

## Toward a better understanding of habituation process to human observer: A statistical approach in *Macaca leonina* (Primates: Cercopithecoidea)

Eva Gazagne<sup>1,2\*</sup>, Alain Hambuckers<sup>1</sup>, Tommaso Savini<sup>2</sup>, Pascal Poncin<sup>3</sup>, Marie-Claude Huynen<sup>1</sup> & Fany Brotcorne<sup>1</sup>

**Abstract.** Habituation allows an observer to closely approach and follow free-ranging animals, as they no longer respond to the observer presence (e.g., through flight, avoidance, display, curiosity). While habituation is implicitly acknowledged as a necessary step before any direct observational studies of primates, there is very little published data on the subject. The aim of this study is to analyse the habituation process over time (17 months) in a wild-feeding troop of northern pigtailed macaques (*Macaca leonina*) inhabiting a degraded forest fragment of the Sakaerat Biosphere Reserve, Thailand. Based on the number of encounters, contact duration with the studied troop, and behavioural responses to the observer recorded ad libitum and via scan sampling, we found statistical evidence of habituation progress over five stages: early, minimal, partial, advanced, and full. The complete habituation process took nearly 13 months. Factors such as the macaques' limited experience of human contact, semi-terrestriality, large ranging patterns, fission-fusion dynamics, unpredictable resource use, as well as reduced native fruit availability in this degraded forest fragment may explain the length of the process. It was only possible to collect ranging and behavioural data from the partial habituation stage, although these data were biased toward adult males and sub-adults, while overestimating movement behaviour over inactivity and social behaviours. Our results highlight the importance of analysing behavioural data of fully habituated groups of primates to limit biases of observer presence, and also of not underestimating the habituation process length. This study provides novel information on the habituation process in macaques and proposes an effective methodology to analyse the habituation process across a wide range of primate species.

**Key words.** habituation process, *Macaca leonina*, multinomial logit regression, Thailand, degraded habitat

### INTRODUCTION

Habituation is a general term used to explain a decrease in response intensity to a recurrent stimulus or set of stimuli. Specifically, animal habituation to the presence of human observers (hereafter referred to as 'habituation process') occurs when repeated and neutral contact between wild animals and humans lead to a reduction in fear and flight responses, and ultimately to the ignoring of observers (Thorpe, 1963; Williamson & Feistner, 2011). The behavioural flexibility of non-human primates (hereafter called 'primates') and their ability to adapt to novel conditions make them especially appropriate subjects for

this type of process (Bertolani & Boesch, 2008; Jack et al., 2008). Although habituation is one of the hallmarks of field primatology (Hanson & Riley, 2017) and widely accepted as the first step toward conducting field research on complex or elusive behaviours, the process is still poorly documented.

The habituation process in wild primates presents many practical and ethical dilemmas, notably an increased risk of disease transmission (Nizeyi et al., 2001; Woodford et al., 2002; Engel et al., 2006), environmental alteration (Fedigan, 2010), induced stress (Woodford et al., 2002), ecological and behavioural modifications (Jack et al., 2008; Klailova et al., 2010; McLennan & Hill, 2010; McDougall, 2012; Nowak et al., 2014), increased facility of poaching and hunting (Jack et al., 2008; Malone et al., 2010), and over-habituation (Fedigan, 2010; Webb & McCoy, 2014). Therefore, relying on habituation to study wild primate species should be carefully considered. Alternative methods to monitor demography, density, ranging, diet, and activity patterns of free-ranging primates without extensive habituation to human observers include the use of indirect observation, such as noting nest sites, food remains, faeces, footprints, and vocalisations (Goldsmith, 2005; Trolliet et al., 2016); distance sampling such as line-transect methods (Marshall et al., 2008); and telemetry devices such as Global Positioning

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<sup>1</sup>Unit of Research SPHERES, University of Liège, Quai van Beneden, 22, 4020 Liège, Belgium; Email: evagazagne@live.fr (\*corresponding author)

<sup>2</sup>Conservation Ecology Program, King Mongkut's University of Technology Thonburi, 49 Soi Tienthalay, 25 Bangkhuntien-Chaithalay Road, Thakham, Bangkhuntien 10150, Thailand

<sup>3</sup>Unit of Research FOCUS, University of Liège, Quai van Beneden, 22, 4020 Liège, Belgium

System (GPS) collars or subcutaneous implants combined with Geographical Information Systems (GIS) (Fedigan et al., 1988; Juarez et al., 2011; Qi et al., 2014; Trayford & Farmer, 2012). However, these methods can perform poorly in densely forested habitats or with unhabituated animals (Spragues et al., 2004; Marshall et al., 2008), and do not allow the simultaneous collection of a wide range of eco-ethological data. Additionally, some of these methods can be invasive (i.e., capturing, darting, collaring, etc.), complex to implement, and carry potential costs to animals associated with risk of injury, behavioural modification, and sometimes death (Juarez et al., 2011; Cunningham et al., 2015; Hopkins & Milton, 2016). For many forest-dwelling species, the habituation process is therefore indispensable to the collection of detailed data on ranging patterns, habitat preferences, foraging strategies, social interactions or organisation, and more complex behaviours (e.g., display, food processing, etc). Collecting direct observational data through primates' habituation can be highly beneficial, and ultimately better contribute to species conservation.

The length of time necessary for habituation ranges from hours to days for prosimians (e.g., *Galago* spp., most nocturnal lemurs); months for orangutans and monkeys (e.g., colobus, guenons, mangabeys, marmosets, muriquis, pottos, patas, siamangs); and years for baboons and African great apes (e.g., bonobos, chimpanzees, gorillas) (Williamson & Feistner, 2011). For instance, in apes and hamadryas baboons, the process seems particularly long and difficult (Kummer, 1995; Bertolani & Boesch, 2008; Klailova et al., 2010). Additionally, terrestrial primates or primates which live in fission-fusion society (i.e., groups split into temporary or permanent subgroups with variable membership, size, and spatial cohesion over time) are known to take longer to habituate than arboreal forest primates or primates living in stable groups (Bertolani & Boesch, 2008; Williamson & Feistner, 2011). In general, species such as macaques (*Macaca* spp.) are usually considered relatively easy to habituate because of their behavioural flexibility; they exhibit rapid adaptive behavioural responses to environmental changes (Williamson & Feistner, 2011; McLennan et al., 2017; Kalbitzer & Chapman, 2018). However, the specific process of macaque habituation with regard to timeline, behavioural changes, and associated risks has not received much attention (Rasmussen, 1991). To the best of our knowledge, there are only three studies on macaque habituation process: an observational and experimental one-year field study by Bernstein (1967) on southern pigtailed macaques (*Macaca nemestrina*) using food provisioning and a trained captive monkey; a study by Schülke et al. (2011) providing monthly contact time and daily travel distance over 11 months of habituation in Assamese macaques (*Macaca assamensis*); and one recent seven-month study by Hanson & Riley (2017) exploring behavioural changes in moor macaques (*Macaca maura*) and human participants using an ethnoprimateology-based methodology.

