ANIMAL BEHAVIOUR, 1999, **58**, 203–209
Article No. anbe.1999.1146, available online at http://www.idealibrary.com on IDEAL

Early development of climbing skills in harvest mice

REIKO ISHIWAKA & TAKAYUKI MŌRI
Zoological Laboratory, Faculty of Agriculture, Kyushu University

(Received 8 May 1998; initial acceptance 2 June 1998;
final acceptance 15 March 1999; MS. number: 5870R)

The tiny harvest mouse, *Micromys minutus*, is skilled at climbing among grasses. Owing to the short lactation period of 15–16 days, young harvest mice need to achieve this climbing skill very rapidly. We examined the early development of five components of climbing behaviour and the final climbing pattern of harvest mice from birth to weaning. During the lactation period, the pups' climbing ability developed rapidly and they were able to climb a vertical bar by the time they first emerged from their nest. Climbing skills were acquired in the following order: hand grasping at 3–7 days; foot grasping at 6–9 days; quadruped stance at 6–11 days; tail prehension at 10–11 days; and righting at 10–12 days. The ratio of foot digit length to foot length was greater in harvest mice than in laboratory mice, *Mus musculus*, indicating a better grasping ability in the former.

The harvest mouse, *Micromys minutus*, spends most of its life in long grass and similar vegetation such as reedbeds, rushes, grassy hedgerows, ditches and bramble patches, cereals, some legumes and other crops. Probably as a result of adaptation to this habitat, adults are small, weighing ca. 8 g. They grasp leaves and stems with their feet and prehensile tail, leaving their hands free for other tasks such as feeding and grooming.

Females give birth to very immature young after a gestation period of 17–19 days (Ishiwaka & Mōri 1998). The lactation period of 15–16 days is also short compared with that of other small rodents (Ishiwaka & Mōri 1998). The pups are similar to those of other species such as house mice, *Mus domesticus*, at birth. They weigh 1–1.2 g and grow rapidly, reaching 4–5 g at weaning (R. Ishiwaka, unpublished data). The hair starts developing 3–4 days postpartum, while the eyes and the auditory meatus open at 8–9 and 9–10 days, respectively. The pups are provided with both milk and regurgitated food by their mother until weaning (Ishiwaka & Mōri 1998). They first emerge from the nest and nibble on solid food at 11–12 days (R. Ishiwaka, unpublished data). In the field, the mother leaves her pups when they are 15–16 days old (Harris & Trout 1991) and lactation almost ceases at this time in the laboratory (Ishiwaka & Mōri 1998).

Because of the short lactation period, it is likely that the pups rapidly develop the ability to climb, despite their immaturity at birth. The strong foot grasping and tail prehension characteristic of harvest mice are not common in murids, however, and thus few data are available on their development (Layne 1959; Altman et al. 1971). The behavioural development of harvest mice was described by Trout (1978), but not in detail. Our aim was to determine whether young harvest mice acquire the ability to move on grass leaves and stems during the short suckling period. We examined the development of five components of climbing: righting; quadruped stance; hand grasping; foot grasping; tail prehension; and the final climbing pattern on horizontal and vertical bars.

Correspondence: R. Ishiwaka, Zoological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan (email: ishiwaka@agr.kyushu-u.ac.jp).

MATERIALS AND METHODS

Subjects

We used harvest mice from a colony established with five wild individuals captured in Saga, Kyushu, Japan, in 1990. After the study, we kept the mice for further research. We housed the mice as breeding pairs in plastic cages, measuring 27 × 16 cm and 20 cm high, in a room with a 14:10 h light:dark photoperiod (lights on at 0830 hours) and kept at 23 ± 1°C. We covered the bottom of the cage with wood chips, and provided rice straw as nest material and shelter. There was sufficient rice straw (ca. 15 cm deep) for the pups to practise climbing in it. Water and food were available ad libitum. The diet was a mixture of canary seeds, Chinese, foxtail and Japanese millet seeds, flax seeds, sunflower seeds and commercial laboratory chow (Oriental Yeast Co. Ltd, Tokyo, Japan). We checked females for pregnancy each day to find pups within 24 h of birth.

