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Lion-tailed macaques show a stable direction and reinforcement of hand preference in simple reaching tasks over several years

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Abstract. Research on hand preference in non-human primates provides information about the evolutionary origin of population-level bias of human handedness. Human hand preference has been shown to remain stable throughout an individual's lifespan. However, the stability of hand preference and its change with age in non-human primates remains questionable. We recorded hand use in lion-tailed macaques (*Macaca silenus*) during simple reaching tasks in three time periods over six years. We tested the effect of age and body posture on the direction and strength of hand preference in 23 observed individuals. In a subsample of 13 individuals followed for two or three subsequent time periods, we assessed the stability of hand preference across study periods. The direction of hand preference was highly stable; we detected no individuals changing from a left- to right-, or right- to left- preference and repeated quantitative measures of hand preference were correlated among subsequent study periods. Hand preference was, however, reinforced in older individuals and an individual's hand preference was stronger in postures with both hands free for foraging. Stable hand preference at an individual level, and its reinforcement over an individual's lifetime, is emerging as a robust finding across the primate order.

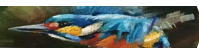
Key words: handedness, hand use, laterality, unimanual behaviour, *Macaca silenus*

Introduction

Lateral bias in behaviour appears to be widespread in vertebrates and includes asymmetry in the use

of sensory organs, jaws, feet, limbs, infant cradling, or whole-body turning (Rogers & Andrew 2002, Schaafsma et al. 2009, Ströckens et al. 2013). A behaviour that has received particular attention

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is the lateralized use of hands for reaching and manipulating food and other objects (Forrester 2017). Interest in this behaviour is mainly fuelled by the fact that humans are characterized by a hand-use bias at the population level; across all human cultures a higher proportion of individuals preferentially use their right hand over their left for a variety of manipulative tasks (Annett 2002).

The hands of non-human primates, like those of humans, are well-adapted for complex manipulative tasks (Preuschoft & Chivers 1993). Preferred use of one hand, either at the individual or population level, has been found across the primate order, i.e. in representatives from most strepsirrhine families (Ward et al. 1993, Milliken et al. 2005, Leliveld et al. 2008, Lhota et al. 2009), New World monkeys (Meguerditchian et al. 2012, Hashimoto et al. 2013, Nelson et al. 2015), Old World monkeys (Meguerditchian et al. 2011, Maille et al. 2013, Regaiolli et al. 2018), lesser apes (Stafford et al. 1990) and great apes (Hopkins et al. 2011), although it appeared not to be a rule for all primate species across a variety of behaviours. The pattern of hand use is affected by several factors: demographic and social characteristics as well as those related to context and behaviours observed (Chapelain & Hogervorst 2009).

Homology of the proximate mechanisms that determine hand preference in non-human primates compared to humans remains a subject of discussion (Fagot & Vauclair 1991, McGrew & Marchant 1997, Fitch & Braccini 2013). In humans, early forms of lateralized behaviours are detectable prenatally; the human foetus preference for one thumb over the other for suckling is consistent with the hand preference in reaching tasks several weeks postpartum (Hepper et al. 1991). In early childhood, human handedness may still be subject to developmental transitions but by the age of three or four years, individual handedness for reaching or grasping, in the sense of the direction, is relatively stable (Michel 2002, Corbetta et al. 2006, Scharoun & Bryden 2014, but see also Porac et al. 1980 or Dellatolas et al. 1991), although, the degree of handedness may continue increasing over time (McManus et al. 1988).

Only a few studies have recorded hand preference across an individual's lifespan in non-human primates. Hand preference in a naturalistic group of captive chimpanzees (*Pan troglodytes*) for a coordinated bimanual (tube) test remained stable

over a period of 11 years, although it only remained stable during a one-year period in the case of a simple unimanual task (Padrell et al. 2019). Adult olive baboons (*Papio anubis*) maintained the same hand preference for unimanual and bimanual food manipulation five years after first testing (Molesti et al. 2016). For gestural communication the time consistency has been demonstrated in adult olive baboons four years after first testing (Meguerditchian et al. 2011). Early-life lateral biases in chimpanzee infants, including hand-to-mouth actions and defensive reaching, predicted hand preferences for a coordinated bimanual task when the individuals were juveniles (Hopkins & Bard 2000). The results of studies that have compared cross-sectional hand preference of individuals of different ages are contradictory. Some studies have shown that hand preferences of non-human primates are reinforced with increasing age across different tasks: experimental tube task (Chapelain et al. 2011), bimanual feeding (Rogers & Kaplan 1996), gesticulation (Hopkins & Leavens 1998), food reaching (Stafford et al. 1990), but others detected no such relationship: tube task (Hopkins et al. 2011), food reaching and tube task (Nelson et al. 2015, Molesti et al. 2016), or even that hand preference diminishes with age, e.g. nut cracking (Boesch 1991). Few studies have revealed a changing population-level hand preference bias with increasing age (Forsythe & Ward 1988, Harrison & Byrne 2000), suggesting that age may influence not only strength but also the direction of hand preference.