Classified as vulnerable by the IUCN (Boonratana et al., 2020), the frugivorous northern pigtailed macaques (*Macaca leonina*) are among the shyest and wildest macaques in

Southeast Asia, and are known to avoid anthropogenic environments. The species is semi-terrestrial, lives in multi-male/multi-female groups characterised by fission-fusion dynamics, and exploits large home range with long daily travels (Choudhury, 2008; Albert et al., 2011; Gazagne et al., 2020a, 2020b). These factors make the northern pigtailed macaques difficult to observe and track in their natural habitat without preliminary habituation. Additionally, in degraded habitats, northern pigtailed macaques are often the last remaining dispersers of large seeds and are therefore important seed dispersal agents (Albert et al., 2014). Ultimately, habituation becomes a prerequisite step to collecting eco-ethological data, able to provide good support for the conservation of the species and for habitat restoration within its ranges.

The aim of this study is to document the habituation over time of a wild-feeding troop of northern pigtailed macaques inhabiting a degraded forest fragment of the Sakaerat Biosphere Reserve ('Sakaerat', hereafter) in Thailand. We identified different stages of habituation based on the number of encounters, contact duration with the studied troop, the macaques' behavioural responses to the observer, and improvement in the data collected, and we analysed the progress of habituation over 17 months. We tested the effect of habituation on the macaques' activity budget and observed age-sex classes, and the effect of the observer search effort on the macaques' detection over time. We present new insights about habituation methods, identify factors influencing the macaques' habituation effectiveness, and discuss the potential drawbacks of habituating free-ranging macaques.

## METHODS

**Study site.** The study was conducted at the Sakaerat Biosphere Reserve located in Northeastern Thailand (14°26' to 14°32'N; 101°50' to 101°57'E) and covering an area of 80 km<sup>2</sup>. With altitude levels ranging between 250 and 762 m above sea level, the reserve is characterised by a monsoonal climate with a wet season (from May to October) and a relatively dry season divided into a cool (from November to February) and a hot (from March to April) period. The mean annual temperature is 25.6°C while the mean annual rainfall of the region is 1,200 mm (Thai Institute of Scientific and Technological Research, 2017). Before its creation in 1977, the reserve had already undergone severe deforestation and forest conversion to plantation forests dominated by exotic species (i.e., *Acacia* spp., *Eucalyptus* spp.), resulting in a significant degradation of the pristine habitat quality and reduced native fruit availability (Gazagne et al., 2020a). Sakaerat is also surrounded by zones of dry-evergreen forest with the hard edge of a five-lane highway, extensive agricultural areas, and human settlements, and includes many roads, making the reserve a fragmented and degraded habitat. The Sakaerat environmental research station within the reserve is run as a facility for ecological and environmental research, but also as a popular science camp for school children. Sakaerat is free of active tourism, but is suffering from plant and mushroom picking and illegal hunting.

**Study troop.** We analysed the habituation process of a wild troop of northern pigtailed macaques (*M. leonina*) never studied before in Sakaerat. Over the study, the troop counted approximately 143 individuals, including 14 adult males, 42 adult females, 10 sub-adults, and 77 juveniles. As usual for this species, the adult sex ratio was biased in favour of females, with three females per male.

**Data collection.** A single observer (EG) performed the habituation process, which took place over 17 months and covered three study periods. We first performed a pilot study of four months (February–June 2015) in order to get familiarised with the study site and to collect preliminary data on macaque presence in the area; we did not gather data on habituation per se during this period. Later on, we collected data on habituation over two study periods (March–August 2016 and January–July 2017: 13 months in total). Adapting stages previously described to characterise habituation progress for western lowland gorillas and chimpanzees (Ando et al., 2008; Bertolani & Boesch, 2008), we defined five different habituation stages over 13 months according to tracking characteristics (e.g., number of encounters, contact duration with the troop), the macaques' ad libitum behavioural responses directed to the observer, and data collection improvement (e.g., pinpoints of resource location, possibility of behavioural scan sampling). Stages are defined as follows: (1) early stage: macaques always flee the observer, short and interrupted tracking; (2) minimal stage: displays directed at the observer, short and interrupted tracking; (3) partial stage: long and interrupted tracking, possibility of scan sampling data collection; (4) advanced stage: reduced displays directed at the observer, full and consecutive tracking; and (5) full stage: individuals apparently ignore the observer, full and consecutive tracking.

Troop location was determined using the location of the previous sighting, walks along existing transects, or by random walks into the forest. To detect macaques, we used visual clues such as feeding or faeces remains, auditory clues such as vocalisation or noise from macaque movement in the vegetation, as well as olfactory clues such as the musky smell of macaques, indicating recent use of an area. To quantify the search effort, we calculated a monthly percentage representing the number of days in the field looking for macaques by the total number of days per month. To quantify the progress in macaque detection, we calculated a monthly percentage of macaque detection based on the number of days with encounters by the number of days in the field. To quantify the duration of troop contact, we recorded the time of encounter with and loss of the macaques. We set the beginning of a visual contact when the macaques became aware of the observer's presence, and the end when they were lost (neither seen nor heard for five minutes) or sleeping at the sleeping site. We also recorded the number of encounters per day to quantify the progress in following the troop without losing it. We recorded the troop's location using track-log data at 15-minute intervals with a handheld GPS (GPSmap 62s, Olathe, KS, Garmin,  $\leq 10$  m error) in order to indicate monthly activity centres used to locate the troop.

While following the troop, we collected behavioural data to estimate how macaque behaviour changed during the habituation process. We collected monthly category of distance (precision of up to five metres) between the troop and the observer, as well as ad libitum behavioural displays directed at the observer (Altmann, 1974). Using scan sampling (Altmann, 1974), it was only possible to collect behavioural data from the partial habituation stage. Therefore, during the partial, advanced, and full habituation stages, we defined the troop activity budget using 30-minute scan samples of all visible troop members, recording age-sex class and activity (i.e., inactivity, movement, feeding, social, and other). Finally, we estimated the demographic composition of the troop by conducting direct count census when the macaques were travelling and crossing the main roads in Sakaerat.

**Data analysis.** We used R version 3.5.0 (R Development Core Team, 2018) for statistical analyses ( $\alpha = 0.05$ ). We ran chi-squared goodness-of-fit tests to compare the monthly average troop age-sex class frequency observed during the scan samples between partial, advanced, and full habituation stages and the known troop demography (used as expected ratio). The corresponding standardised residuals were calculated to determine which observed frequencies significantly differed from the expected ones (Agresti, 2007).