Evaluation of Climbing Skills

We first observed a preliminary group of 17 pups to establish the criteria to evaluate the development of climbing skills. To remove the pups, we had to damage the nest, and this caused considerable stress to the mothers. We minimized this disturbance by removing a litter of pups only every other day. We thus had two groups of litters: an even-day group first observed on the day of birth, and an odd-day group first observed 1 day postpartum. The even-day group involved three males and four females from four litters, and the odd-day group six males and four females from four litters. This level of disturbance had no serious consequences such as females killing or abandoning their pups. For each pup we observed the following skills each time we removed it from the nest: righting, quadruped stance, hand and foot grasping, tail prehension and climbing behaviour on horizontal and vertical bars.

Righting

We categorized the reaction of pups when we placed them on their sides.

(1) The pup showed no attempt to right its position.

(2) The pup tried to right its position but was not able to do so within 10 s, or was not able to keep the quadruped posture for more than 5 s even if it was able to right within 10 s.

(3) The pup was able to right within 10 s and to keep the quadruped stance for 5 s or more.

(4) The pup was able to right immediately.

Quadruped stance

We placed the pup on a horizontal plate on its abdomen and classified its posture.

(1) The pup's four limbs could not prevent it from turning laterally, so it was forced to lie on its side.

(2) The pup's four limbs prevented it from turning laterally. However, the hindlimbs did not support the body, and the pup's venter touched the plate (juvenile stance).

(3) The pup maintained the adult quadruped stance and its hindlimbs held its venter above the plate.

Grasping

Each pup was held by its tail, and we rubbed the palm of a hand or the sole of a foot with the tip of a pair of tweezers (3.7 mm wide, 0.6 mm thick; Fig. 1).

(1) No response was detected.

(2) The pup extended the digits of the hand or foot.

(3) The pup grasped the tip of the tweezers, but released it if the tweezers were moved away from the pup.

(4) The pup kept hold of the tip of the tweezers when the tweezers were moved away from the pup.

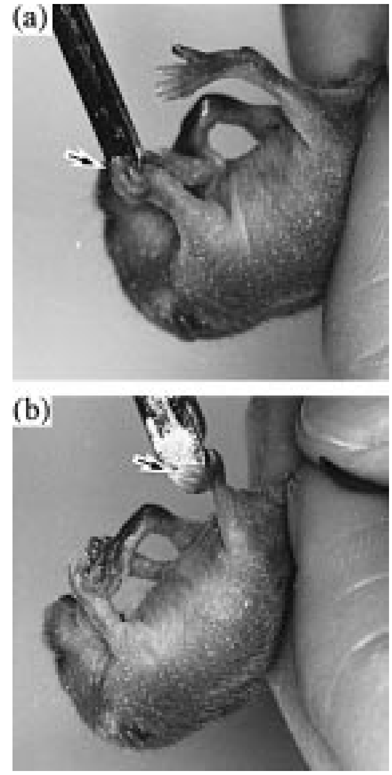

Figure 1. The hand or foot grasping test. Pups 5 days old are shown grasping the tip of a pair of tweezers with (a) the hand and (b) the foot (see arrows).

Tail prehension

The tail of the harvest mouse winds up in a reflex action, especially when the mouse loses its balance. Accordingly, we observed the response of the tail when the pup was held upside down by its dorsal skin, head and chest with the tip of its tail touching a bar made of bamboo (3 mm in outer diameter; Fig. 2). Since an elaborate movement of the tail tip is very important for tail prehension, we focused on the tail tip to assess the development of the prehension.

(1) No response of the tail was detected.

(2) The tail coiled over one-third to three-quarters of the perimeter of the bar.

(3) The tail coiled over more than three-quarters of the perimeter of the bar.

Bar holding and climbing

We placed the pups on a horizontal or vertical bar made of bamboo (3 mm in diameter) ca. 20 cm above a foam pad.

(1) The pup failed to hold on to the horizontal bar for 10 s.

(2) The pup held on to the horizontal bar (with its body crossed on the bar) for at least 10 s (Fig. 3a), but not the vertical bar for 10 s.

(3) The pup was able to hold on to the vertical bar for 10 s or more, but did not climb it.

(4) The pup was able to climb on the vertical bar 3 cm or more (Fig. 3b).

