



Ontogeny of locomotion in mouse lemurs: Implications for primate evolution

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ABSTRACT

The environment of juvenile primates is very challenging. They have to forage and move on the same substrates as adults do and escape the same predators, despite their immature state. In this study, we explore the developmental strategies that may provide effective locomotor abilities early in life. This could provide new insights into the selective pressures acting on juvenile primates and into evolution of primate locomotion. We conducted an ontogenetic study of 36 arboreal gray mouse lemurs from birth to adulthood (6 months of age). The investigated parameters were, for both limbs, (1) grasping behavior during locomotion (i.e., grip postures), (2) grasping performance (i.e., pull strength), and (3) motor coordination (i.e., rotarod test). Our results show that 8-day-old babies are able to climb substrates of various slopes and diameters outside of their nest. Although juveniles cannot successfully complete a motor coordination test before 30 days of age, young individuals display relative pull strengths that are very high or even on par with adults, guaranteeing stability on narrow substrates. These powerful grasps highlight the importance of the grasping function for these juveniles that are not carried and move independently on arboreal substrates shortly after their first week of life. Moreover, the pedal grasping provides a secure grasp on all substrates across ontogeny; however, manual secure grasps decrease during development, being highly used only shortly after birth on vertical and narrow substrates. These results first suggest different functional roles of the hands and feet, with the hind limbs ensuring body balance on the substrates, freeing the upper limbs for manipulation. They further show vertical and narrow branches to be especially challenging, requiring strong grasps, which suggests that they may drive the evolution of strong grasping abilities in primates.

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1. Introduction

The environment of juvenile primates is very challenging. They have to forage and move on the same substrates as adults do and escape the same predators. Juvenile primates have, however, a physiological immature state, they are not 'miniature adults' as highlighted by Young and Shapiro (2018). During their development, they experience changes in body shape and behavioral transitions (Young and Heard-Booth, 2016; Druelle et al., 2017a; Boulinguez-Ambroise et al., 2019). These developmental changes make them a very relevant model to understand the evolutionary transitions from a nonspecialized to a specialized arboreal animal. Indeed, an ontogenetic approach allows one to simultaneously

quantify the changes in different parameters and relations that may exist between them. To reveal relations between form and function, it is essential to better understand the selection pressures driving the phenotypic differences between species (Byron et al., 2015; Young and Shapiro, 2018). In primates, understanding the relations between grasping form and function is relevant to understanding the anatomical specializations of the prehensile extremities which likely originated early on in their evolution (Russo and Young, 2011; Boulinguez-Ambroise et al., 2019; Thomas et al., 2016; Druelle et al., 2017a; Young and Shapiro, 2018). However, different species can share the same morphology but display different behaviors, and a shared behavior can be induced by different morphologies (Pouydebat et al., 2008, 2009, 2014). A longitudinal intraspecific, or ontogenetic, approach may consequently help better understand how the relationships between form and function develop during ontogeny and how different functional requirements imposed by the substrates animals move on may drive the evolution of the underlying anatomy and function.

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Moreover, the physiological and behavioral changes that occur during the development may be more significant than the differences observed between species (Young and Shapiro, 2018), increasing our resolution to identify relations between form and function.

Recent ontogenetic studies on locomotion in primates have demonstrated variability in terms of the morphology of the prehensile system and grasping behavior during growth (Sarringhaus et al., 2014; Young and Heard-Booth, 2016; Druelle et al., 2017a,b; Poindexter and Nekaris, 2017; Patel et al., 2018; Boulinguez-Ambroise et al., 2019). Relatively larger extremities have been documented in juvenile Javan slow lorises (*Nycticebus javanicus*; Poindexter and Nekaris, 2017) and juvenile rhesus monkeys (*Macaca mulatta*; Patel et al., 2018). Moreover, a greater anatomical mechanical advantage of both the triceps and biceps brachii has been observed in young capuchin monkeys (*Sapajus apella* and *Cebus albifrons*) than in adults (Young, 2005). Behavioral transitions have been also observed, for example, in chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla beringei beringei*), the juveniles of which display a much more arboreal locomotor repertoire than adults (Doran, 1992, 1997; Sarringhaus et al., 2014). However, very few studies have related the changes in anatomy to transitions in behavior (but refer to the studies of Druelle et al., 2017a; Boulinguez-Ambroise et al., 2019). To better establish relations between grasping form and function, several authors have pointed out the need to integrate the development of grasping performance (Young and Shapiro, 2018). Boulinguez-Ambroise et al. (2019) investigated the development of grip strength in young gray mouse lemurs (*Microcebus murinus*). They found relative near-maximal levels of grasping strength associated with relatively longer hind limbs in juveniles, suggesting selection on grasping performance. Their study, performed on individuals aged one and a half month, three months, and six months, shows the importance of collecting performance and locomotion data as soon as possible after birth. In the first weeks of life, arboreal locomotion is very challenging for juveniles, whose neuromuscular system is still not fully developed at this point in time.

