old, 52 days old and 53 days old, respectively),

Group 4 – late lactation (weaning), (two individuals with young of 68 days old and 70 days old, respectively),

Group 5 – unsuckled nipple in the pouch of a female with young suckling other nipples (four individuals with young of 36 days old, 39 days old, 52 days old and 53 days old, respectively).

The mass of the young, and of the mammary tissue, was obtained for some of the above females and for others at particular times throughout the lactation period as part of a separate study.

The proportions of the three types of fibres present in the ilio-marsupialis muscles in non-lactating females, and in lactating females at different stages of lactation, were analysed by Fisher exact test. All statistical analyses were two-tailed, and significance was accepted at the  $P < 0.05$  level.

## Results

### Morphology

The ilio-marsupialis muscle of each side of the body originates on the anterior iliac spine and is closely associated

with the lumbar musculature before passing through the inguinal canal to lie superficial to the abdominal musculature dorsal to the pouch area (Fig. 1). There, each muscle divides into four branches, each of which can be traced to the base of one of the four nipples on the corresponding side of the pouch. In serial histological sections, each branch can be seen to fan out from beneath the nipple and insert on to the skin of the pouch and that of the nipple itself. Strands of ilio-marsupialis muscle can be seen in a section through a nipple and the adjacent pouch skin in Fig. 3. There is an obvious increase in the size of the muscle and of its branches to suckled nipples during lactation, but this increase is difficult to quantify because the muscle cannot be dissected from tendon to tendon.

#### Muscle fibre-type profiles

Fig. 4 shows the stages at which the groups of muscle samples were taken and the appearance of the pouch together with, in females suckling young, major events during the suckling period. The number of fibres examined and the number of animals they were obtained from, together with the average percentage of fibre type per animal ( $f_A$ ) and the

percentage of fibre type per total fibres ( $f_T$ ) in each of the five groups are given in Table 1. It is important to note that, despite the variation in the number of fibres investigated per animal (2–9), the average percentage of  $f_A$  was close to the fraction of  $f_T$  examined for each group. This indicates that the inter-animal variability in the fibre composition of the ilio-marsupialis muscle is relatively small. The largest difference between  $f_A$  and  $f_T$  values was found in Group 5 (unsuckled nipples), where fibres were taken from animals at very different stages of lactation. This may account for the higher inter-animal variability. Nevertheless, even in this group, there was consistency in the sense that no slow-twitch fibres were found in any of the four animals examined. Given the close values between  $f_T$  and  $f_A$  in Table 1, only the  $f_T$  values for the fast-twitch, slow-twitch and composite fibres are presented in Fig. 5.

In Group 1, the majority of fibres (71%) were classified as composite, with only 22% showing fast and 7% showing slow activation characteristics. The fibre-type profile of this group represents the steady-state (control) condition of the ilio-marsupialis muscle in females not suckling young. In muscles associated with suckled nipples (Groups 2, 3 and 4), the proportion of composite fibres was less than in Group 1. In Group 2, there was a greater proportion of fast fibres (75% versus 22%,  $P < 0.05$ ) and a lower proportion of composite fibres (25% versus 71%,  $P < 0.05$ ) than in Group 1. Further shifts in fibre-type composition were seen in Groups 3 and 4. In Group 3, there was a significant increase in the proportion of slow fibres (39% versus 0%,  $P < 0.05$ ) and, consequently, a lower proportion of fast fibres (39% versus 75%,  $P < 0.05$ ) than in Group 2. In Group 4, when weaning of the young is in progress, the proportion of composite fibres was lowest (6%), with most fibres being classified as either fast (69%) or slow (25%). These results show that the principal effect on the ilio-marsupialis muscle during progression of the lactation period is the transformation of composite fibres associated with suckled nipples into either fast-twitch or slow-twitch fibres. In our view, the fibre type transformation in both directions is not caused by one stimulus, i.e. suckling, but rather by changes in the load placed on the ilio-marsupialis muscle associated with growth of the young and changes in their behaviour (see Discussion). The change first seen was from composite to fast-twitch fibres and, later in the lactation period, it was also to slow-twitch fibres. Fibres in Group 5 (associated with unsuckled nipples in the pouch of females with young suckling other nipples) showed a similar fibre-type profile to those in Group 1 (Fig. 5). No significant difference ( $P = 0.39$ ) was found between the types of fibres identified in Groups 1 and 5, which suggests that