Development of Climbing Skills

We examined the development of climbing skills in another set of litters from birth to weaning. As before, we

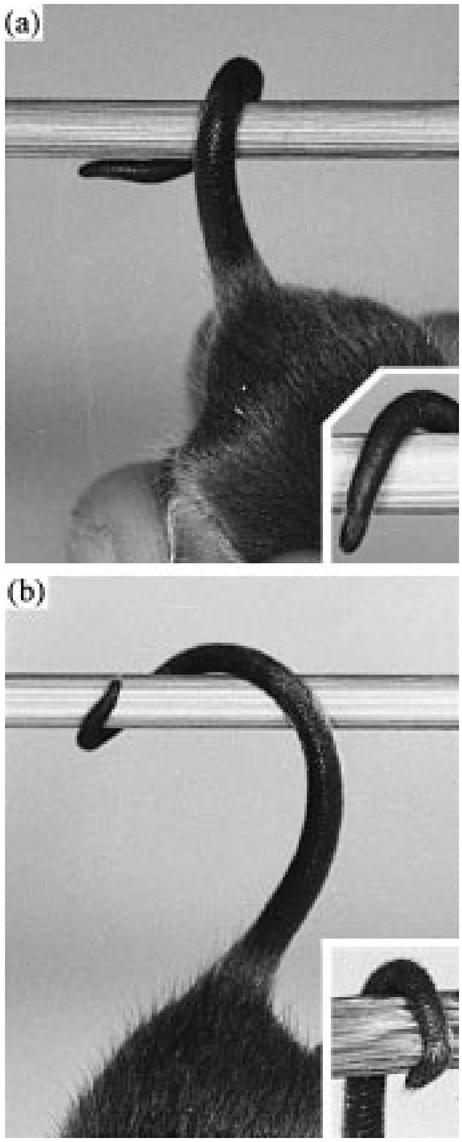

Figure 2. The tail prehension test. The tail of a pup at (a) 7 days and (b) 10 days of age, when the pup was held upside down with its tail touching a bar. The tip of the tail is not in contact with the bar at 7 days (a, inset), but is at 10 days (b, inset).

used even-day and odd-day groups. The even-day group involved nine males and 13 females from six litters, and the odd-day group involved nine males and seven females from five litters. We tested the pups' climbing skills according to the criteria described above. We present the results as percentages of pups achieving each category as a function of age.

We did not use a mixed longitudinal/cross-sectional design, for two reasons. First, it was difficult to identify and mark pups, for example by ear or toe clipping, because the pinnae of the pup adhere to its head until 3 days of age, and the toes are very important for climbing in this species. Second, we wanted to avoid stressing the lactating females. In addition, repeated tests on the same pups may affect the development of the skills we examined. A litter effect may have affected our results, because we had to compare litters rather than siblings within a litter. However, variation between litters appeared to be small, and even- and odd-day groups involved six and five litters, respectively. Therefore, this effect would be minor.

Anatomical Observations

Separation of the digits

We examined the separation of digits in pups of different ages, in another set of litters. We used even-day and odd-day groups as before. The even-day group involved five males and nine females from six litters, and the odd-day group involved seven males and five females from five litters. We recorded the age when the digits started to separate and when separation was complete. We regarded the start of separation as when more than one-quarter of the length of any digit was free from the others.

Ratio of foot digit length to foot length

We recorded the ratio of the length of the foot digits (FDL) to the foot length (FL) in 10 adult harvest mice and 10 adult laboratory mice, *Mus musculus* (five males and five females in each species). Using electric callipers, we measured the length between the base and the tip of each digit (FDL) and the length between the heel and the tip of the third digit (FL) to the nearest 0.01 mm.

Statistical Analyses

As we could not sex pups 0–5 days old, we used a Meddis nonparametric ANOVA to examine whether there was a sex difference in the development of skills from 6 days of age to the day when all the pups achieved the skill. We used a specific analysis because of the different sample sizes between the sexes. There were no significant sex differences: righting ($Z=1.11$, $N_1=63$, $N_2=73$, NS); quadruped stance ($Z=0.38$, $N_1=54$, $N_2=60$, NS); hand grasping ($Z=0.00$, $N_1=18$, $N_2=20$, NS); foot grasping ($Z=0.58$, $N_1=36$, $N_2=40$, NS); tail prehension ($Z=0.50$, $N_1=54$, $N_2=60$, NS); and bar holding and climbing ($Z=0.03$, $N_1=54$, $N_2=60$, NS). We therefore pooled values of males and females for further analyses. We used a two-tailed Mann–Whitney *U* test to test the difference in mean FDL/FL ratio between species.

RESULTS

Development of Climbing Skills

Behavioural components

We detected weak attempts to right in 10.3% of 0-day-old pups and 19.1% of 1-day-old pups (Fig. 4a). These pups moved aimlessly when placed on their side. They struggled to right using both forelimbs and hindlimbs. If they chanced to roll on to the venter, they were able to drag themselves a short distance, but soon toppled over. Temporary righting was achieved by 50% of the 3-day-old pups. Some pups could right themselves straight away at 7 days and all pups could do this by 12 days.