Hand preference in non-human primates may also be affected by the body posture adopted during an observed behaviour. The postural origins theory (MacNeillage et al. 1987, MacNeillage 2007) suggests that human handedness evolved from manual laterality of an ancestral arboreal primate, who used the right hand for postural support and left hand for visually guided tasks. Several authors have demonstrated that hand preference is stronger when primates adopt more demanding body postures, compared to easier or habitual ones (upright posture: Larson et al. 1989, Hashimoto et al. 2013; upright posture and hanging: Blois-Heulin et al. 2006; leaning to moat: Forsythe & Ward 1988), due to different sensorimotor demands of alternative body postures. However, other authors have suggested that complex postures that require use of one hand for postural support, depending on the substrate structure, may decrease the preference (bamboo processing: Stafford et al.



1993; arboreal ant fishing: Marchant & McGrew 2007). A possibility is that when foraging in a complex environment requiring the adoption of a stable body posture, the choice of hand for the manipulation of food may be constrained. Finally, some authors have found no effect of body posture on the strength of hand preference (tapping and probing with fingers: Lhota et al. 2009).

Macaques (genus *Macaca*) represent one of the most comprehensively studied groups of non-human primates; several studies on hand preference have been conducted using this genus (reviewed in Regaiolli et al. 2018). In all macaque species studied so far, significant differences in hand preference have been reported at an individual level, but only two studies shown a right- or left- hand bias in hand use at the population-level. Barbary macaques (*Macaca Sylvanus*) exhibited significant right-hand bias for bimanual (complex tube) task but not for a unimanual task (Regaiolli et al. 2018), whereas rhesus macaques (*Macaca mulatta*) more frequently used the left hand for both types of task (Westergaard et al. 1997). The lion-tailed macaque (*Macaca silenus*) represents one of the most ecologically divergent macaque species. Their diet includes a wide range of plants (fruits, seeds, fungi, sprouts) invertebrates and vertebrates. Small insects are often extracted by breaking off tree bark or breaking dead branches and they have been reported to use tools (Hohmann 1988, Westergaard 1988). This species is the most arboreal of the genus, spending most of their time in the upper or middle strata of the forest (Santhosh et al. 2015). They, therefore, represent a suitable model species for addressing the postural origins theory of laterality. Despite this, only one study has focused on hand preference in this species and followed four individuals during the manufacture and use of probing tools, with three of the individuals proving markedly left-handed (Westergaard 1991).

In the present study we assessed hand preference in captive lion-tailed macaques (*Macaca silenus*) while performing a simple reaching for food task. It has been argued that hand preference may depend on the type and complexity of the manual action (Fagot & Vauclair 1991, Rogers 2009), i.e. complex behavioural tasks are more likely to elicit stronger and more stable hand preference than simple behavioural tasks (Hopkins et al. 2007). However, hand preference for the most frequent components of foraging behaviour, such as simple reaching, may have a more profound effect on foraging

efficiency and, in turn, individual fitness of the animals. Further, this behaviour may bring valuable insights into understanding hand preference across the primate radiation (Papademetriou et al. 2005), since it occurs frequently as a part of natural behaviour in most primates and in many other animals. It can easily be studied across all possible settings including studies in the wild as well as on zoo primates kept in coherent groups, where food competition, dominance relationships among group members, as well as husbandry and welfare concerns, may not allow for individual experimental testing.

We used repeated observations of the same individuals over a six-year period to assess the stability of their hand preference. We predicted that a significant right-hand preference would not change to a significant left-hand preference or vice versa in subsequent periods of observation, in homology to the stable preference that has been previously reported in humans. We combined a longitudinal and a cross-sectional approach to test for the effect of age on quantitative measures of strength and direction of hand preference. We predicted that the strength of hand preference would increase with age, while we did not expect any effect on directionality. To assess the relative importance of age compared to other factors that may influence hand preference, we also tested cross-sectionally the effect of body posture on the strength and direction of hand preference. We predicted that body posture would influence the strength of hand preference, without specifying which position (one-free-hand or two-free-hands) should increase the strength of preference.