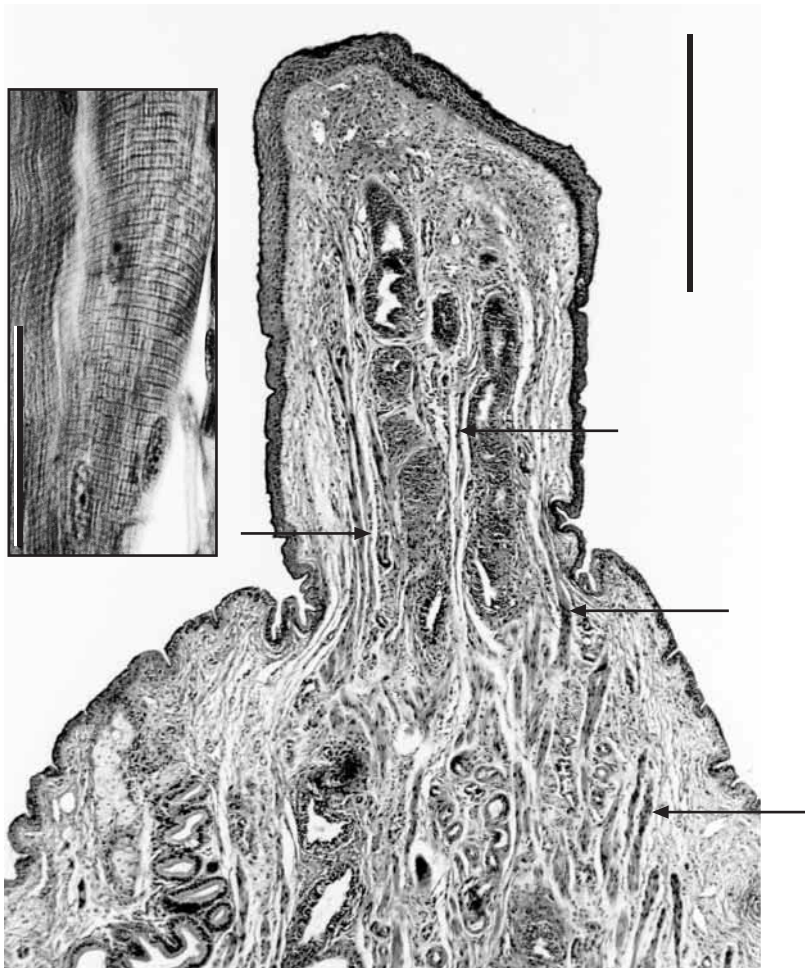


Fig. 3. Sagittal section through a nipple and the adjacent pouch skin showing strands of ilio-marsupialis muscle (arrows). Scale bar, 0.5 mm. Insert: strands of ilio-marsupialis muscle at a higher magnification. Scale bar, 50  $\mu$ m.

Table 1. The number of fibres, the number of animals, the average percentage of fibre type per animal ( $f_A$ ) and the percentage of fibre type per total fibres ( $f_T$ ) in each group examined

| Group | No. fibres<br>(no. animals) | Fast-twitch |         | Composite |         | Slow-twitch |         |
|-------|-----------------------------|-------------|---------|-----------|---------|-------------|---------|
|       |                             | % $f_A$     | % $f_T$ | % $f_A$   | % $f_T$ | % $f_A$     | % $f_T$ |
| 1     | 14 (2)                      | 21          | 22      | 73        | 71      | 6           | 7       |
| 2     | 12 (3)                      | 72          | 75      | 28        | 25      | 0           | 0       |
| 3     | 18 (3)                      | 37          | 39      | 19        | 22      | 44          | 39      |
| 4     | 16 (2)                      | 69          | 69      | 7         | 6       | 24          | 25      |
| 5     | 17 (4)                      | 52          | 41      | 48        | 59      | 0           | 0       |

Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.

lactation *per se* has no effect on fibre-type profile; rather, it is the demand on the muscles associated with individual nipples that is related to changes in fibre-type profile.