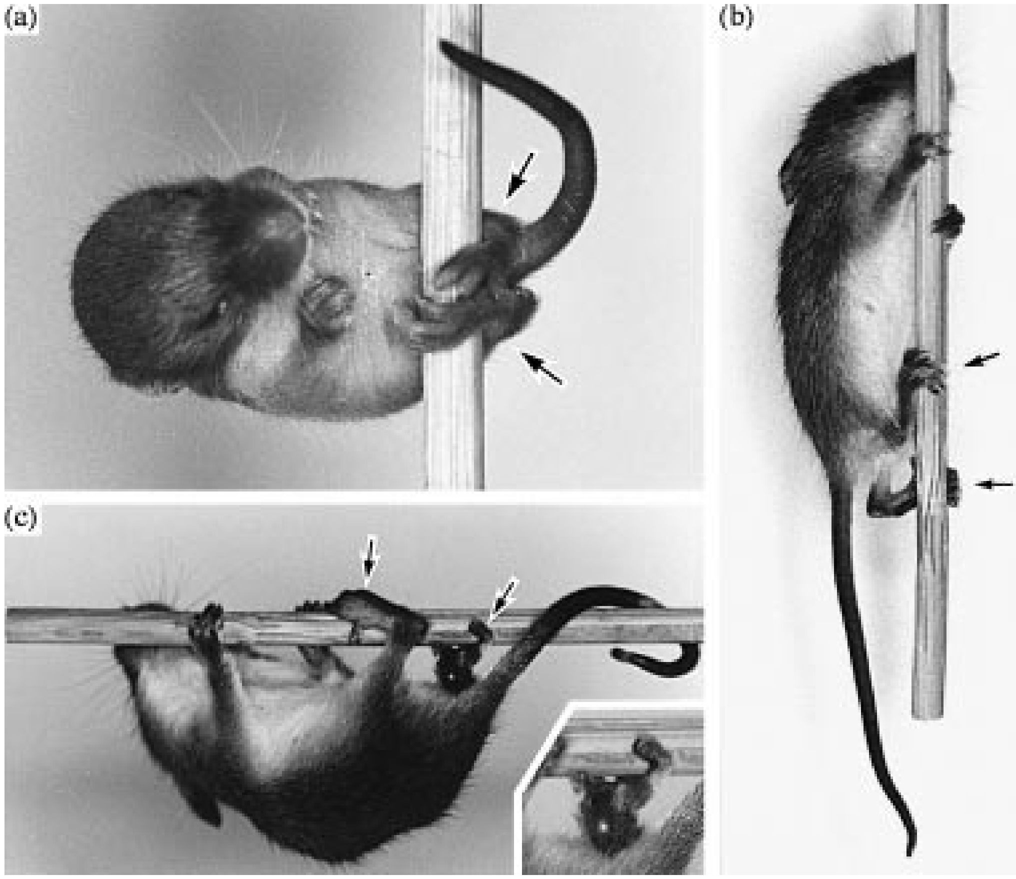

Figure 3. The bar-holding and climbing test. (a) A 7-day-old pup holding its body horizontally, supported by its hindlimbs (see arrows). Note that the photograph is taken from above, not from the side. (b) A 10-day-old pup grasping a vertical bar with its feet (see arrows). (c) A 10-day-old pup grasping a horizontal bar. The fifth digit of the left foot acts as another first digit (see the right-hand arrow and inset), whereas the first digit is opposite to the other digits in the right foot (see the left-hand arrow).

Since neonatal pups were not able to support themselves with their four limbs, their abdomen touched the plate until 2 days of age (Fig. 4b). More than half of the 3-day-old pups (64.7%) used their limbs to avoid turning laterally. By 5 days, all the pups achieved a juvenile quadruped stance in which each heel stuck out from the trunk in the dorsal view. The adult-like quadruped stance first emerged at 6 days and was fully achieved by 11 days.

More than 85% of the 0-day-old pups could extend their digits or grasp weakly with their hands, and 22.7% of them used their feet for grasping (Fig. 4c, d). All the pups showed either weak or strong grasping with hand and foot at 3 days. The percentage of pups grasping strongly increased dramatically thereafter. All the pups grasped strongly with their hands at 7 days. Strong foot grasping developed gradually, and was established by 9 days.