Methods

Subjects

This study was based on 374 hours of observations of two groups of captive lion-tailed macaques held at Ostrava Zoo and Plzeň Zoo. Data were collected during three different study periods (S1-S3): (S1) August-October 2009, 18 individuals were followed by B. Kuběňová; (S2) July and September 2013, 13 individuals were followed by V. Tomanová; (S3) June and August 2015, nine individuals were followed by V. Tomanová. Altogether, 23 different individuals were observed (males = 9, females = 14). We followed all individuals older than one year, which is the age of weaning for this species, (Lindburg et al. 1989). Captive lion-tailed macaques reach sexual maturity at the age

of four years in females and five years in males in North American zoos (Lindburg et al. 1989). Our sample, therefore, included individuals that can be classified as juveniles and adults (see Table 1). Although the species is reported to live for up to 40 years in captivity (Weigl 2005), the oldest individual in our study, aged 25 years, already appeared old. Due to death, age restrictions and animal transfers to/from different facilities, we could not observe all individuals during all of the study periods. Subsequently, nine individuals (males = 2, females = 7) were observed during two, and four individuals (males = 0, females = 4), during all three periods.

All individuals were born in captivity and had been socially housed since birth. In both zoos, enclosures were divided into indoor and outdoor quarters, equipped with platforms, ropes and branches; individuals of both groups could freely move within and between the quarters. In Ostrava Zoo, individuals were fed four times each day with granules or cereals, fruit, raw and cooked vegetables, a protein-rich meal (small pieces of cooked chicken or fish, mealworms, cottage cheese, yogurt), boiled potatoes, or pasta. In Plzeň Zoo, individuals were fed three times each day with fresh vegetables and fruit, mealworms, boiled potatoes, pasta, and a mixture of oatmeal, yogurt, and honey. Fruit and vegetables were cut into approximately $3 \times 3 \times 5$ cm pieces, e.g. apples were halved.

Data collection

Data were collected using a focal-animal sampling method (Altmann 1974). Individuals were selected in random order for observation and were observed for eight hours each, starting with the first feeding of the day (Ostrava Zoo at 7:30, Plzeň Zoo at 8:00) and ending with the last feeding of the day (Ostrava Zoo at 15:30, Plzeň Zoo at 16:00). This time corresponded with the groups' active period. There was a 30-minute break in data collection around noon, when macaques were mostly resting. Each individual was observed at least twice per study period (S1, S2, S3).

We recorded spontaneous hand use during normal feeding on a typical diet for the animals. We define feeding as an individual grasping food and placing it into its mouth. We recorded the hand that picked up a piece of food. Usually, individuals used the same hand to pick up the food from the ground and put it into the mouth, which resulted in one record

in our data. On rare occasions, when individuals used one hand to pick up food from the ground and the other to place it into mouth, we made one record for each hand, to distinguish the situation from the single hand usage. If the individual used one hand successively, in a "series" (bouts: McGrew & Marchant 1997) of rapid mutually dependent actions (e.g. when quickly picking up food items one by one with one hand only, whilst standing on their three other limbs without changing posture), we made only one record. The series was considered complete if the individual: 1) began to perform another activity (excluding scratching), 2) changed body posture; or 3) moved to another location and stopped feeding (Harrison & Nystrom 2010). A new independent record was recorded if the individual stopped feeding for at least two seconds.

Feeding that occurred on the ground or on platforms

The macaques were free to move in the enclosure where the food was distributed. The position of the food chosen in relation to the focal individual was not recorded and selection of food items in all directions was recorded. Most of the postures adopted by the macaques during feeding sessions were undemanding in terms of positional support. In some positions one or both hands were not in contact with the substrate. We therefore recorded the individual's posture as either one-free-hand or two-free-hands. The one-free-hand posture is defined as a posture involving the individual standing on three limbs or clinging (e.g. to the vertical parts of platforms, ropes or bars) and one hand is used to pick up a food item and the second is used for postural support. The two-free-hands posture is defined as when the individual uses one hand for feeding while the second is relaxed (e.g. when sitting or, rarely, standing bipedally). Age was extracted from the birth records of each zoo.

Hand preference assessment

We used two methods to describe individual hand preference (Hopkins 1999). First, we calculated the z-score following the formula $z = [x - (np)] / \sqrt{(npq)}$, where x represents the number of right-handed responses, n the total number of responses and p and q are the expected right-handed and left-handed responses based on the null hypothesis, $p = q = 0.5$. The z-score indicates whether the distribution of left- and right-hand use differs from chance and permits classification of individuals as left-preferent ($z < -1.96$), right-preferent ($z > 1.96$)

Table 1. Individual characteristics and hand preference from throughout the observational study periods.