In this study, we investigated the ontogeny of locomotion in the gray mouse lemur (*M. murinus*) to document the relations between grasping performance and grasping behavior. The gray mouse lemur is a small-sized arboreal primate (Cheirogaleidae) from Madagascar. Omnivorous and nocturnal, it lives in the fine terminal branches of trees—an ecological niche which likely requires strong grasping abilities (Martin, 1972; Charles-Dominique, 1977; Petter, 2010). A main interest of this model for an ontogenetic study is the fast growth it experiences. Mouse lemurs begin independent exploration outside of the nest at the age of 2 weeks, are weaned at 3 months, and adult at 6 months of age (Colas et al., 1999). From birth to adulthood, we quantified behavioral (grip postures on substrates) and performance traits (hand and foot pull strength, motor coordination). We report manual and pedal postures during horizontal walking and upward climbing on both narrow and wide substrates as substrate orientation and slope are known to influence posture (Reghem et al., 2012). Given the observations of Boulinguez-Ambroise et al. (2019) documenting relatively near-maximal levels of grasping strength in one-month-old mouse lemurs and their early independent explorations outside of the nest (Colas et al., 1999), we predict (1) juveniles to display relative hand and foot grasping strengths similar to those of adults from the first week onward. As body proportions change during growth (Druelle et al., 2017a,b; Boulinguez-Ambroise et al., 2019), changes in motor coordination are required. We thus predict (2) the acquisition of balance to occur later than grasping ability. Moreover, young mouse lemurs are not carried by the mother (the mother carries babies in her mouth only while escaping predators), and begin exploration shortly after their

first week of life (Colas et al., 1999). Thus, we expect (3) that juveniles will be able to climb and walk on various substrates shortly after birth. Taking into account the strong grasping strength and the likely lack of motor coordination, we expect them to display (4) behavioral transitions in their grasping postures during locomotion, with more powerful grasps in juveniles than in adults (Reghem et al., 2012). Finally, according to previous studies on mouse lemurs (Reghem et al., 2012; Toussaint et al., 2015), we expect (5) the diameter and the slope of the substrate to affect the use of grip postures. The validation of these hypotheses would bring new elements to our understanding of the selection pressures that have driven the ontogeny and evolution of grasping ability in primates. These results would further allow a better understanding of the potentially different functional roles between grasping hands and feet.

2. Materials and methods

2.1. Animal subjects and care

All our subjects (*M. murinus*) were descendants from wild individuals caught along the southwestern coast of Madagascar fifty years ago. They were born and raised in captivity in the colony of the UMR 7179 (CNRS/MNH) of the Muséum National d'Histoire Naturelle (Brunoy, France, Agreement F91-114-1). We longitudinally followed up a sample of 36 individuals (18 females and 18 males) during their growth. Until weaning, the young mouse lemurs were housed in the nursery with their siblings and their mother in 66 × 50 × 60 cm enclosures. At three months of age, after weaning, young mouse lemurs were placed in larger cages (167 × 60 × 70 cm) with two to seven individuals of the same age. Animal keepers enriched the cages with fresh leafy branches and wooden nest boxes. Animals were fed three times a week with pieces of fruit and a mixture made of condensed milk, Blédine (cereals and milk mixture), egg yolk, and gingerbread. Water was provided ad libitum. The temperature was maintained around 25 °C, the humidity was maintained at 40%, and the photoperiod was set at a 14 h light and 10 h darkness cycle for the summer-like season and a 10 h light and 14 h darkness cycle for the winter-like season. We identified animals, thanks to an ear tag. The Ethics Committee of the Museum National d'Histoire Naturelle approved all measurements. The research adhered to the legal requirements of the European Union (Directive 2010/63/EU).

2.2. Performance measurements

We investigated grasping performance in our sample of young mouse lemurs ($n = 36$) by measuring pull strength of both hands and feet at the ages of 8, 15, 30, 45, 90, and 180 days. We performed the measurements using a Kistler three-dimensional force platform previously used for adults (Thomas et al., 2016) and young mouse lemurs (Boulinguez-Ambroise et al., 2019). The animal repeatedly gripped a small iron bar and was then pulled away horizontally from it. We selected an iron bar of a small diameter (8 mm) so that individuals of all ages can wrap their fingers around it. The small iron bar was fixed on a piezoelectric force platform (Kistler squirrel force plate, ±0.1 N; Winterthur, Switzerland), which was connected to a charge amplifier (Kistler charge amplifier type 9865). Forces (N) were recorded at 1 kHz during 60 s sessions. We extracted the maximal pull strength from the three-dimensional force recording using Bioware software (Kistler, Winterthur, Switzerland).

2.3. Motor coordination test

We tested for motor coordination in 28 young mouse lemurs at the ages of 8, 15, 30, and 45 days by using an accelerating rotarod

(Mouse RotaRod NG, model 47650, by UgoBasile; Fig. 1). We were not able to test all of our original 36 individuals as it was possible to test only a limited number of animals per day. The experiment consisted in putting the animal on a rotating cylinder with progressive acceleration (Deacon, 2013). The rotarod performance is the time spent on the rod before the animal falls or grips to the cylinder during 3 rotations; maximal duration is fixed at 5 min (Languille et al., 2015; Pifferi et al., 2018; J. Royo, unpublished data). We first performed a 1 min habituation trial at a low and constant speed of rotation (5 rotations per minute [rpm]). After 1 min rest, we carried out the test. The test was composed of five consecutive sessions separated by 1 min rest in the nest box. During each



Figure 1. Schematic representation of the rotarod (Mouse RotaRod NG, model 47650, by UgoBasile). The animal walks on a rotating cylinder with progressive acceleration. The two circular panels prevent the animal jumping out of the way. A tub is placed with a padded cushion under the rod to secure the animal in case of a fall (the height from the tub to the base of the rod is 21 cm) so that the animal would not be hurt.

session, the cylinder was put in rotation with a progressive acceleration from 10 to 30 rpm in 300 s.