The tail did not show any prehensile reflex at birth. Weak tail prehension was observed in 9.1% of the 1-day-old pups, and in all pups by 4 days (Fig. 4e). Strong tail prehension was seen in more than half the pups at 10 days, and in all pups at 11 days.

Final climbing pattern

The younger pups usually hung on to a horizontal bar with three limbs (both hindlimbs and a forelimb), or occasionally just with both hindlimbs, with their whole body crossing the bar (Fig. 3a). If they could not use their hindlimbs, younger pups fell on to the foam pad. By contrast, older pups held the horizontal bar with both hands and feet, with their body parallel to the bar (Fig. 3c). The pups that succeeded in holding the horizontal bar with hands and feet were always able to hold the vertical bar (Fig. 3b). Pups 0–2 days old failed to hold on to the horizontal bar, but 17.7% held on to the horizontal bar at 3 days (Fig. 4f). All the pups achieved this skill by 7 days of age. Holding on to the vertical bar was achieved by 31.3% of the 7-day-old pups and nearly one-tenth of the 8-day-old pups successfully climbed on it. Vertical bar climbing developed dramatically thereafter, and was achieved by all pups at 11 days. The majority of pups were not able to climb the horizontal bar by the time they were weaned.

Anatomical Observations

Hand and foot digits

The digits of all limbs were attached to each other at birth but began to separate at 1 day. The hand digits were separated at 3 days. Foot digits started to separate by 3 days, and this was completed at 7 days. The joint of the