ID	group	sex	age in first observation (years)	2009				2013				2015			
				HI	z-score	R/L/ Ambi	n	HI	z-score	R/L/ Ambi	n	HI	z-score	R/L/ Ambi	n
Wapi	O	F	2.1	-	-	-	-	0.00	-0.07	A	231	0.06	1.01	A	252
Vidisha	O	F	2.2	-	-	-	-	0.04	0.67	A	271	0.03	0.86	A	305
Madlenka	P	F	1.5	-	-	-	-	0.04	0.70	A	290	0.00	-0.07	A	233
Pavčina	P	F	3.5	-	-	-	-	-0.03	-0.47	A	292	0.02	0.27	A	226
Puttur	O	M	1.3	-0.01	-0.26	A	381	-	-	-	-	-	-	-	-
Lenka	P	F	1.8	0.09	2.06	R	479	-	-	-	-	-	-	-	-
Orissa	O	F	2.3	0.06	1.10	A	398	0.10	1.89	A	383	-0.05	-0.87	A	295
Pepík	P	M	3.0	0.08	2.05	R	644	-	-	-	-	-	-	-	-
Nadu	O	F	3.4	0.15	3.12	R	422	0.22	3.96	R	330	0.20	3.29	R	270
Mudi	O	F	4.1	0.02	0.39	A	644	0.13	2.65	R	386	0.11	2.01	R	322
Josefka	P	F	4.3	0.04	0.90	A	404	-	-	-	-	-	-	-	-
Mandurái	O	M	5.1	0.18	4.10	R	493	-	-	-	-	-	-	-	-
Kérála	O	F	6.0	0.18	3.32	R	337	0.22	4.41	R	407	-	-	-	-
Hakim	O	M	6.5	0.21	3.34	R	262	-	-	-	-	-	-	-	-
Hasan	O	M	6.3	0.14	2.61	R	368	-	-	-	-	-	-	-	-
Hadim	O	M	7.4	0.10	1.75	A	334	-	-	-	-	-	-	-	-
Dáda	O	F	14.5	-0.27	-3.06	L	131	-0.17	-3.64	L	471	-	-	-	-
Nagar	O	M	13.5	-	-	-	-	-0.27	-6.28	L	540	-0.31	-5.14	L	273
Vikina	P	F	12.3	0.01	0.29	A	422	-0.15	-2.70	L	344	-	-	-	-
Norton	O	M	12.5	-0.16	-3.47	L	443	-	-	-	-	-	-	-	-
Wuppi	P	M	16.9	-0.13	-2.16	L	263	-0.31	-5.76	L	352	-	-	-	-
Mirunka	P	F	19.2	0.06	0.96	A	280	-	-	-	-	-	-	-	-
Bětká	O	F	24.6	-0.20	-4.77	L	581	-0.26	-5.78	L	483	-0.25	-4.41	L	305

Note: HI = handedness index, O = Ostrava Zoo, P = Plzeň Zoo, R = right-preferent, L = left-preferent, Ambi = ambipreferent.



or ambipreferent ($-1.96 < z < 1.96$) (McGrew & Marchant 1997). Second, the handedness index (HI) of an individual was calculated using the formula $HI = (R - L)/(R + L)$, where R represents the number of times the right hand was used and L the number of times the left hand was used. The HI describes both the direction of the preference, left-preferent individual < 0 and right-preferent individual > 0 , and also the strength of the preference which corresponds to the absolute value ABS (HI). However, it does not indicate statistical significance between the numbers of times the right or left hand is used and hence individuals cannot be categorized as left- or right-preferent or ambipreferent.

Both the HI and z-score were calculated for each individual, in each time period, separately for two-free-hands and one-free-hand postures and merging the data from both postures.

Data analysis

To assess group level handedness, we first used a binomial test to compare the number of right- and left-preferent individuals categorized based on the z-score. As explained, this approach ignores ambipreferent individuals (McGrew & Marchant 1997). Thus, we also used an alternative method with a Wilcoxon signed-rank test to assess whether the mean HI differed from zero. For these analyses, we used the HI and z-score calculated from the last observation period each individual was followed ($n = 23$), combining data from both body postures.

To evaluate the stability of hand preferences we first compared hand preference of each individual during two or three study periods (see Table 1), also irrespective of body posture. An individual was considered to have a stable hand preference if it was categorized the same way (left-handed, right-handed or ambipreferent) during at least two observational periods. Second, we performed Spearman correlation of HI between seasons. For individuals studied for all three periods, only the first two seasons were included in the analysis to have comparable data with individuals studied for two periods only. The preference was considered stable if the correlation was significant at the 95% confidence level.