2.4. Grasp types: locomotion

We tested 21 of the 36 individuals for quadrupedal locomotion under four conditions: on a (1) wide horizontal substrate, (2) narrow horizontal substrate, (3) wide vertical substrate, and (4) narrow vertical substrate. As some females gave birth on the same days, we had to reduce the sample for these experiments: it was only possible to test a limited number of animals per day. The substrates were wooden sticks of 2 cm (wide) and 1 cm (narrow) in diameter and 50 cm in length, fixed at both ends within a Plexiglas enclosure (50 × 25 × 25 cm). We ensured that the individuals were able to wrap their digits around the narrow substrate but were not able to do so with the wide substrate. As soon as the animals moved along the branch, we filmed them using a camera (SONY Handycam DCR-SR75) and zoomed in to characterize the types of grasps used in the different conditions. The animals were tested in each condition at the age of 8, 15, and 30 days. Video analyses were conducted using software QuickTime player, version 10.4 (Apple Inc., Cupertino, CA, USA). For analysis, we recorded the ten last grasps of each sequence for both hands and feet, when the animals were engaged in movement: vertical upward climbing or horizontal walking going from the one end to the other end of the substrate. Grasps displayed when the animal was static or hanging were not taken into account. To characterize the different grasps, that is, the position of the digits relative to the substrate, we used the classification followed by Reghem et al. (2012). They identified four categories (Fig. 2), in our study species, based on the postural axis of the hand (Cartmill, 1974; Reghem et al., 2012).

2.5. Data analysis

Pull strength To investigate grasping performance across ontogeny, we tested the effect of age and sex on pull strength and ran a generalized linear mixed-effects model (family = Gaussian, link = identity) with pull strength (i.e., hand or foot pull strength) as our variable to be explained, age and sex as fixed variables, and the identity of the individual as a random effect. As we performed measurements on a longitudinal sample and as body mass impacts pull strength (Zablocki-Thomas et al., 2018), we scaled performance to body mass before analyses. We divided the force (N) by the product of the body mass (kg) and the standard gravitational acceleration (9.81 m/s²) as described by Hof (1996). Foot pull strength data were also log₁₀-transformed to meet the assumptions of normality of the residuals. We ran analyses of variance (type 3) to test the statistical significance of the models. For a better understanding of the comparison of the grasping performance across ontogeny, we also expressed the performance at 8 days relative to the performance at the older developmental stages (scaled data).

Motor coordination The rotarod performance is the time spent walking on the rotating rod. We consequently scaled the scores. If the session ended with a three rotations grip, we subtracted from the score the time spent gripping. If the individual only gripped (falling before three rotations or gripping during three rotations), we reduced the score to zero. Finally, if the animal reached the maximal duration, we kept 300 s as the score. As we are measuring performance, we kept the maximal score of the five sessions for each developmental stage.

Locomotion behavior We first calculated for each locomotion condition (i.e., the four substrate types) the percentage with standard deviation for each grasp type, considering all age categories. This illustrates which grip postures were used in which locomotion

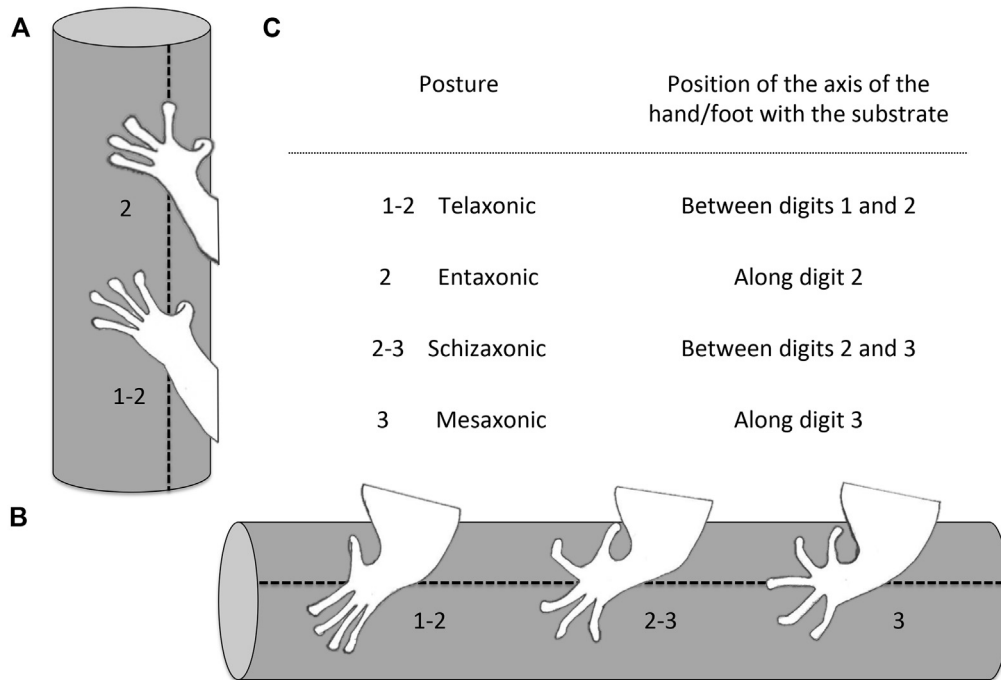


Figure 2. Illustration of grasping postures (hand of *Microcebus murinus*) on vertical (A) and horizontal (B) substrates. Postures (C) differ according to the position of the digits relative to the substrate (digit 1 is the thumb/hallux, digit 2 is the index, and so on). In the telaxonic, or secure grasp, the thumb/hallux is fully opposed to the lateral digits.

condition. We then calculated the proportion of each grip posture for each individual and each condition: each substrate and each developmental stage (i.e., at the age of 8, 15, and 30 days). Proportions were arcsin transformed to meet assumptions of normality and homoscedasticity. We tested effect of age as the predictor of behavior by running a generalized linear mixed-effects model (family = Gaussian, link = identity) for each substrate types with the grip posture as the explained variable, the age as fixed effect, and the identity of the individuals as a random effect. We ran analyses of variance to test the statistical significance of the models.