To test the effect of habituation progress on macaque activity budget and whether this effect varied between age-sex classes, we used a multinomial logit regression model with random intercepts (Fahrmeir et al., 2013). Outcome variables were behaviour categories (i.e., inactivity, movement, feeding, social, and other). Fixed explanatory factors were age-sex classes (i.e., adult males, adult females, sub-adults, and juveniles) and time divided into the last three habituation stages (partial, advanced, and full habituation) as well as the pairwise interactions. As data were not independent, the identity of the scan sample was included as a random effect. The model was computed using a Bayesian Markov chain Monte-Carlo method with the R package MCMCglmm (Hadfield, 2010). The length of the chain was 40,000 (argument 'nitt'); the discarded values of the Markov chain were 15,000 (argument 'burnin'); and the interval at which the results were stored was 20 (argument 'thin'). We then used the model to calculate the posterior distribution of the probability (i.e., relative frequency) to observe a specific activity according to age-sex class over habituation stages and examined the overlaps of the 95% credible intervals.

Finally, we looked for trends in the search effort and the progress of macaque detection over habituation stages and tested for possible relationships between these two variables. To do this, we took into consideration the potential serial autocorrelation in the data (Hartmann et al., 1980; Gage & Lewis, 2013). We evaluated the serial autocorrelation of the log of each time series (i.e., search effort and progress of macaque detection) by fitting a linear regression model with a lag 1 value as explanatory variable and time (for possible trend), and subsequently applied the Durbin-Watson test for autocorrelation of the residuals with function *dwt* of the R *car* package (Fox & Weisberg, 2011). As trend test, we

Table 1. Typical behavioural responses of *Macaca leonina* to the observer's presence during the habituation process in Sakaerat Biosphere Reserve.

Behaviour	Definition
Flight	Rapid, panicked departure coupled with alarm or fear vocalisations. If individuals are in trees, they drop to the ground like ripe fruits and flee in all directions at full speed.
Avoidance	Individuals are relatively calm and silent, and disappear quickly but still stay in the area.
Curiosity	Individuals watching or glancing at the observer. They can move to get a clearer view or approach the observer.
Barking	Loud screaming, emitted by males and associated with aggressive or fear behaviours. Males often bark hidden from the observer in the vegetation.
Display	Vocalisations and displays (e.g., repetitive jumping on a branch, branch-shaking, branch-kicking, yawns) directed at the observer.
Puckering	Fixedly looking the observer with raised eyebrows, scalp pulled backward, ears flattened, neck extended, and with lips forward (compressed and protruded); potentially associated with intimidation.
Ignore	No discernible response while obviously having noticed the observer.

considered the P value of t-test produced for time coefficient in the regression model. We then tested for correlation between the residuals of both time series using the Pearson correlation coefficient.

## RESULTS

**Description of habituation stages.** The monthly results about tracking characteristics and behavioural responses to the observer, which we used to define the five habituation stages over 13 months, are detailed in Tables S1 and S2 (Supplementary Material).

During early habituation (March to June 2016), the percentage of days that had at least one encounter with the troop was 44% (i.e.,  $N = 43$  days with encounters for  $N = 97$  days in the field) (Fig. 1). The mean number of encounters per day was  $2 \pm 1.2$  SD ( $N_{\text{tot encounter}} = 81$ ). We could follow the macaques for relatively short and varying periods of time per contact (mean duration = 63 min, range: 2–323 min) and per day (mean cumulative duration = 122 min, range: 7–659 min) (Fig. 2), only from large distances ( $> 50$  m) and often based on auditory cues (e.g., contact calls, movements in the vegetation, etc.). The first reactions of individuals during encounters were flight, i.e., rapid, panicked departure coupled with alarm or fear vocalisations. If individuals were in trees, they dropped to the ground (like ripe fruits) and fled in all directions at full speed. After the panic movement, the macaques usually moved away silently on the ground, making them very hard to locate or follow (especially because of the dense forest understories at the study site). To find the macaques again, the observer looked for them following a Fibonacci spiral pattern (i.e., spiral that gets wider from its origin) from the loss point. During this early habituation stage, the macaques seemed to be under stress; the observer tried to avoid surprising and sudden movements that might frighten the animals and would have constituted a negative experience. For the same reason, when the observer was seen by an animal, a calm attitude, even crouching, was adopted

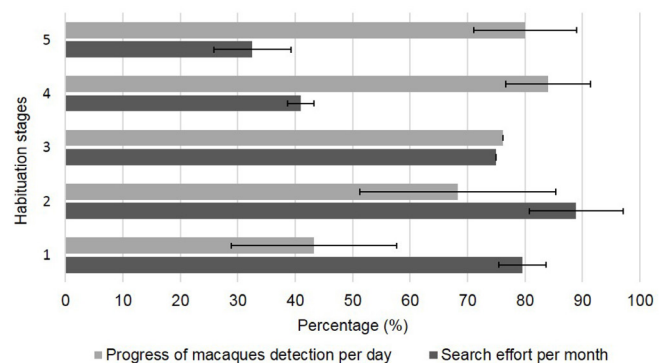


Fig. 1. Progress of macaque detection based on the number of days with encounters and the number of days in the field, and evolution of the search effort per month over the five habituation stages: early (1), minimal (2), partial (3), advanced (4), and full (5).

(van Krunkelsven et al., 1999). We avoided direct staring at the macaques and rather privileged indirect observation using binoculars.

During the minimal habituation stage (July to early August 2016, and 10 days in January 2017 after a six-month interruption), the troop became easier to find and the percentage of days that saw encounters rose to 68% (i.e.,  $N = 28$  days with encounters during  $N = 41$  days in the field) (Fig. 1). The mean number of encounters per day remained unchanged at  $2 \pm 1.1$  SD ( $N_{\text{tot encounter}} = 51$ ). Similar to the early habituation stage, we could follow the macaques for an average of 69 min per encounter (range: 6–497 min) and for an average of 125 min per day (mean cumulative duration, range: 7–719 min) (Fig. 2). However, the distance between the observer and the troop decreased; we could follow the macaques from approximately 50 to 20 metres and were able to locate the first sleeping sites of the troop. In addition, macaques started to address curiosity and agonistic behaviours toward the observer, e.g., display, barking, puckering behaviour (or pucker, see definitions in Table 1 and Supplementary Material—Note on puckering behaviour; Fig. 5). These behaviours were only performed by adult and