To test if the strength and direction of hand preference are affected by age and body posture we used linear mixed models (LMMs) with the lme4 package (Bates et al. 2015) in R 3.1.1 (R Core

Team 2020). We fitted one model with the absolute value of HI and one model with the actual HI as a response variable. Repeated observations of some individuals were accounted for by using individual ID as a random factor in the model, thus repeated observations were not treated as independent in the analysis and our evaluation of the age effect thus combines a longitudinal and transverse approach. As fixed factors we included: age and body posture (one-free-hand, two-free-hands), study period (S1, S2, S3), and group (Plzeň Zoo, Ostrava Zoo). To control for unequal number of hand use records per individual, we added the sum of records (hand uses) per individual into both models, and a polynomial of the sum in the second model as fixed factors, since it might be expected that a higher number of records would lead to higher absolute values of HI, and thus actual values of HI closer to 1 or -1 . We fitted full models and counted the 95% confidence interval of the predictors using the *confint* function. The predictor was considered significant when the interval did not include zero. We checked the models for linearity, homogeneity of variance, the normal distribution of the model residuals and collinearity. All the criteria were met. The variance inflation factor (VIF) values were all < 1.25 .

Ethical note

Our study was observational and non-invasive. All zoos involved in this study are members of the European Association of Zoos and Aquaria, maintaining high quality husbandry conditions and welfare. All procedures performed in this study adhered to the European Union regulations (Directive 2010/63/EU) and legal requirements of the Czech Republic.

Results

Population-level preference

In total, our dataset consisted of 14,787 records. We recorded between 64 and 184 (median = 166) records per individual, posture and period, which we used to calculate the z-score and HI (Table 1). Binomial tests, based on the z-score, revealed no difference between the number of left-preferent and the number of right-preferent individuals ($P = 0.79$; 95% CI = 0.289, 0.823; Estimate = 0.571). The result of the one-sample Wilcoxon signed-rank test indicated that the distribution of the HI did not differ from the normal distribution ($P = 0.657$; 95% CI = -0.032 , 0.050) suggesting an absence of hand preference at the population-level.

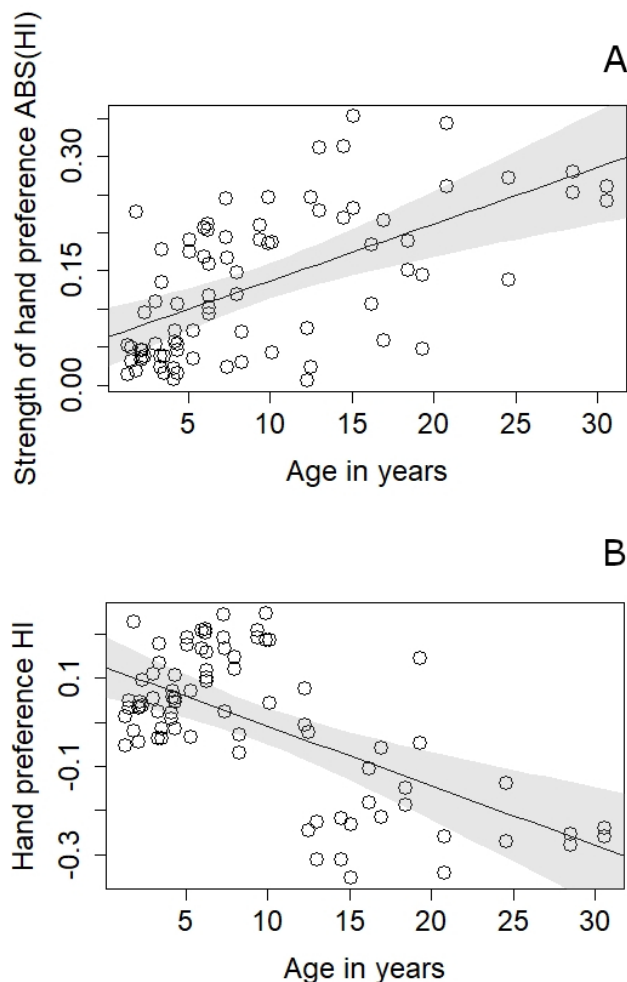


Fig. 1. Effect of age on the strength of (A) absolute hand preference ABS (HI) and (B) hand preference HI. Fitted line is in black; grey area represents the 95% confidence region of the corresponding LMM. Each point represents one value measured in one individual per period per posture ($n = 80$ observations of 23 individuals). See Methods on the details of the LMM used to treat the repeated, non-independent observations of the same individuals.