3. Results

3.1. Pull strength development

Hand pull strength (Wald chi-square value = 1.22, $p = 0.268$), relative to body mass, did not vary according to the different

developmental stages (Fig. 3). Foot pull strength (Wald chi-square value = 15.57, $p < 0.001$) showed a significant difference across ontogeny (Fig. 3). However, pull strength at 8 days was already greater than 80% of the strength at 6 months for both hands (92.7%) and feet (84.9%). Very young mouse lemurs displayed near-maximal levels of grasping strength, relative to their body mass. We found no significant effect of sex. Means of raw and scaled data are provided in Table 1.

3.2. Motor coordination acquisition

At very young ages (8 and 15 days old), baby mouse lemurs were unable to walk on the rotarod. A transition occurred when mouse lemurs were 30 days old: they were all able to walk on the rotating rod, and some of them almost ($220 \text{ s} < \text{scores} < 300 \text{ s}$) reached the maximal score ($n = 4$) or reached ($n = 1$) it. When at the age of 45 days, almost all finished the test (Fig. 4). The few individuals ($n = 3$) who did not reach the maximal score at the age of 45 days were

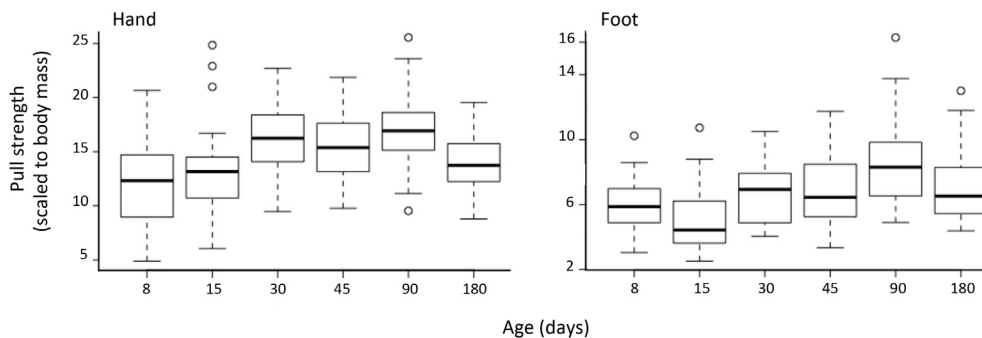


Figure 3. Box plots of the ontogeny of the relative hand and feet pull strengths (data are scaled to body mass) during the 6-month development of *Microcebus murinus*. The box plots are made of a vector containing the 1st quartile (Q1, box lower 'hinge'), the median (bold horizontal line), the 3rd quartile (Q3, box upper 'hinge'), and the adjacent values (whiskers). The length of the whiskers is calculated from the interquartile range (IQR = $Q3 - Q1$): $Q1 - 1.5 \times \text{IQR}$ (lower whisker), $Q3 + 1.5 \times \text{IQR}$ (upper whisker). The number of babies in the litter explains the higher variability in the two first stages as it affects the milk consumption and thus the rate of weight gain.

Table 1

Summary detailing the longitudinal development of pull strength in 36 young mouse lemurs (*Microcebus murinus*). Raw and scaled data for both hand (HPS) and foot (FPS) and body mass are provided. Table entries are means \pm SD. Scaled data are the force (N) that we divided by the product of the body mass (kg) and the standard gravitational acceleration (9.81 m/s²). The relative performance at 8 days is also expressed in percentage of the relative performance at the older development stages.

Age (days)	Body mass (g)	HPS (N)	HPS (scaled)	Relative HPS at 8 days (%)	FPS (N)	FPS (scaled)	Relative FPS at 8 days (%)
8	13.1 \pm 3.4	1.6 \pm 0.5	12.9 \pm 4.7	100	0.8 \pm 0.2	6.1 \pm 1.7	100
15	19.3 \pm 4.3	2.4 \pm 0.7	13 \pm 4.1	99	0.9 \pm 0.2	5 \pm 1.9	121
30	30.3 \pm 5.4	4.6 \pm 0.9	15.7 \pm 3.3	82	1.9 \pm 0.6	6.4 \pm 1.9	94.7
45	40.1 \pm 5.6	5.9 \pm 1.4	15 \pm 3.2	85.6	2.6 \pm 0.8	6.7 \pm 2.1	90.8
90	59 \pm 8.1	9.8 \pm 2.1	17 \pm 3.4	75.7	4.9 \pm 1.5	8.6 \pm 2.7	71.1
180	89.2 \pm 20	11.8 \pm 2.2	13.9 \pm 3	92.7	6.2 \pm 2	7.2 \pm 2.1	84.9

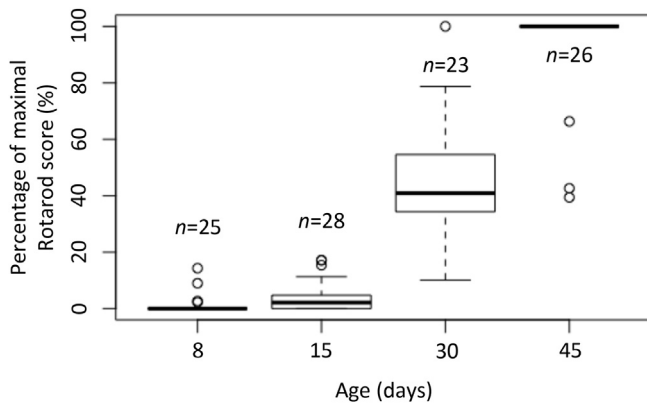


Figure 4. Box plots of the evolution of balance performance (quantified by the rotarod test) during the development of *Microcebus murinus*. Juveniles were able to walk on the rotating rod when they were 30 days old. The observed variability at this stage can be explained by a litter effect: there can be up to four mouse lemurs in the same litter. Consequently, they do not have equal access to maternal milk, which makes them grow at different rates. The maximal score is fixed at 300 s, the rotating rod reaching 30 rotations per minute.

tested five days later, and all made it. Small sample variations between stages are due to a few individuals that refused to perform the test, at some points, by jumping repeatedly from the device as soon as they were put on it.