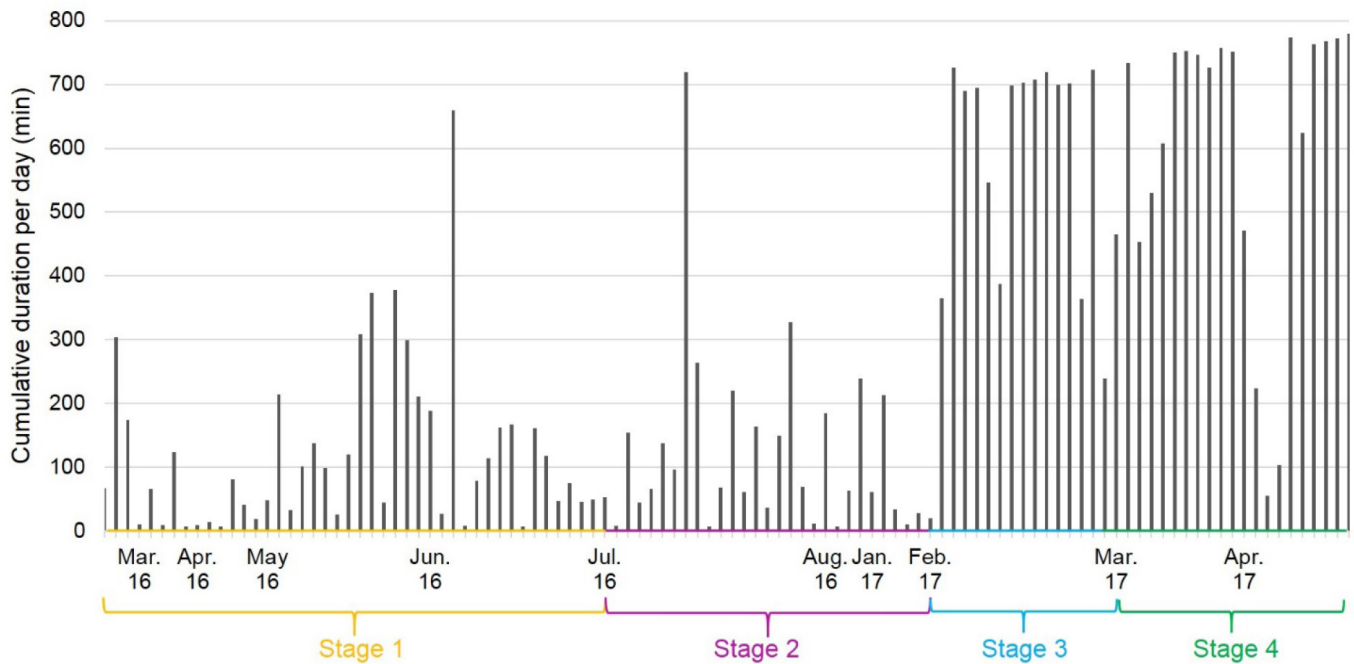


Fig. 2. Cumulative duration of encounters per day, while following the Sakaerat troop (N = 108 days of tracking), during the early (Stage 1), minimal (Stage 2), partial (Stage 3), and advanced (Stage 4) habituation stages. Full habituation (Stage 5), corresponding to full and consecutive days of tracking, is not presented in the figure.

sub-adult males and by some juveniles, while females and most juveniles still fled or avoided the observer (Table 1). When individuals displayed signs of curiosity or agonism toward the observer, the latter kept a neutral attitude to avoid any positive or negative interaction with the animals. Upon finally observing some females (most of them being in oestrus) in January 2017, the females began to act curious and to direct vocalisations toward the observer (Table S2).

During the partial habituation stage (February 2017), the percentage of days that had encounters with the troop was 76% (i.e., N = 16 days with encounters during N = 21 days in the field) (Fig. 1). The average duration of troop monitoring increased to 449 min per encounter (range: 20–726 min) and 562 min per day (cumulative duration, range: 20–726 min) (Fig. 2). The mean number of encounters per day diminished with an average of  $1 \pm 0.5$  SD ( $N_{\text{tot encounter}} = 20$ ), which reflects the decreased probability of losing the troop after having found it. It was possible to follow the troop for full days (N = 10). We could also estimate quite precisely the location of feeding trees or sleeping sites (based on macaque location in the evening and the next morning). The tracking distance of the troop decreased to approximately 20 metres. Adult and sub-adult males were the first to approach to within 5–10 metres from the observer and to occasionally display puckering behaviour, threats, and intimidations towards the latter. A specific type of intimidation display exclusively performed by adult and sub-adult males has been called branch-kicking. This intimidation display consisted of kicking a tree branch with one of the two hind legs while holding onto other branches or the tree trunk with the forelegs (cf. Modahl & Eaton, 1977), and was performed while the observer was standing or passing under the tree crown, resulting in branches (mainly dead) dropping from above the observer. Some individuals from all age-sex classes

started to ignore the observer (Tables 1, S2). From this partial habituation stage, we were able to collect behavioural data via scan sampling.

In the advanced habituation stage (March to April 2017), the percentage of days on which there were encounters with the troop was 84% (i.e., N = 21 days with encounters during N = 25 days in the field) (Fig. 1). The probability of losing the troop stayed unchanged with a mean of  $1 \pm 0.7$  SD encounter per day ( $N_{\text{tot encounter}} = 26$ ), and we could follow macaques for six consecutive full days from sleeping site to sleeping site. The average duration of troop monitoring was 485 min per encounter (range: 21–780 min) and 600 min per day (cumulative duration, range: 55–780 min) (Fig. 2). We could follow the troop from less than 20 metres away, and sub-adult males and juveniles frequently approached to within 1–2 metres of the observer. At this stage, individuals mainly displayed curiosity, puckering behaviour, avoidance, or ignored the observer altogether (Table S2).