Stability of hand preference

We did not detect any change from left to right, or right to left hand preference based on the z-scores among the three study periods. From 13

individuals, who were followed for two or three subsequent periods, 11 individuals maintained the same hand preference in all studied periods (see Table 1). Only one individual switched from ambipreference to right-preference, and one individual switched from ambipreference to left-preference between the first and second (but not between second and third) study periods. Further, there was a strong correlation between HIs taken for the same individual in two subsequent study periods ($\rho = 0.85$, $P < 0.001$).

Effect of age and body posture on the strength of hand preference

Across all study periods, ABS (HI) varied between 0.02 and 0.35 (mean = 0.15, SD = 0.10) and between 0.01 and 0.31 (mean = 0.12, SD = 0.09) for two-free-hands and one-free-hand postures respectively. The strength of hand preference appeared to be affected by age, body posture and group identity (Table 2). The model predicted that an increase in age of one year increased the ABS (HI) by 0.4–1.1% (95% CI). Individuals had between 1.4–5.8% stronger preference in a two-free-hands than in a one-free-hand posture. Individuals belonging to Plzeň group had by 0.7–9.7% weaker preference than Ostrava group (Table 2, Fig. 1A).

Effect of age and body posture on the direction of hand preference

The HI across all study periods (S1, S2, S3) varied between -0.35 and 0.25 (median = 0.04) and between -0.31 and 0.21 (median = 0.02) for two-free-hands and one-free-hand posture respectively. From all model predictors, only age affected the HI. The LMM predicted that an increase of age by one year decreased the HI by 0.8–1.9% (95% CI), suggesting that the older an individual the greater the probability they use their left hand (Table 3, Fig. 1B).

Table 2. Results of linear mixed models assessing predictors of strength of hand preference expressed by ABS (HI). $n = 80$ lines of 23 individuals, each line represents one value per individual per period per posture. See Methods for details of the LMM used to handle the repeated, non-independent observations of the same individuals. Significant results are in bold.

ABS (HI)	Estimate	SE	2.5%CI	97.5%CI
age	0.007	0.002	0.004	0.011
posture (two-free-hands)	0.036	0.011	0.014	0.058
study period (S2)	0.007	0.021	−0.032	0.046
study period (S3)	−0.019	0.024	−0.046	0.026
group (Plzeň)	−0.052	0.024	−0.097	−0.007
sum of records	−0.0001	0.0001	−0.0004	0.0001

Table 3. Results of the LMM to assess predictors of hand preference (expressed by HI). n = 80 lines of 23 individuals, each line represents one value per individual per period per posture. See Methods for details of the LMM used to handle the repeated, non-independent observations of the same individuals. Significant results are in bold.

HI	Estimate	SE	2.5 %CI	97.5%CI
age	-0.014	0.003	-0.019	-0.008
posture (two-free-hands)	-0.006	0.015	-0.035	0.023
study period (2)	-0.018	0.034	-0.084	0.047
study period (3)	-0.015	0.038	-0.089	0.058
group (Plzeň)	-0.032	0.043	-0.113	0.050
sum of records	-0.096	0.108	-0.302	0.109
sum of records (polynomial)	-0.098	0.099	-0.294	0.088

Discussion

At the individual level, 60.9% of the subjects showed significant hand preference in one or the other direction in a simple food reaching task. The situation in which most individuals significantly prefer using their right or left hand but with no significant preference at the population level, corresponds to Level 2 of the framework proposed by McGrew & Merchant (1997), where Level 1 represents a situation in which most individuals are ambipreferent and with no population-level preference, while at Level 5 the majority of individuals are fully lateralized and with a significant population-level preference. Level 2 hand preference for simple unimanual reaching has been previously reported for several other simiiformes (black-headed spider monkey *Ateles fusciceps*: Nelson et al. 2015; squirrel monkey *Saimiri sciureus*: Meguerditchian et al. 2012; common marmoset *Callithrix jacchus*: Hashimoto et al. 2013; olive baboon: Molesti et al. 2016; more examples: Papademetriou et al. 2005) and strepsirrhine (indri *Indri indri*: Rigamonti et al. 2005; ring-tailed lemur *Lemur catta*: Shaw et al. 2004; gentle lemur *Hapalemur griseus*: Stafford et al. 1993; mouse lemurs *Microcebus murinus* and *Microcebus lehilahytsara*: Leliveld et al. 2008). A relatively smaller proportion of studies have reported a right- or left-hand bias at the population level for simple reaching tasks, spanning the primate order (for review see Papademetriou et al. 2005).