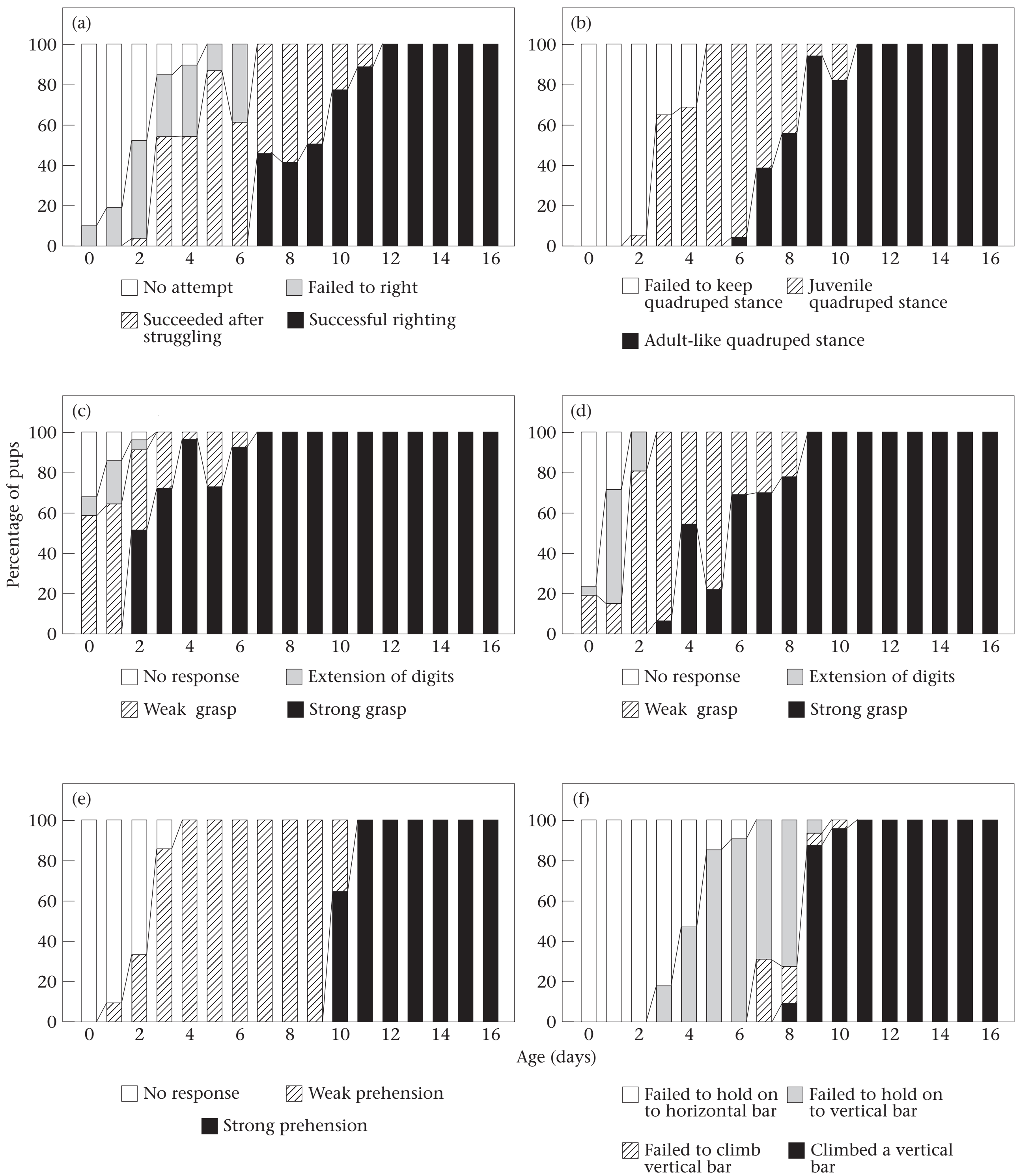

Figure 4. Percentage of pups that achieved each level of six climbing skills as a function of age. N=22 for the even-day group and N=16 for the odd-day group. (a) Righting; (b) quadruped stance; (c) hand grasping; (d) foot grasping; (e) tail prehension; (f) bar holding and climbing.

base of the fifth digit is loose enough to make a right angle with the fourth digit, and it reached the adult form by 9–10 days. It was often used instead of the first digit, supporting the side of a bar opposite to the side supported by the other four digits, making the grip firmer (arrows in Fig. 3c).

Foot digit/foot length ratio

The FDL/FL ratios of adult harvest mice, especially those of the fourth and fifth digits, were significantly larger than those of laboratory mice (Fig. 5; Mann–Whitney U test, two tailed: first digit: U=83, N_1=N_2=20, P<0.01; second digit: U=96, N_1=N_2=20, P<0.01; third

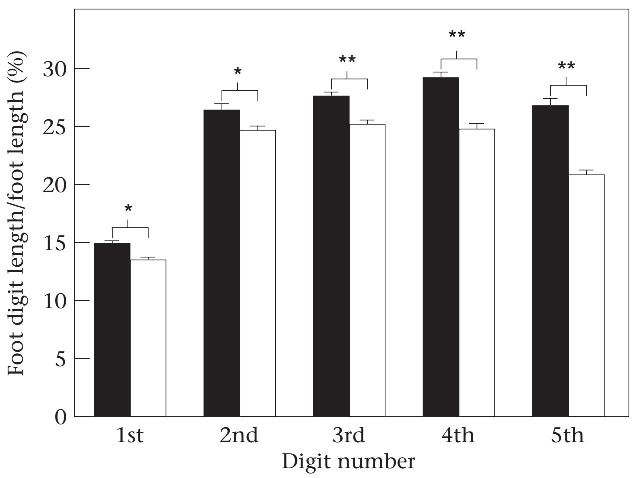

Figure 5. The ratio of the lengths of the foot digits and of the foot in harvest mice (■) and laboratory mice (□). Vertical bars show SE. **P*<0.01; ***P*<0.001. *N*=10 for each species (five males and five females).

digit: $U=76$, $N_1=N_2=20$, $P<0.001$; fourth digit: $U=30$, $N_1=N_2=20$, $P<0.0001$; fifth digit: $U=22$, $N_1=N_2=20$, $P<0.0001$).

DISCUSSION

The development of the righting response in harvest mice began earlier and took longer than the other skills, the pups going through a phase of toppling over before achieving the adult form. Despite this slow development, more than 80% of the pups over 9 days of age achieved the adult-like quadruped stance. Immediate righting probably required well-developed neural control. The earliest skills to appear were strong hand and foot grasping, suggesting they were the most important components of the climbing skill. The achievement of tail prehension by 11 days suggests that pups acquire all the skills they need for climbing grasses by the time they leave the nest. Our results indicate that the development of hand and foot grasping, the muscle mass of the hindlimbs, and interlimb coordination are the main phases in the attainment of climbing skill in harvest mice.

The separation of foot digits occurs nearly in parallel with that of hand digits in most murids, for example, *Rattus exulans* (Wirts 1973; Brooks & Htun 1980), *Praomys natalensis* (Meester 1960; Baker & Meester 1977) and *Mus musculoides* (Anadu 1976). In contrast, in harvest mice, the foot digits separated later and took longer than the hand digits. This is probably related to the large FDL/FL ratio compared with laboratory mice. The delayed and extended digit separation would explain the slower development of strong foot grasping than that of hand grasping, in addition to the longer developmental period of the hindlimbs' neural control (see below).