Finally, during the full habituation stage (May to July 2017), the mean percentage of encounters per day was 80% (i.e., N = 24 days with encounters during N = 30 days in the field) (Fig. 1), and the observer did not lose the troop (number of encounters = number of days with encounters). We could follow the troop for more than seven full consecutive days from sleeping site to sleeping site. After almost 10 months of habituation, all individuals of the troop mostly ignored the observer and could be considered fully habituated (Table S2). The following distance of the troop ranged between 1 and 20 metres and we could approach individuals at 1–2 metres. Only juveniles sometimes displayed curiosity and puckering behaviours, and a few females kept their distance (but we could still observe them at 5 to 10 metres). During the full habituation stage, we could collect detailed behavioural

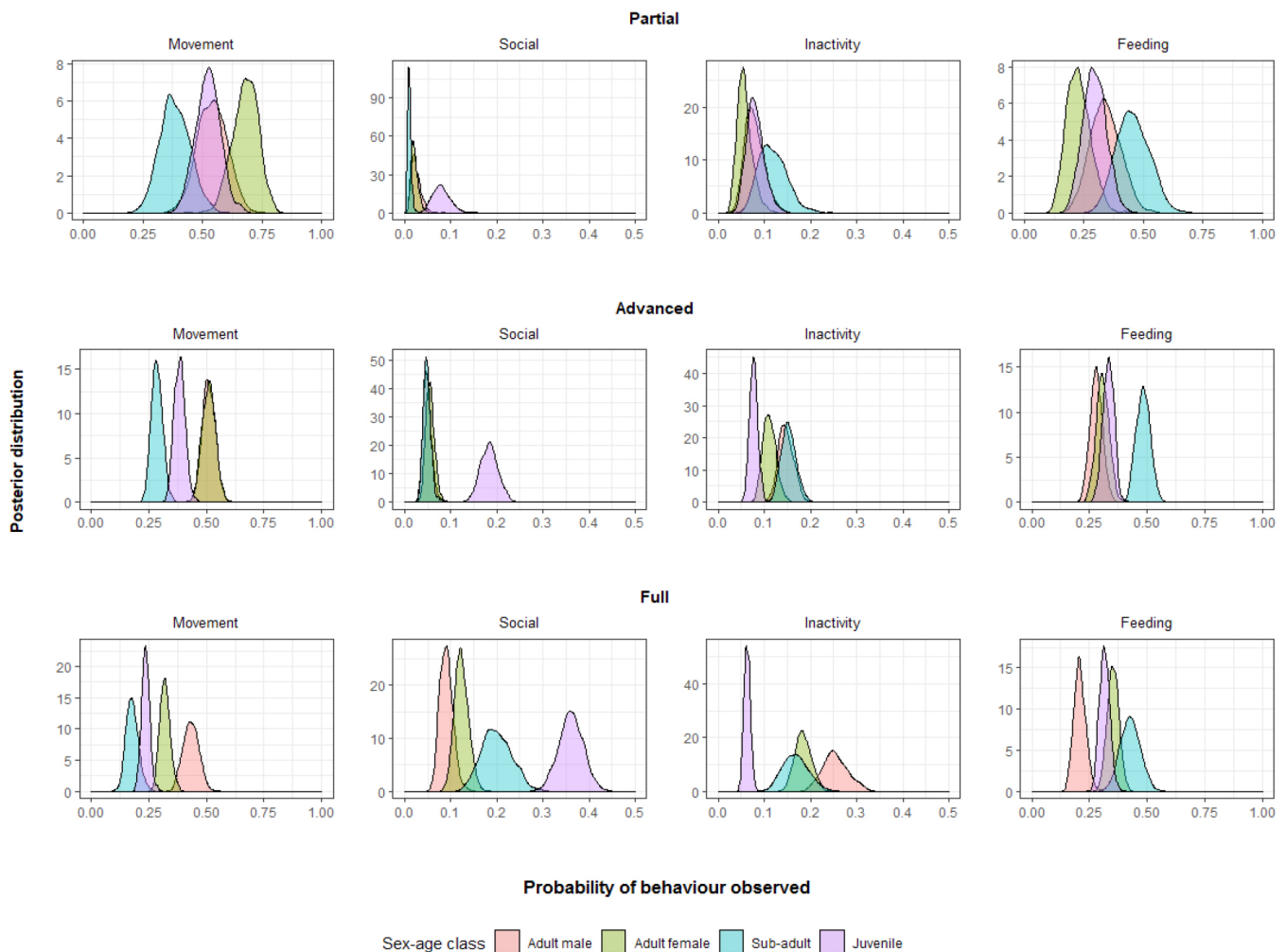


Fig. 3. Posterior probability distributions of movement, social, inactivity, and feeding behaviour observations for each habituation stage (i.e., partial, advanced, and full habituation) according to age-sex class (i.e., adult male, adult female, sub-adult, and juvenile), computed with the multinomial logit model.

patterns such as a complex food processing behaviour, the ‘leaf-wrap’ (i.e., a type of tool use sensus Panger et al., 2002), whereby the macaque wraps an item in a leaf (usually noxious caterpillars) before rubbing the leaf containing the item against a solid substrate (e.g., tree branch or rock).

#### Behavioural changes of each age-sex class over time.

Regarding the overall activity budget, the multinomial model revealed that movement behaviour significantly diminished and social behaviour significantly increased for adult females, sub-adults, and juveniles through the last three habituation stages (Fig. 3, Table 2). Unlike sub-adults and juveniles, adult classes were inactive significantly more often as habituation progressed. Although adult females were observed feeding more often and adult males less often over the habituation periods, general posterior probabilities remained similar. Other behaviour stayed relatively unchanged with habituation progress (Table 2).

During the partial habituation stage, adult males and sub-adults were represented in scan samples significantly more than expected based on the troop composition, while we observed adult females and juveniles significantly less than expected (chi-squared test:  $\chi^2 = 62.86$ ,  $df = 3$ ,  $P < 0.001$ ;

Fig. 4). With regard to the behaviours, the multinomial model showed that adult females were observed in movement more often than other age-sex classes and significantly more often than sub-adult males (Table 2, Fig. 3). The probability of observing social behaviour in juveniles was significantly higher than in other age-sex classes. Regarding feeding, inactivity, and other behaviours, the overlap between age-sex classes was more important, although we observed sub-adult males more often (Table 2, Fig. 3).

During the advanced habituation stage, age-sex classes were still significantly biased toward adult males and sub-adults in comparison to adult females and juveniles (chi-squared tests:  $\chi^2 = 35.133$ ,  $df = 3$ ,  $P < 0.001$ ; Fig. 4). In general, we observed inactivity behaviour more often during the advanced habituation stage. Moreover, individuals moved less but were more likely to engage in social behaviour (Fig. 3). There was a significantly higher probability of observing social behaviour in juveniles than in other age-sex classes. Juveniles were observed moving significantly less than adult males and females, but significantly more than sub-adults (Table 2, Fig. 3). Probabilities of observing feeding behaviour were quite similar for adult males, adult females, and juveniles, while significantly higher for sub-adults (Table 2).

Table 2. 95% posterior density intervals calculated with the multinomial logit model for each behaviour category over habituation stages, according to the age-sex class. Significant differences (i.e., with no confidence interval overlap) between age-sex classes for a given habituation stage are in bold; significant differences between habituation stages for a given age-sex class are highlighted in grey.