The lack of significant population-level hand preference in our study may not be conclusive. A population-level bias may become detectable with increasing complexity of the observed task (Hopkins et al. 2007) and/or with increasing sample size. Studies on Barbary macaques

(Regaiolli et al. 2018) and white-faced capuchins *Cebus capucinus* (Meunier & Vauclair 2007) showed a hand preference at the individual level for simple reaching tasks and at the population-level for a coordinated bimanual task. Right-handedness at the population-level appears to only be detectable with a large sample size, as concluded by Hopkins et al. (2011) after increasing the number of gorillas (*Gorilla gorilla*) tested in their study. Therefore, for our study it is inconclusive whether there is a population-level bias or not. The proportion of right-handed individuals (57.1%) in our study is almost identical to the proportion of right-handed baboons (57.7%) in a sample of 189 individuals studied in a complex bimanual task by Molesti et al. (2016). With our smaller sample size, however, the same proportion of right-handed individuals does not lead to a statistically significant result. The absolute values of HIs observed in our study were low, even though the majority of animals showed a statistically significant hand preference. Molesti et al. (2016) reported similarly low absolute values of HIs for both unimanual and bimanual tasks in a population of olive baboons, but with statistically significant hand preference at the individual (both tasks) and population (unimanual task) level.

These findings call into question the biological and evolutionary significance of hand preference in some species. It is possible that a strong hand preference is a handicap rather than advantage during competitive feeding in a group of macaques or baboons. In a zoo setting, macaques typically use both hands during feeding on small (grain-sized) food items. While one hand picks up a piece of food and moves it to their mouth, the other hand simultaneously reaches for another piece of food (S. Lhota, pers. observ.). Furthermore, individuals



may increase the efficiency of competitive foraging by using the hand that is closer to the food item, as has been reported in baboons (Meunier et al. 2011). A strong preference for one hand would most likely decrease foraging efficiency, putting the animal at a disadvantage in competition for food with other group members. Therefore, it is possible that the statistically significant, yet rather weak, hand preference observed in some primates represents a by-product of other lateralized functions of the brain, combined with an acquired motor habit, while natural selection favours ambipreference for simple, repetitive, unimanual foraging tasks. Laterality is a multimodal trait; that is: different tasks and requirements may lead to different hand use or specialisation. Keeping in mind that hand preference for gestural communication may be influenced by different neural substrates, we may speculate that the right-handed preference for gestures in baboons may have been freed from such selection (Meguerditchian et al. 2010, 2011). It does not involve foraging tasks, making the issue of competition and intake rate irrelevant.

The long-term stability of hand preference at the individual level, as observed in our study, has also been reported for unimanual and bimanual manipulation in olive baboons (Molesti et al. 2016), and for gestural communication in olive baboons (Meguerditchian et al. 2011) and chimpanzees (Meguerditchian et al. 2010). It is, therefore, one of the few robust results within the often-conflicting literature on non-human primate hand preference. In our study, none of the animals switched from left- to right-handed preference, or vice versa, and none of switched from either hand preference to ambipreference; the only changes we recorded were from ambipreference to a left or right-hand preference. These results show greater stability in hand preference compared to the larger sample of olive baboons (Molesti et al. 2016), for which the comparable unimanual (but also for the more complex bimanual) task, some baboons switched their hand preference within five years between two testing periods. A possible reason that Molesti et al. (2016) observed some individuals switching hand preference and we did not is due to: 1) Molesti et al. (2016) sampled more individuals than we did, and consequently had a higher probability of capturing individuals with unusual developments in their hand preference, or 2) the limited number of data points collected for some baboons (as few as five observations) which may have resulted in a small-sample bias.

In our study, age significantly influenced both strength and direction of hand preference. Strength of hand preference (measured as an absolute value of HI) was higher in older individuals. This result corresponds with findings in several previous cross-sectional studies on non-human primates (Stafford et al. 1990, Rogers & Kaplan 1996, Hopkins & Leavens 1998, Chapelain et al. 2011, Hopkins et al. 2011), but contradicts others. Numerous studies on various tasks detected no effect of age on the strength of hand preference, even though some involved large sample sizes (Hopkins et al. 2007, 2011, Molesti et al. 2016). In the context of these negative findings, we speculate that the weak HIs observed in our study, which tended to increase with age, may reflect a motor habit, which is formed and gradually fixed during an individual's lifetime, and that some of individuals may remain ambipreferent. However, we cannot rule out other explanations, e.g. the low task complexity used in our study (Fagot & Vauclair 1991, Prieur et al. 2019).