3.3. Locomotion grasping repertoire development

Overall, mouse lemurs used predominantly telaxonic (digits 1 and 2) and entaxonic (digit 2) manual grasps on vertical substrates, whereas they used predominantly schizaxonic (digits 2 and 3) and mesaxonic (digit 3) manual grasps on horizontal substrates (with very anecdotal uses of 2 and 3–4 grasps; Fig. 5). For both narrow (Wald chi-square value = 19.59, $p < 0.001$) and wide (Wald chi-square value = 9.99, $p < 0.01$) vertical substrates, the proportion of 1–2-type grasps decreased across development, whereas the proportion of 2-type grasps increased (Wald chi-square value = 33.9, $p < 0.001$; Wald chi-square value = 18.75, $p < 0.001$, respectively; Fig. 6). The proportion of 2–3-type grasps decreased (Wald chi-square value = 18.84, $p < 0.001$), whereas the proportion of 3-type grasps increased (Wald chi-square value = 47.8, $p < 0.001$) across the development for walking on narrow substrates (Fig. 7). The use of 2–3- and 3-type grasps did not change significantly across development for walking on wide substrates (Fig. 7). We observed a unique posture for pedal grasping, maintained during development: the hallal secure grasp with the hallux being in opposition with all the lateral digits.

4. Discussion

In our general hypothesis, we expected the grasping behavior during locomotion to vary throughout ontogeny in relation to the development of grasping performance. Our results assessed our five predictions.

Our first prediction suggested that strong grasping performance would be acquired very early after birth. We found relative grasping performance to be very high or even on par with adults in our first developmental stage (i.e., 8 days of age). In fact, relative hand maximal pull strength did not vary across the development: at the first week of life, it reached 92% of the strength of six-month-old individuals (it is to be noted, however, that absolute performance does increase dramatically with age). Relative foot maximal pull strengths did increase across the development, but eight-day-old individuals already displayed near-maximal levels of grasping strength, reaching 85% of the adult performance. These results match with those of and complete the study of Boulinguez-Ambroise et al. (2019), which measured grasping performance in 1-and-half-month-, three-month-, and six-month-old mouse lemurs. Furthermore, they found a positive relationship between size of the hind limbs and foot grasping performance and found relatively longer hind limbs in juveniles. This suggests that the relatively longer limbs of juveniles may permit young primates to compensate for their immature musculature, allowing them to display high grasping abilities very early after birth. This further suggests selection on grasping performance early in life. The longer limbs of juveniles may be explained by growth allometries: limbs are made of many different segments growing at different rates causing differences in bone proportions across the development (Young and Shapiro, 2018). Strong grasping performance at young ages may also be enabled by greater mechanical advantage in juveniles. For example, the forelimb musculature of young capuchin monkeys (*S. apella* and *C. albifrons*) displays high anatomical mechanical advantage relative to adults (Young, 2005). Having a high anatomical mechanical advantage decreases the amount of muscle force required to set the limbs in motion (Young and Shapiro, 2018). This feature may consequently offset musculoskeletal immaturity and permit strong grasping performance early in development. However, the ontogenetic decrease of the limb muscle anatomical mechanical advantage is not shared across primates (Fellmann, 2011, 2012). More studies on the ontogenetic changes in mechanical advantage of the muscles important in grasping are needed to better understand whether they drive the relatively high levels of grasping performance early in life.

Our second prediction stated that the full acquisition of motor coordination would occur later than for grasping performance. Our results validated this hypothesis. Motor coordination is commonly assessed by the rotarod test in small rodents (Deacon, 2013). We carried out this test in our young mouse lemurs, whose size and weight are appropriate for the device. Our subjects became