The development of the hindlimbs in harvest mice matched that of the forelimbs. The forelimbs were used for support in more than half the pups by 3 days. At the same time, the pups started to support their body with their hindlimbs. In contrast, early development of the forelimbs compared with the hindlimbs is reported in laboratory rats, *Rattus norvegicus* (Altman & Sudarshan 1975), and mice (Fox 1965). Forelimbs are necessary to orientate or to punch when suckling milk. Consequently, in other rodents, the quadruped stance is initiated by the forelimbs supporting the body and completed with support from the hindlimbs (Altman & Sudarshan 1975). Suckling pups in laboratory rats (Altman & Sudarshan 1975) and mice (Fox 1965) both crawl and pivot their bodies but harvest mouse pups rarely do this (R. Ishiwaka, unpublished data) probably because their hindlimbs provide more support.

Harvest mouse and rat pups hold on to horizontal bars in different ways. The hindlimbs of the harvest mouse pup were important for horizontal bar holding, whereas a suspended young rat tends to grasp a horizontally extended string or wire with its forepaws (Altman et al. 1971). In rats, grasping is followed by tensing of the shoulder muscles, attempts at pull-ups with the forelimbs, and the synergistic support of the body with the hindlimbs (Altman & Sudarshan 1975). Despite the difference in climbing object used in our tests and those of Altman et al. (1971) and Altman & Sudarshan (1975), it is clear that the hindlimbs of a young harvest mouse are extremely functional and strong compared with the young rat's hindlimbs. This is consistent with the early emergence of supporting hindlimbs seen in the development of the quadruped stance. The powerful hindlimbs of young harvest mice are probably related to their strong foot grasping.

In harvest mice, although the hindlimb's strength rapidly increases, its neural control does not. A phase of toppling over, in which pups only temporarily righted themselves, continued from 2 to 11 days before the adult form was achieved. The extended development of hindlimb neural control may delay adult-like immediate righting. In contrast, in laboratory mice, the pup tries to right itself with its forelimbs and head without using its hindlimbs (R. Ishiwaka, unpublished data). This prevents the pup turning laterally once righting is successful. Toppling behaviour has not been reported in other species, except for *Reithrodontomys humulis* (Layne 1959) in North America which lives in a similar habitat to that of the harvest mouse. Well-developed muscle mass with immature neural control would produce this behaviour.

Harvest mouse pups could not climb along a horizontal bar by the time they were weaned, indicating the limb muscles continue to develop beyond the lactation period. Horizontal bar climbing is not likely to be as essential as vertical bar climbing for living among grasses.

The prehensile tail of the harvest mouse functions as an extra limb during climbing, as in some primates and in *R. humulis* (Layne 1959), and is likely to be important for rodents living on grasses. Rodents often use their tail to help them balance while climbing. However, the harvest mouse holds on to grasses with the prehensile tail, especially when it is not able to use its hands for holding on. The achievement of tail prehension by 11 days shows that this essential skill is acquired by the time the pup starts to explore.

The foot digits of the harvest mouse, especially the fourth and fifth digits, were relatively long compared with those of the laboratory mouse. Such long fourth and fifth digits and the wide angle between them should provide a firm support on the vertically standing stems characteristic of grassland vegetation.

In general, the body systems of rodents change dramatically during the first 3 weeks after birth: morphology is modified by allometric growth (Peters 1983; Bertram & Biewener 1992; Blumberg-Feldman & Eilam 1995); the neural control of locomotion is expanded to the supraspinal centres (Viala et al. 1986); and muscle mass, muscle fibre physiology and metabolism all change (Oron 1990). Harvest mouse pups underwent extreme changes for nearly 2 weeks postpartum to become skilful at climbing. In several other rodent species, the pups go through a similar period of about 10 days between the emergence of the adult gait and the first exploration from the nest, regardless of the type of adult gait or the duration of the postnatal period (Eilam 1997). By contrast, harvest mouse pups had almost no period for practising before they emerged from the nest.