	Habituation stage					
	Partial		Advanced		Full	
<b>Movement</b>						
Quantiles	2.5%	97.5%	2.5%	97.5%	2.5%	97.5%
Adult male	0.419	0.661	0.457	0.566	<b>0.370</b>	<b>0.497</b>
Adult female	0.575	0.777	0.459	0.568	0.282	0.367
Sub-adult	0.268	0.512	<b>0.243</b>	<b>0.334</b>	0.131	0.234
Juvenile	0.425	0.624	0.340	0.432	0.203	0.273
<b>Social</b>						
Quantiles	2.5%	97.5%	2.5%	97.5%	2.5%	97.5%
Adult male	0.011	0.050	0.034	0.069	0.066	0.120
Adult female	0.010	0.040	0.039	0.075	0.096	0.154
Sub-adult	0.004	0.019	0.034	0.066	0.140	0.266
Juvenile	<b>0.048</b>	<b>0.120</b>	<b>0.147</b>	<b>0.221</b>	<b>0.311</b>	<b>0.413</b>
<b>Feeding</b>						
Quantiles	2.5%	97.5%	2.5%	97.5%	2.5%	97.5%
Adult male	0.221	0.467	0.228	0.333	<b>0.161</b>	<b>0.261</b>
Adult female	0.141	0.331	0.254	0.360	0.303	0.400
Sub-adult	0.330	0.594	<b>0.428</b>	<b>0.541</b>	0.344	0.512
Juvenile	0.207	0.391	0.284	0.374	0.277	0.359
<b>Inactivity</b>						
Quantiles	2.5%	97.5%	2.5%	97.5%	2.5%	97.5%
Adult male	0.045	0.124	0.117	0.179	0.203	0.311
Adult female	0.031	0.095	0.086	0.140	0.152	0.220
Sub-adult	0.070	0.184	0.124	0.185	0.119	0.225
Juvenile	0.048	0.121	0.061	0.095	<b>0.050</b>	<b>0.076</b>
<b>Other</b>						
Quantiles	2.5%	97.5%	2.5%	97.5%	2.5%	97.5%
Adult male	0.003	0.037	0.008	0.021	0.007	0.024
Adult female	0.004	0.026	0.010	0.024	0.012	0.028
Sub-adult	0.011	0.063	0.020	0.041	0.014	0.045
Juvenile	0.010	0.036	0.017	0.032	0.018	0.032

During the full habituation stage, observed frequencies of the age-sex classes collected during scan sampling no longer varied significantly from expected frequencies based on the troop composition (chi-squared tests:  $\chi^2 = 1.494$ ,  $df = 3$ ,  $P = 0.684$ ; Fig. 4). Regarding the activity budget, for all age-sex classes, the probability of observing movement was significantly less than for other habituation stages. Sub-adults and juveniles moved significantly less than adults, and adult males moved significantly more (Table 2, Fig. 3). Conversely, all age-sex classes performed social behaviour significantly more often than during other habituation stages. Posterior distributions of feeding behaviour were quite similar to the advanced habituation stage, although adult males were observed feeding significantly less during the full habituation stage. Finally, both adult classes were observed to be inactive more often than sub-adults and juveniles. While juveniles

stayed inactive significantly less often than other classes, they engaged significantly more often in social behaviour (Table 2, Fig. 3).

**Trends in search effort and detection.** Overall, we found a significant effect of the time on the search effort, with the latter decreasing over habituation stages (estimate =  $-0.046 \pm SE 0.02$ ,  $P = 0.049$ ). While the progress of macaque detection increased over the habituation stages, we did not find a significant effect of the time on the detection progress (estimate =  $0.014 \pm SE 0.027$ ,  $P = 0.604$ ) (Fig. 1). Finally, we did not find a significant correlation between the two-time series (i.e., the search effort and the progress of macaque detection) (Pearson correlation:  $r_p = 0.076$ ,  $P = 0.824$ ,  $N = 13$ ).

## DISCUSSION

Habituation allows an observer to approach closely, collect complex behavioural data, and follow free-ranging primates, because they no longer respond to the observer's presence (e.g., through fear, flight, intimidation display). Yet, despite the importance of this process in primatology, very few empirical studies are available. We defined five different stages (early, minimal, partial, advanced, and full) characterising the progression of the habituation process of a large troop of northern pigtailed macaques in Thailand. We recorded statistical evidence of habituation progress. It took close to 13 months of effort to fully habituate the troop, which contradicts previous consideration stating that flexible and adaptable species such as macaques are relatively easy to habituate.

**Habituation of the northern pigtailed macaques.** During the onset of the habituation process (early and minimal habituation stages), we dedicated considerable search effort to maximising the probability of encounter. Macaques were hard to find and track because of their skittish behaviour and specific flight tactic (Table 1), which was also observed in other non-habituated groups of northern pigtailed macaques in Thai wet-evergreen, dry-evergreen, or dry-dipterocarp forests (T. Savini, pers. obs.). However, habituation progress, coupled with our improved tracking skills and knowledge about the troop's habits, allowed the search effort to decrease significantly while maintaining the proportion of troop detection and contact duration, which remained quite similar over the latter stages as the macaques became easier to find and to follow without being lost.

Interestingly, the interruption to fieldwork from early August 2016 to mid-January 2017 did not impact habituation progress. After more than six months of effort, during the partial habituation stage, we were able to collect behavioural data through scan sampling. However, behavioural data collected during the partial and advanced habituation stages were biased toward the most conspicuous individuals (i.e., sub-adult and adult males), while a large majority of sighted individuals moved away to avoid the observer instead of staying engaged in their activities. As frequently observed in other primate species, males were habituated more quickly than females (Fossey, 1983; Tutin & Fernandez, 1991; Cipolletta, 2003; Doran-Sheehy et al., 2007; Klailova et al., 2010). In Sakaerat, females tended to stay away from the observer until later in the minimal habituation stage, although oestrus females were habituated faster. As mentioned by Bertolani & Boesch (2008), who studied habituation in chimpanzees characterised by a fission-fusion social system, this difference in response between females could be explained because fertile females tend to associate with adult males who have more frequent contact with the observer. In addition, non-oestrus females may well be carrying or protecting young and therefore have a lower tolerance toward human followers (they are warier and tend to retreat to safety). After 10 months, during the full habituation stage, we observed a clear shift in the proportion of individuals sighted during scan sampling corresponding to troop demography (Fig. 4). At this stage, macaques mainly