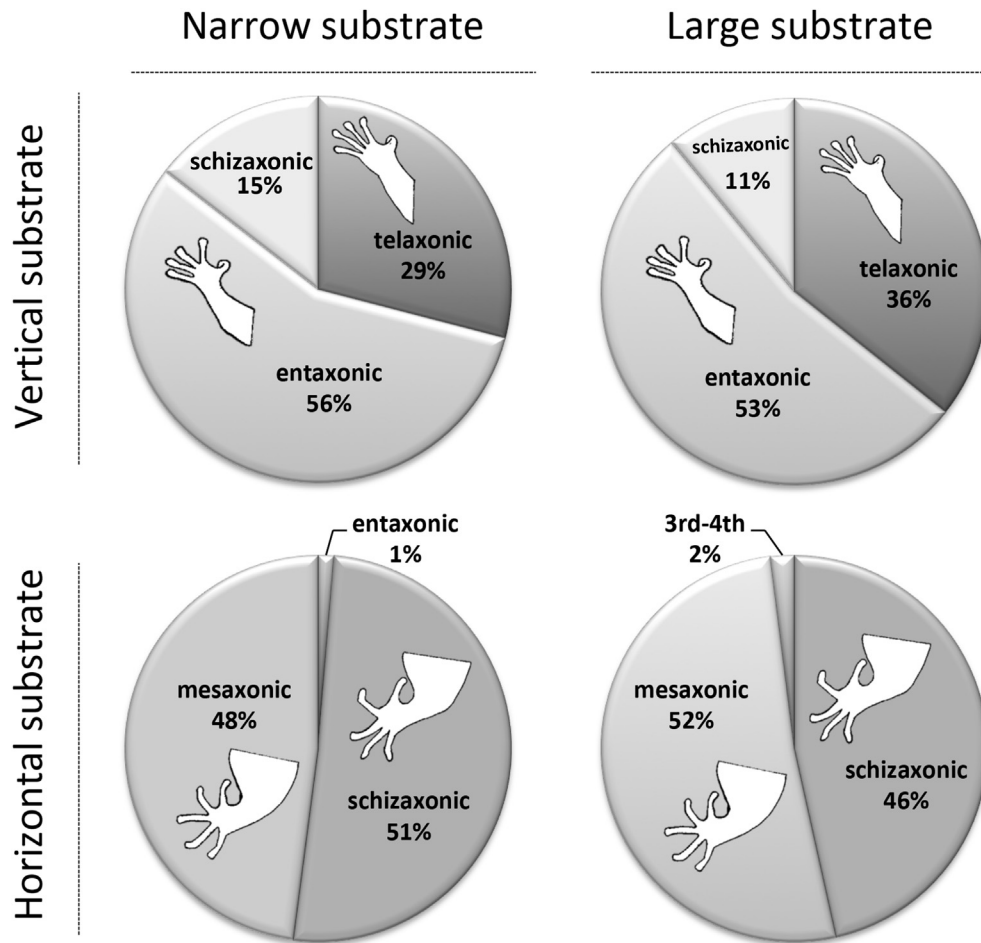


Figure 5. Overview of the use of the different hand postures during climbing and quadrupedal walking (i.e., horizontal) on both narrow and wide substrates in *Microcebus murinus* (all age-groups considered).

successful in walking on the rotating rod at 30 days of age and finished the full test at 45 days of age. However, the baby mouse lemurs were successful at climbing on vertical substrates and walking on horizontal substrates as soon as they are 8 days old, validating our third prediction. Well-coordinated movements are observed in many tetrapod neonates, whose nervous system, including peripheral nerves, can be functional even before the appearance of locomotor behaviors (Carrier, 1996). The early onset of climbing and walking indicates a relatively functional and mature nervous system shortly after birth. The lack of motor coordination revealed by the rotarod test, namely, the difficulty to maintain balance and posture, may then be explained by the process of growth itself. Indeed, Carrier (1996) highlighted the problem of the integration of sensory input, neural control, and motor output during growth. He first evoked the ontogenetic changes of the musculoskeletal system that lead to changes in motor output. But also, altered sensory fields during the development make a correct perception difficult and may cause difficulties in the control of balance and position (Knudsen and Knudsen, 1985; Carrier, 1996). However, the control of balance and position seems to be an important parameter for efficient locomotion on narrow branches, which suggests adaptations that offset this lack of motor coordination and improve the performance of juveniles. The strong grasping performance we found shortly after birth may be one of these adaptations. High strengths in the hand and foot grasps may ensure the grip on the substrate during moving and overcome

unbalanced motions that could lead to a fall. Although most studies focus on selection on adult life stages, selection acts from the beginning and may be even stronger at the vulnerable juvenile stages; the study of juvenile development may thus better account for the selection pressures driving the evolution of form and function in adults (Carrier, 1996; Young and Shapiro, 2018). The validation of our three first predictions highlights the crucial function of grasping in primates involving the very fast acquisition of powerful grasping as early as the first days of life. Our next predictions refer to the potential selection pressures that may have driven these strong grasping abilities in primates.

Our two last predictions stated that ontogenetic changes in grasping postures would occur during quadrupedal locomotion, with an effect of the diameter and the slope of the substrate. Our results validated these hypotheses. We observed telaxonic (digits 1 and 2) and entaxonic (digit 2) postures only on vertical substrates, whereas the mesaxonic grasping (digit 3) occurred only on horizontal substrates. The schizaxonic grasps (digits 2 and 3) were used on both vertical and horizontal substrates. This use of different types of grasps between vertical and horizontal substrates, with more ulnarly deviated postures (digits 1 and 2 and digit 2) on vertical substrates, asserts to the effect of substrate orientation on manual grasping, which tallies with previous studies on hand postures during quadrupedal locomotion (Cartmill, 1974; Lemelin and Schmitt, 1998; Reghem et al., 2012). Indeed, according to hand posture, the axis of the substrate will fall between or along the