As predicted, young harvest mice were able to climb before the end of the short suckling period. This species builds its breeding nest in the stalk zone above the ground. The pups, therefore, have to be able to move around within the vegetation by the time of their first exploration. As grasses grow in the spring and wither in the autumn every year, and as they are easily flattened by wind and rain, they provide only a short-term breeding habitat. Harvest mice therefore need a short lactation period and must rapidly become proficient at climbing. The species' short gestation period and rapid sexual maturation support this hypothesis (Ishiwaka & Mōri 1998). Because of the short lactation period, females must provide their pups with large amounts of nutrients each day (Demment & Van Soest 1985). Regurgitation feeding of the pups is one of the tactics this species uses to resolve this problem (Ishiwaka & Mōri 1998). Various characteristics of the harvest mouse are thus explained by its adaptation to living among grasses.

Acknowledgments

We thank Y. Masuda of Kyushu University, T. Sakata of Senshu University of Ishinomaki, and J. M. Bedford of Cornell University Medical College for their support, advice and comments throughout our study. S. Shiraishi commented on our observation and analyses. We are grateful to A. K. Turner and the referees for their helpful comments on the manuscript.

References

Altman, J. & Sudarshan, K. 1975. Postnatal development of locomotion in the laboratory rat. *Animal Behaviour*, **23,** 896–920.

Altman, J., Anderson, W. J. & Strop, M. 1971. Retardation of cerebellar and motor development by focal x-irradiation during infancy. *Physiology and Behavior*, **7,** 143–150.

Anadu, P. A. 1976. Observations on reproduction and development in *Mus musculoides* (Rodentia, Muridae). *Mammaria*, **40,** 177–186.

Baker, C. M. & Meester, J. 1977. Postnatal physical and behavioural development of *Praomys* (*Mastomys*) *natalensis* (A. Smith, 1834). *Zeitschrift für Säugetierkunde*, **42,** 295–306.

Bertram, J. E. A. & Biewener, A. A. 1992. Allometry and curvature in the long bones of quadrupedal mammals. *Journal of Zoology*, **226,** 455–467.

Blumberg-Feldman, H. & Eilam, D. 1995. Postnatal development of synchronous stepping in the gerbil (*Gerbillus dasyurus*). *Journal of Experimental Biology*, **198,** 363–372.

Brooks, J. E. & Htun, P. T. 1980. Early post-natal growth and behavioural development in the Burmese house rat, *Rattus exulans*. *Journal of Zoology*, **190,** 125–136.

Demment, M. W. & Van Soest, P. J. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalists*, **125,** 641–672.

Eilam, D. 1997. Postnatal development of body architecture and gait in several rodent species. *Journal of Experimental Biology*, **200,** 1339–1350.

Fox, M. W. 1965. Reflex-ontogeny and behavioural development of the mouse. *Animal Behaviour*, **13,** 234–241.

Ishiwaka, R. & Mōri, T. 1998. Regurgitation feeding of young in harvest mice, *Micromys minutus* (Muridae, Rodentia). *Journal of Mammalogy*, **79,** 1911–1917.

Harris, S. & Trout, R. C. 1991. Genus *Micromys*. In: *The Handbook of British Mammals*. 3rd edn (Ed. by G. B. Corbet & S. Harris), pp. 223–239. Oxford: Blackwell Scientific.

Layne, J. N. 1959. Growth and development of the eastern harvest mouse, *Reithrodontomys humulis*. *Bulletin of the Florida State Museum. Biological Science*, **4,** 59–82.

Meester, J. 1960. Early post-natal development of multimammate mice *Rattus* (*Mastomys*) *natalensis* (A. Smith). *Annals of the Transval Museum*, **24,** 35–52.

Oron, U. 1990. Proteolytic enzyme activity in rat hindlimb muscles in fetus and during postnatal development. *International Journal of Developmental Biology*, **34,** 357–360.

Peters, S. E. 1983. Postnatal development of gait behaviour and functional allometry in the domestic cat (*Felis catus*). *Journal of Zoology*, **199,** 461–486.

Trout, R. C. 1978. A review of studies on captive harvest mice (*Micromys minutus* (Pallas)). *Mammal Review*, **8,** 159–175.

Viala, D., Viala, G. & Fayein, N. 1986. Plasticity of locomotor organization in infant rabbits spinalized shortly after birth. In: *Development and Plasticity of the Mammalian Spinal Cord* (Ed. by M. E. Goldberger, A. Gorio & M. Murray), pp. 301–310. Padova: Liviana Press.

Wirts, W. O., II 1973. Growth and development of *Rattus exulans*. *Journal of Mammalogy*, **54,** 189–202.