### Discussion

It is accepted that mammalian skeletal muscle is a dynamic tissue that undergoes adaptive changes in response to altered functional demands (Pette and Staron, 1997, 2001; Pette, 1998). Muscle tissue is therefore not composed of fixed percentages of different types of fibres but instead exhibits fibre-type plasticity (or adaptability) that takes place in response to altered physical demands placed on the muscle. Numerous studies have demonstrated such plasticity of the muscle fibres. For example, fast-to-slow and slow-to-fast transitions in myosin heavy chain isoforms have been shown to occur in response to altered neuromuscular activity resulting from chronic low-frequency stimulation (Leeuw and Pette, 1993), endurance training (e.g. Klitgaard et al., 1990) and hindlimb suspension (Stevens et al., 2000). In

the present study, we present evidence that the fibre-type profile of the branches of the ilio-marsupialis muscle in the marsupial *Sminthopsis douglasi* changes when the nipples are suckled and in a manner dependent on the age of the suckling young.

In the absence of suckling young, the ilio-marsupialis muscle consists predominantly of composite fibres (71%). By comparison, only 28% of the fibres from the soleus muscle of the stripe-faced dunnart *Sminthopsis macroura*, a smaller congener of the species used in this study, were composite (Wilson and Stephenson, 1990). A high proportion of composite fibres in a muscle normally indicates a more

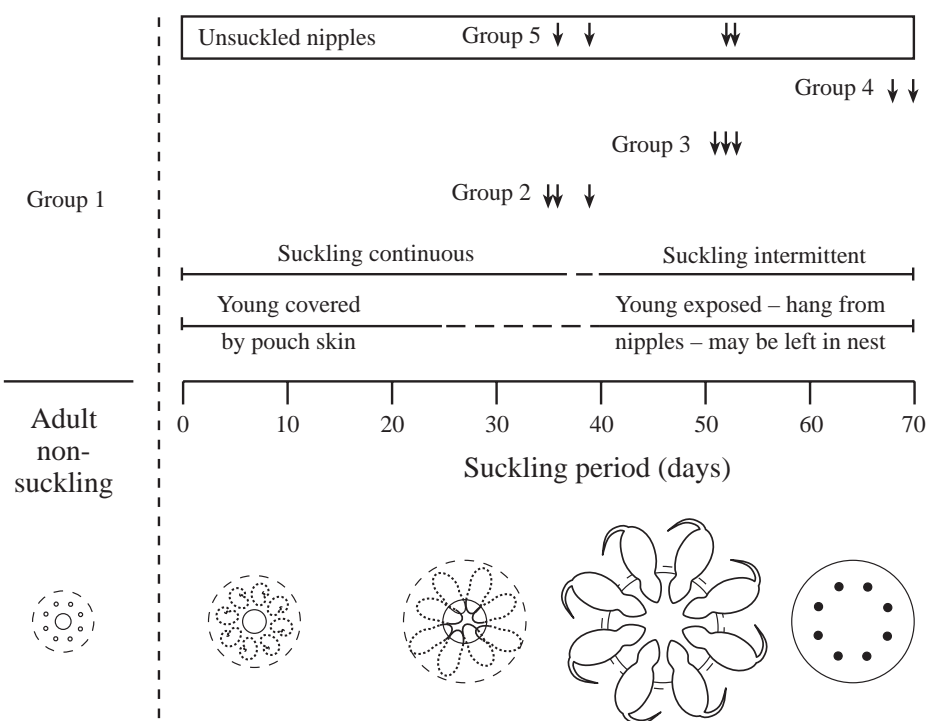


Fig. 4. The groups of ilio-marsupialis muscle samples taken from the animals used in this study are shown in relation to the schematic appearance of the pouch in both non-suckling adults and in females suckling young, together with events during the suckling period. Arrows indicate the ages of the young in the samples taken from lactating females. Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.

complex functional role of that muscle (Stephenson, 2001). When the nipples are not suckled, the branches of the muscles are small, suggesting little or no functional role other than perhaps to maintain the tone of the pouch skin fold. The high proportion of composite fibres in these muscles would enable them to adapt to changes in demand during the suckling period.