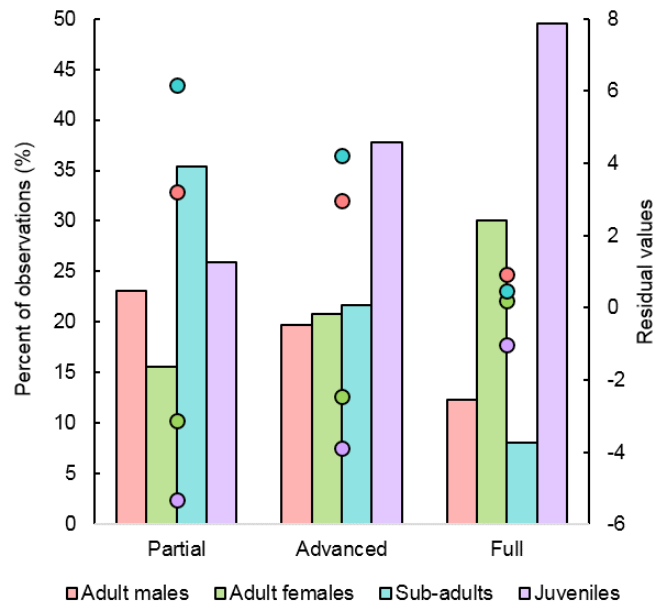


Fig. 4. Proportions of age-sex classes recorded during scan samples during partial, advanced, and full habituation stages. Chi-squared tests were performed for each habituation stage with the theoretical results based on troop demography ( $N_M = 14$ ,  $N_F = 42$ ,  $N_{Sub-A} = 10$ ,  $N_{Juv.} = 77$ ): adjusted residuals (dots) were significant when their absolute value exceeded 2.

ignored the observer and the troop activity budget no longer seemed biased by the observer's presence; individuals stayed engaged in their social behaviours or inactive instead of moving away. However, feeding and other behaviours did not significantly change with habituation progress. During this last stage, behavioural variations we recorded between age-sex classes no longer seemed to be due to observer avoidance, but rather corresponded to commonly observed variations in Cercopithecoidea. Adult males were observed feeding significantly less but moving more and staying more in the inactive state, probably as they patrolled or monitored their environment (Isbell & Young, 1993; Masi et al., 2009; Shanee & Shanee, 2011). Additionally, while juveniles were significantly less inactive, they were performing significantly more social behaviours (e.g., playing, grooming, or social mounting) than adult and sub-adult individuals, which is commonly described in primates (e.g., Back et al., 2019, for *Sapajus* sp.; Jaman & Huffman, 2013, for *Macaca mulatta*; Prates & Bicca-Marques, 2008, for *Alouatta caraya*; Shanee & Shanee, 2011, for *Oreonax flavicauda*; Wallace, 2001, for *Ateles chamek*). These results highlight the importance of analysing behavioural data of fully habituated groups of primates to limit biases of observer presence.

Habituation in social groups may be driven by social learning (Samuni et al., 2014). During habituation, we often observed a domino effect between individuals: if one individual flees, all the other individuals also flee. The opposite is evenly true; habituated individuals act as catalysts, speeding up habituation for the rest of the group. It is therefore possible for habituation to spread from a few individuals to an entire subgroup or population (Turner, 2005). In the Sakaerat troop, adult females had major importance in the habituation process. When some females failed to display



a fear response toward the observer, the other members of the troop rapidly followed (cf. partial habituation stage). Overall, we found that habituation of this troop of northern pigtailed macaques was long, following not a linear, but rather “sawtooth” pattern with rapid changes (Fig. 2). This result is expected for large social groups characterised by fission-fusion dynamics. During our study periods, the large troop (approximately 143 individuals counted) often split into several subgroups at feeding or sleeping sites, and there were a few times when they remained split for consecutive days. For example, we could follow two or three subgroups in one day, and encounter a different subgroup composed of less habituated individuals the next day. As the observer is not in regular contact with the same subgroup, and consequently the same individuals, habituation of such a large troop can take a considerable amount of time.

**Factors that may influence macaque habituation effectiveness.** Time required to habituate primates is taxon-specific, but also depends on habitat-based factors and on the primates’ previous experience with humans (Williamson & Feistner, 2011). Primates with previous experience of hunting are harder to habituate than primates with neutral experience with humans. Conversely, provisioning is often considered a positive stimulus to primates and therefore tends to speed up the habituation process (Bertolani & Boesch, 2008). While it took only four months to fully habituate a semi-provisioned troop of northern pigtailed macaques ranging around the visitor centre in the large and pristine Khao Yai National Park (KYNP; Albert et al., 2013), it took six months to fully habituate the wild-feeding troop inhabiting Mo Singto forest in KYNP (José-Domínguez et al., 2015). Beyond food reinforcement, previous experience of encountering and seeing humans has an influence on habituation. In KYNP, the macaques were accustomed to regularly seeing tourists, which can partially explain the shorter amount of time required to habituate the wild-feeding KYNP troop compared to that for the Sakaerat troop. Additionally, the KYNP semi-provisioned troop came regularly to the same spot to check for human food, and frequently reused the same sleeping trees (Albert et al., 2011), allowing the observer to find them easily. In contrast, predicting wild-feeding troop locations is very difficult in space and time, and a lot of the habituation effort is devoted to finding the troop.

In fact, a crucial aspect of the habituation of forest-dwelling primates is finding them. The difficulty can be exacerbated by several factors such as large home range, long travel distance, spatio-temporal variation of resource distribution and availability (i.e., low predictability), little reuse of sleeping sites, large group size, fission-fusion social system, full or semi-terrestriality (e.g., inconspicuous movement on the ground), and dense forest habitat (i.e., lack of visibility) (Williamson & Feistner, 2011). Our study troop and site exhibited all of these characteristics, which can be even more severe in a degraded forest fragment (Gazagne et al., 2020a, 2020b). Habitat degradation, which often results in reduced availability and quality of food resources, modified temporal and spatial distribution of food resources, and loss of sheltering or sleeping locations, can significantly affect

primates’ space use, activity budget, social organisation, and health status, and can have a drastic effect on their survival (de Almeida-Rocha et al., 2017; Estrada et al., 2017; McLennan et al., 2017; Kalbitzer & Chapman, 2018). We assume that macaques living in the degraded forest of Sakaerat with sub-optimal native fruit resources (Gazagne et al., 2020a) are under higher stress than conspecifics inhabiting the pristine KYNP, which also partially explains their longer habituation process. Moreover, the el Niño climate phase in 2016 caused an unusual drought in Sakaerat and significantly decreased fruit availability (E. Gazagne, unpublished data), which likely stressed the macaques and did not ease the habituation process.