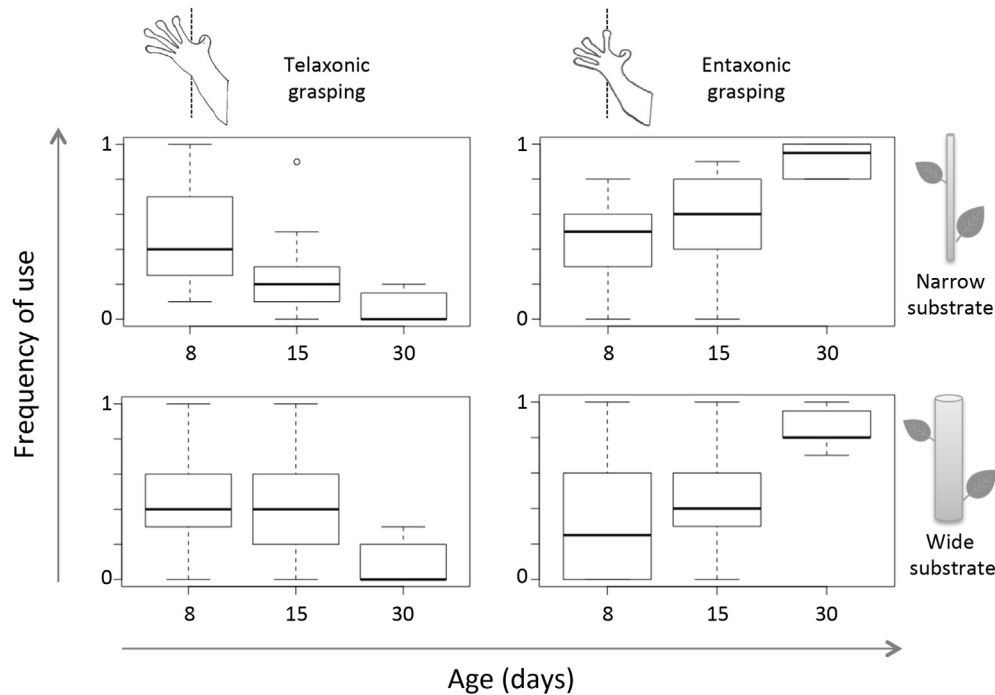


Figure 6. Box plots of the use of telaxonic (digits 1 and 2) and entaxonic (digit 2) manual grasping at three developmental stages (i.e., at the age of 8, 15, and 30 days) in *Microcebus murinus* during climbing on vertical narrow (1 cm) and wide (2 cm) substrates. The box plots are made of a vector containing the 1st quartile (Q1, box lower 'hinge'), the median (bold horizontal line), the 3rd quartile (Q3, box upper 'hinge'), and the adjacent values (whiskers). The length of the whiskers is calculated from the interquartile range ($IQR = Q3 - Q1$): $Q1 - 1.5 \cdot IQR$ (lower whisker), $Q3 + 1.5 \cdot IQR$ (upper whisker).

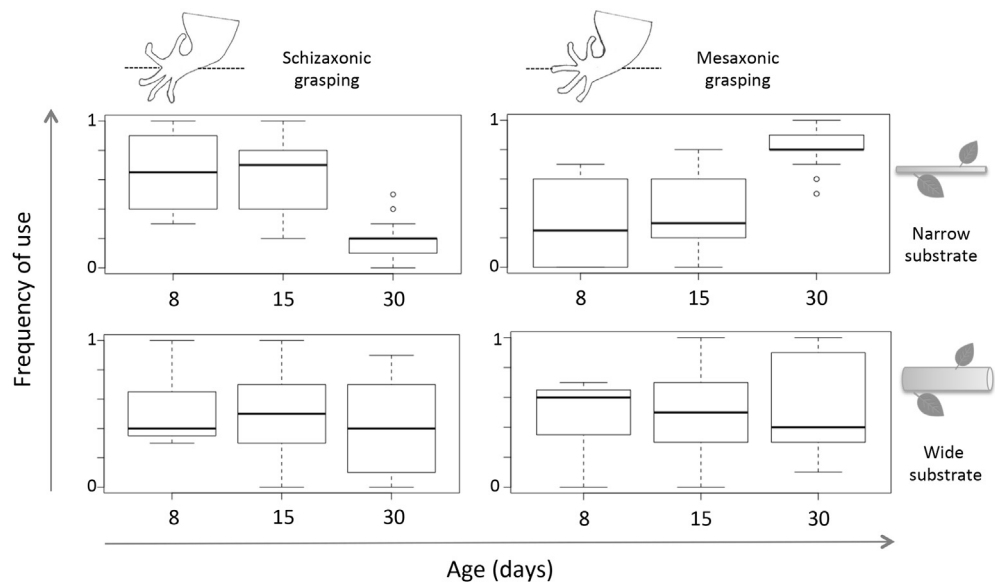


Figure 7. Box plots of the use of schizaxonic (digits 2 and 3) and mesaxonic (digit 3) manual grasping at three developmental stages (i.e., at the age of 8, 15, and 30 days) in *Microcebus murinus* during quadrupedal walking on horizontal narrow (1 cm) and wide (2 cm) substrates. The box plots are made of a vector containing the 1st quartile (Q1, box lower 'hinge'), the median (bold horizontal line), the 3rd quartile (Q3, box upper 'hinge'), and the adjacent values (whiskers). The length of the whiskers is calculated from the interquartile range ($IQR = Q3 - Q1$): $Q1 - 1.5 \cdot IQR$ (lower whisker), $Q3 + 1.5 \cdot IQR$ (upper whisker).