In the presence of suckling young, the fibre-type profile of the ilio-marsupialis muscle changed. For approximately the first 40 days of the lactation period, the young are suckled continuously (the attachment phase), and during this time

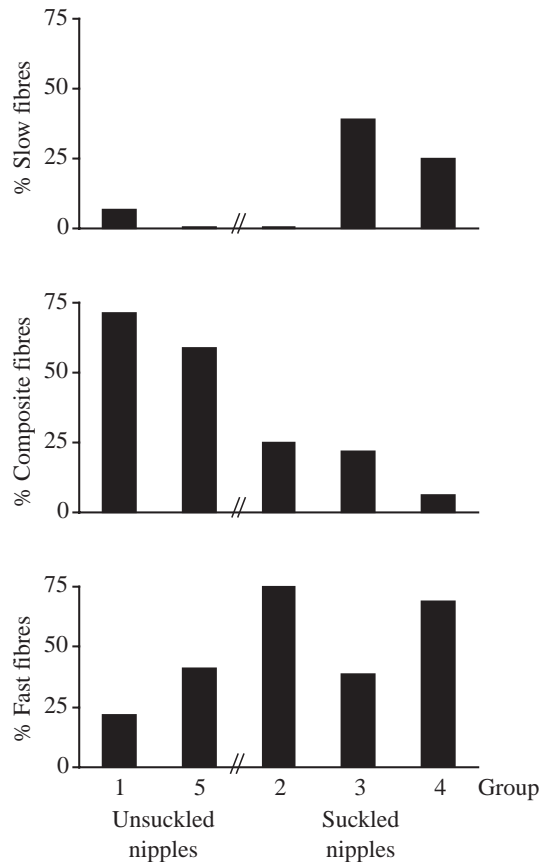


Fig. 5. The relative proportions of fast-twitch, composite and slow-twitch muscle fibres in populations of fibres (*fr*) dissected from muscles associated with the nipples in each of the five groups studied. Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.

individuals increase in mass from 15 mg at birth to as much as 2 g. The mass of the mammary tissue also increases, from approximately 300 mg to 2 g. Towards the end of the attachment phase, the pouch skin fold no longer covers and provides protection and support for the young. They become increasingly exposed and finally hang down from the nipples (Figs 1, 4). Late in the attachment phase (Group 2; 35–39 days), it was found that the percentage of composite fibres had decreased and the percentage of fast-twitch fibres had concomitantly increased. At this stage, the mother would have to carry her young (total mass of approximately 16 g, or one-third of her own mass) in the pouch when out foraging. To prevent them from dragging on the ground, the mother would need to be able to hold them close to her body but perhaps only for short periods while actually moving in search of food. The shift at this stage to fast-twitch fibres, which are usually associated with dynamic muscular activity, would presumably facilitate intermittent retraction of the young, but more observations on the behaviour of animals at this time are required to support this explanation.

Growth of the young accelerates after day 40, with 51–53-day-old young (Group 3) each weighing as much as 6 g, and 68–70-day-old young (Group 4) weighing 15–20 g. These older young are not carried in the pouch when the mother is out foraging, but there is an increase in the mass of mammary tissue up to approximately 10 g (15–20% of maternal body mass at the beginning of lactation). The most pronounced change in fibre-type composition of the ilio-marsupialis muscles associated with suckled nipples in Groups 3 and 4 is an increase in the proportion of slow-twitch (fatigue-resistant) fibres. This may occur in response to the need to support a greater mass of mammary tissue and, although there may be little need to hold the young close to her, at least some of the mass of the young while they are suckling in the nest. Studies on captive females with young of these ages have shown that the young will relinquish the nipples following vibration, for 5–60 s, of the mother's abdominal muscles and possibly also the ilio-marsupialis muscles. The major difference between fibre-type profiles in the ilio-marsupialis muscles of Groups 3 and 4 is the transformation of composite (uncommitted) fibres into fast-twitch fibres. The presence of a high proportion of fast-twitch fibres supports a dynamic function for them at these stages.

Griffiths and Slater (1988) considered that the role of the ilio-marsupialis muscle in the 'pouchless' dasyurid marsupials that they examined was to haul the young up tightly into the pouch area and so protect them from injury. In *Antechinus stuartii* (the species in which they illustrated that anaesthesia reduced the ability of the mother to hold her young close to the body) and other 'pouchless' dasyurids, the young are exposed (i.e. not enclosed by pouch skin) from the time they are born. It would be of interest to examine the fibre-type composition of the ilio-marsupialis muscles of 'pouchless' species to determine what changes occur throughout the suckling period for comparison with species such as *S. douglasi*. Such a study might help to provide a better understanding of the function of the ilio-marsupialis muscles in 'pouched' and 'pouchless' marsupials.

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