**Habituation to specific traits and over-habituation.** Throughout this study, the observer remained constant in their appearance, with the same clothing (bright red shirt visible in the forest), hat, and rucksack. As macaques have good memory recognition of objects and faces (Pascalis & Bachevalier, 1998), we aimed to enhance the macaques’ short-term “trait-based” recognition of the observer. Beyond this trait-based habituation, the generalisation of habituation to other humans can be detrimental to primate populations. With the loss of fear towards humans, animals are vulnerable to approach by hunters and poachers. It was common, after habituation of the Sakaerat troop, to observe adult males, sub-adults, or a few juveniles addressing curiosity or displays towards other scientists and mushroom poachers, while females avoided them (i.e., corresponding to the minimal stage of habituation). This, of course, raises some concerns. We assume that if macaques in Sakaerat remain in infrequent contact with humans, avoidance of other humans can be sustainable, which would help this population to persist (i.e., put them at lower risk). In contrast, the circumstances of the macaques in KYNP, which are frequently in contact with humans and often food-provisioned, lead to over-habituation, i.e., an extreme state of habituation in which macaques have not only lost fear of humans but also include humans in social interactions or food source reliability (Webb & McCoy, 2014). Over-habituation not only impacts primates’ diet, activity budgets, and ranging patterns, but can also promote human-primate conflicts, posing problems for local human populations, ecotourism programmes, and primate conservation, with negative consequences for both parties (Priston & McLennan, 2013; Sengupta et al., 2015).

## CONCLUSION

It is now well documented that the pros and cons of habituation should be carefully considered, yet few studies on forest-dwelling primates such as macaques have detailed the habituation process itself. However, habituation is a necessary step for the collection of eco-ethological data; it is indispensable for sound management of the conservation of vulnerable and unknown species. Our study statistically demonstrated that the habituation of a large troop of *Macaca leonina* with fission-fusion dynamics to a human observer requires considerable time investment (13 months) in a degraded forest fragment characterised by reduced resource

availability and little previous experience with humans. For most primates, the key to habituation success is constancy, knowledge of the study site, and common sense (Williamson & Feistner, 2011). In order to make this process more accessible to field researchers, we encourage the reporting of as much information as possible on habituation progress in a wide range of primate species.

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## SUPPLEMENTARY MATERIAL

Table S1. Record of the monthly frequency and duration of encounters with a troop of *Macaca leonina* during the habituation process in Sakaerat Biosphere Reserve (Thailand). Percentage of macaque detection is based on the number of days with encounters and the number of days in the field. Cumulative contact duration is the sum of all contact durations in a tracking day. A description is provided of the macaque tracking characteristics over the five habituation stages: early (1), minimal (2), partial (3), advanced (4), and full (5).

Stage	Month	Number of encounters	Number of days with encounters	Number of days in the field	Detection of macaques (%)	Mean contact duration per day (min)	Mean cumulative contact duration per day (min)	Tracking characteristics of the troop
1	Mar 2016	18	8	24	33.3	42	95	Short, interrupted, mainly via auditory cues.
	Apr 2016	7	6	23	26.1	24	29	
	May 2016	27	14	24	58.3	85	171	
	Jun 2016	29	15	26	57.7	66	127	
2	Jul 2016	36	19	29	65.5	73	140	Short, interrupted, with increased visual contact. Identification of first sleeping site locations (by triangulation).
	Aug 2016	10	4	4	100	49	124	
	Jan 2017	5	5	8	62.5	70	70	
3	Feb 2017	20	16	21	76.2	449	561	Long, with few interruptions (e.g., 10 full days). Precise estimation of sleeping site centre. Possibility of behavioural data collection via scan sampling.
4	Mar 2017	14	11	12	91.7	520	661	Long and full consecutive days, with few interruptions.
	Apr 2017	12	10	13	76.9	488	612	
5	May 2017	9	9	13	69.2	742	742	Full consecutive days following the troop (without interruption). Possibility of complex behavior collection.
	Jun 2017	7	7	8	87.5	748	748	
	Jul 2017	8	8	9	88.9	763	763	

Table S2. Evolution of macaque behavioural response to the observer’s presence (data collected ad libitum) according to age-sex classes (M: adult males, F: adult females, S: sub-adults, J: juveniles) during the habituation process of a troop of *Macaca leonina* in the Sakaerat Biosphere Reserve (Thailand). The habituation progress is defined over five stages: early (stage 1), minimal (stage 2), partial (stage 3), advanced (stage 4), and full (stage 5). Grey boxes indicate when the behaviour was observed. See Table 1 for a definition of these behavioural responses.

Month	Stage	Flight				Avoidance				Curiosity				Display				Puckering				Ignore			
		M	F	S	J	M	F	S	J	M	F	S	J	M	F	S	J	M	F	S	J	M	F	S	J
March 2016	1	█	█	█	█																				
April 2016		█	█	█	█																				
May 2016		█	█	█	█																				
June 2016		█	█	█	█																				
July 2016	2		█		█	█	█	█	█	█		█	█	█	█	█	█	█		█	█				█
August 2016			█		█	█	█	█	█	█		█	█	█	█	█	█	█		█	█				█
January 2017			█		█	█	█	█	█	█		█	█	█	█	█	█	█		█	█				█
February 2017	3						█			█	█	█	█	█	█	█	█			█	█	█	█	█	█
March 2017	4						█			█	█	█	█	█	█	█	█			█	█	█	█	█	█
April 2017							█			█	█	█	█	█	█	█	█			█	█	█	█	█	█
May 2017	5						█			█	█	█	█	█	█	█	█			█	█	█	█	█	█
June 2017							█			█	█	█	█	█	█	█	█			█	█	█	█	█	█
July 2017							█			█	█	█	█	█	█	█	█			█	█	█	█	█	█

**Note on puckering behaviour.** During the habituation process we observed puckering behaviour (also called pucker, pucker face, flehmen, or lips forward-ears back-neck extended), which is described as compressed and protruded lips with retracted eyebrows, forehead, and ears (Bobbitt et al., 1964; Bernstein, 1967; Maestriperi, 2005). This behaviour is mainly displayed by pigtailed macaques, although it has been only described in southern pigtailed macaques (*Macaca nemestrina*) so far (Bernstein, 1967; Maestriperi, 1996, 2005; Oettinger et al., 2007). Throughout this study, this behaviour was frequently performed by adult males, sub-adults, and juveniles, independently of their social status and in a variety of social contexts that were either affiliative or agonistic (e.g., approach-retreat interactions, grunt, chase, social mount, bouts of playing), as also observed by Maestriperi (2005). When addressed to the observer, we interpreted this behaviour as a potential intimidation display (i.e., distance-reducing function; Maestriperi, 1996), or at the simplest level a way to assert “I am looking at you” (cf. Oettinger et al., 2007). This behaviour might be a way to draw attention to the sender while not necessarily implying a threat, but it is still poorly understood, especially in northern pigtailed macaques. The pucker is a visual display that probably evolved in pigtailed macaques to facilitate cooperation between individuals when silence or stealth is advantageous (Oettinger et al., 2007).



Fig. 5. Adult males (left and right pictures) and a juvenile male (central picture) from the Sakaerat troop performing puckering behaviour.