In addition, the cross-sectional analysis also showed an effect of age on HI, which is a measure of the direction of hand preference. Older animals showed a lower tendency toward right-handedness (within the sample that showed an overall, though non-significant, tendency for a right-hand preference). This result seemingly contrasts with the finding that the direction of hand preference remains stable, i.e. there are no shifts from right-to-left preference. However, it might be possible that the shift from ambipreference to left preference occurs more gradually in individual monkeys over their lifespan compared to the shift from ambipreference to right-preference in other individuals. These different rates of development would result in a relatively higher expression of left-hand preference among older individuals.

We also cautiously speculate that animals with a reverse hand preference may develop their hand preference more slowly compared to animals that fit into the prevailing pattern at the population-level. This scenario would predict increasing left-hand preference in older monkeys, apes, and humans. However, it is not clear at what age this change might happen. For example, juvenile vervet monkeys (*Cercopithecus aethiops*) were right-handed, and adults more left-handed in two natural foraging tasks (Harrison & Byrne 2000). In chimpanzees, a greater frequency of right-handedness in juveniles decreased in adulthood,



which also indicates a relative shift toward left-handedness (Boesch 1991). In contrast, Chapelain et al. (2011) showed a significantly higher tendency to right-handedness among adults compared to juvenile bonobos (*Pan paniscus*) for a coordinated bimanual task, while Rogers & Kaplan (1996) described a shift from ambipreference in bimanual feeding in juvenile orangutans (*Pongo pygmaeus*) to a right-hand preference in adult females. While we cannot rule out interspecific differences, or an effect of different tasks, it is important to note that in our study age was measured as a continuous rather than a discrete variable. It is possible that the tendency toward increased left-handedness in lion-tailed macaques follows the slow process of ageing in adult individuals rather than relatively fast maturation and an abrupt change during early life.

The lack of a detectable population-wide hand preference bias in lion-tailed macaques in our study does not support the postural origins hypothesis (MacNeilage et al. 1987, MacNeilage 2007), which predicts left-bias for simple reaching tasks for quadrupedal arboreal primates. Further, posture did not influence the direction of hand preference. However, the preference (in either direction) was stronger when the animal assumed a two-free-hands (typically sitting) posture with both hands available for feeding. It is not easy to compare this finding with other studies, as various classifications or descriptions of postures combine the availability of one or both hands with other physical demands on posture. In squirrel monkeys, the unimanual reaching while clinging (with only one hand free to reach) elicited a right-hand preference, while bimanual tasks when sitting did not elicit any hand preference in this species (Meguerditchian et al. 2012). Two strepsirrhine species showed stronger hand preference in relatively demanding postures with both hands free, i.e. a bipedal standing posture (Northern lesser galago *Galago senegalensis*: Larson et al. 1989) or a particular demanding sitting posture (black lemur *Lemur macaco*: Forsythe & Ward 1988). The gentle lemurs showed stronger hand preference when feeding from a dish compared to the more positionally demanding species-specific feeding on bamboo shoots (Stafford et al. 1993). We suggest future studies attempt to separate the physical demands of maintaining posture from the availability of one or both hands for manipulative tasks, because

these two aspects of positional behaviour may influence hand preference differently. A posture requiring use of one hand for positional support may potentially impose restrictions on the choice of hand to reach for food or other objects, thereby decreasing the strength of the preference.

Overall, the results of our study show that the majority of captive lion-tailed macaques prefer using their right or left hand for simple feeding tasks. The direction of this preference is stable and is reinforced over the individual's lifetime. This pattern is consistent with findings of previous studies. We speculate that the most likely proximate mechanism responsible for this pattern is motor learning during the lifetime of the animal that may not necessarily have a specific adaptive value. However, the evolutionary significance of this phenomenon, the underlying role of inborn lateralization of neural substrates, the effect of a demanding (arboreal) posture, and the effect of age on the direction of this hand preference, remain unclear. Further study is required to address these questions using a larger sample size among additional species where other tasks, with different cognitive and manipulative demands, are used that allow titration of these hypotheses.

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Author Contributions

B. Kuběnová, M. Konečná, V. Blažek and S. Lhota conceived the idea; B. Kuběnová and V. Tomanová collected and analysed the data; B. Kuběnová, S. Lhota and M. Konečná wrote the manuscript.

Data Availability Statement

The data that support the findings of this study are available in the FigShare Digital Repository: *Kubenova_data_laterality_toShare.csv* at <http://dx.doi.org/doi:10.6084/m9.figshare.19164884>.

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