lateral rays of the hand. The axis of the hand is thus progressively deviated from the substrate axis, which involves an ulnar deviation, with the hand being oriented toward the ulna at the wrist joint (Reghem et al., 2012). This ulnar deviation is particularly pronounced in the telaxonic grasp (digits 1 and 2), whose hand-substrate axis falls between the first and the second ray. In contrast, the mesaxonic grasping along the third ray is in a more

neutral posture of the hand and does not involve a deviation of the ulna. Lemelin and Schmitt (1998) observed that the use of ulnarly deviated hand postures follows substrate preference in seven primate species. The highly arboreal species displayed the most deviated manual postures on both poles and on the ground, whereas highly terrestrial species displayed small deviations. The adaptations to the challenges of arboreal locomotion should be

greater in young mouse lemurs, whose balance is not yet fully developed. From the age of 8 days until 30 days, which corresponds to the achievement of a successful rotarod test, our study showed an ontogenetic decrease in the use of telaxonic grasps (digits 1 and 2) on both narrow and wide substrates. Manual secure grasping (i.e., telaxonic grasps) was commonly used (around 40%) shortly after birth and then decreased during growth, whereas the use of the less deviated entaxonic posture (digit 2) increased to become largely predominant. Regarding horizontal substrates, we observed an additional effect of diameter on the use of hand postures. On narrow horizontal substrates, the use of schizaxonic grasping prevailed (>60%) in the first 2 weeks of life and then decreased, being replaced by the nonulnarly deviated mesaxonic posture. However, there was no change in the proportions of hand postures used on wide horizontal substrates, with a preference for mesaxonic grasping. On the one hand, these findings highlight the strong constraints imposed by vertical arboreal locomotion. For climbing, young mouse lemurs privileged the most ulnar-deviated hand posture (i.e., telaxonic, also called secure grasp). The secure grasp recruits the whole palm of the hand and constitutes a strong pincer, with the thumb being in opposition with the fingers. This manual posture, in association with the high relative grasping strength we documented at these ages, likely enables a powerful forelimb action. These physiological and behavioral adaptations may help overcome the lack of motor coordination and enable efficient arboreal locomotion. Ontogenetic changes in limb postures as behavioral compensation were also observed in squirrel monkeys (*Saimiri boliviensis*). Younger individuals display more extended forelimbs than older ones, enabling them to maintain joint posture with a reduced muscle force requirement (Young, 2009). On the other hand, the difference we observed between narrow and wide horizontal substrates emphasizes the challenge of locomotion on narrow branches. In contrast with the wide horizontal substrate, the narrow one involved a behavioral adaptation overcoming the lack of balance at young ages. With regard to these results, the verticality and the narrowness of arboreal substrates seem to be strong drivers of the selection on grasping ability in primates. The question of whether primate grasping hands and feet evolved under the same selection pressures or not is discussed in the following section and highlights how our results may contribute to a better understanding of the role of grasping in primate evolution.

Although the acquisition of balance led to less deviated postures in the hands, the feet only displayed secure pedal grasping. Secure pedal grasping (i.e., hallucal grasping) is also used by other small nonprimate mammals moving on fine branches (Youlatos, 2008, Youlatos et al., 2018; Byron et al., 2011; Urbani and Youlatos, 2013): namely, the woolly opossum (*Caluromys philander*), the feathertail glider marsupial (*Acrobates pygmaeus*), rodents such as the harvest mice (*Micromys minutus*), and also mice raised in a simulated fine-branch arboreal niche. Indeed, hallucal grasping ensures balance and safety for arboreal locomotor behaviors such as climbing and walking (Urbani and Youlatos, 2013). The maintenance of the secure grasping as a pedal posture during mouse lemur development suggests different functional roles between the hands and feet and emphasizes the role of anchoring ensured by grasping feet. This more substantial role of the feet in primate locomotion is also suggested in other studies. Red ruffed lemurs (*Varecia rubra*) rely more on hind limb than on forelimb grasps during arboreal quadrupedal locomotion, with toe flexors being activated more and longer than the finger flexors (Patel et al., 2015). In addition, ontogenetic data on locomotor development in baboons (*Papio anubis*) revealed a correlation between changes in foot proportions and the time spent in arboreal behaviors, whereas hand proportions were uncorrelated (Druelle et al., 2017a). Moreover, even if their relative ulna length is similar across ages, young

mouse lemurs display relatively longer hind limbs (i.e., tibia and metatarsus) than adults, limb length being positively related to grasping performance (Boulinguez-Ambroise et al., 2019; Thomas et al., 2016). The relatively high foot grasping performance observed in one-month-old juveniles enabled a strong hold on branches, permitting the use of adult bimanual grasping strategies needed to capture mobile prey (Boulinguez-Ambroise et al., 2019). The differences observed between manual and pedal grasping extremities suggest they evolved in different selective contexts, with a division of function. Both hands and feet are recruited for arboreal locomotion, but grasping feet seem to have a more substantial role in locomotion, ensuring anchor and balance on narrow substrates, freeing the forelimbs for other functions (Cartmill, 1985). Specifically, the use of the hands (vs. the mouth) in prey grasping increases with a decrease in the diameter of the substrate in *M. murinus* (Toussaint et al., 2013). Thus, grasping hands may have also undergone foraging selective pressures as they enable predation and manipulation of resources in the arboreal narrow terminal branch milieu (Rolian et al., 2010; Toussaint et al., 2015; Young and Heard-Booth, 2016; Boulinguez-Ambroise et al., 2019).

5. Conclusions

Baby mouse lemurs are not carried by the mother; they move independently on arboreal substrates outside of the nest as soon as they are ten days old. Their efficient arboreal locomotion, shortly after birth, is enabled by morphological and behavioral adaptations that overcome immaturity. Fast acquisition of powerful grasping turns out to be crucial for mouse lemurs whose motor coordination matures later. The ontogenetic changes we observed in the present study emphasize the selection pressures acting in the ecological arboreal niche. Specifically, narrow and vertical substrates appear to have been strong drivers of selection on grasping ability in primates. Furthermore, hands and feet appear to have different functional roles and have likely evolved under different ecological constraints (locomotion and manipulation, respectively). Further ontogenetic studies on the development of hind limbs and forelimbs in both primate and nonprimate arboreal mammals are needed to better understand this division of labor and the associated grasping features.

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