

Temperament and Social Hierarchy in a Colony of Juvenile Male Southern Pig-Tailed Macaques (*Macaca nemestrina*) at the Primate Research Centre, IPB University

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Abstract

The use of southern pig-tailed macaques (*Macaca nemestrina*) as animal models requires adherence to animal welfare principles and the 3Rs (replacement, reduction, and refinement). This study aimed to characterise the temperament of individual juvenile pig-tailed macaques and to determine the social hierarchy structure within a captive colony to support the development of appropriate management and training strategies. Temperament assessment was conducted through behavioural observations using a standardised ethogram during 20-minute focal sampling sessions, four sessions per day over 14 consecutive days. The subjects were four juvenile males, approximately 3 years old, housed in colony cage GM 2/4 at the Research Animal Facility at Lodaya (RAFL), Primate Research Centre, IPB University. We recorded 2,838 behavioural events across five temperament categories (aggressive, neutral, affiliative, submissive, and anxious). A one-way ANOVA with Tukey's HSD post hoc test revealed that affiliative behaviours were significantly more frequent than those in other categories ($p < 0.05$). Three individuals exhibited predominantly affiliative temperaments, while one exhibited an aggressive temperament. Social hierarchy positions (high, middle, and low) were established by summing the frequencies of aggressive–submissive interactions among the four individuals. One individual occupied the high rank, one the middle rank, and two the low rank. Notably, one low-rank individual that had been hand-reared displayed elevated frequencies of anxious behaviours, including stereotypic pacing. These findings provide a behavioural baseline for refining colony management and training protocols consistent with the 3Rs framework.

Keywords: aggressive–submissive interaction, colony management, *Macaca nemestrina*, social hierarchy, temperament

1. Introduction

Indonesia harbours a remarkably high diversity of primates, with at least 11 genera and 59 species distributed across the archipelago (Roos *et al.* 2014). Among these, the southern pig-tailed macaque (*Macaca nemestrina*) is of particular conservation concern. The IUCN Red List reclassified this species from Vulnerable to Endangered in 2022 due to ongoing population declines driven primarily by habitat conversion for plantations, mining, and large-scale development projects, compounded by forest degradation and extreme weather events (Ruppert *et al.* 2022). Despite its global endangered status, *M. nemestrina* is not legally protected under Indonesian national law (Ministerial Regulation P.20/MENLHK/SETJEN/KUM.1/6/2018) and is listed under CITES Appendix II.

Pig-tailed macaques serve important ecological roles as frugivorous seed dispersers that contribute to forest regeneration (Sohara *et al.* 2023; Tsuji and Su 2018). In biomedical research, they are valued as animal models for their genetic, anatomical, physiological, and behavioural similarities to humans

(NASEM 2023). Furthermore, pig-tailed macaques are favoured over other macaque species because of their comparatively cooperative temperament, ease of habituation to humans, and trainability (Sussman *et al.* 2013; Cole 1957).

The use of pig-tailed macaques as animal models must comply with animal welfare principles and the 3Rs framework—replacement, reduction, and refinement (Yurista *et al.* 2017). Refinement entails modifying experimental procedures to improve animal welfare and minimise pain and distress (Intan and Khariri 2020). A key approach to implementing refinement is to create environments that allow macaques to meet their physical and social needs. Achieving this goal requires an understanding of temperament profiles and social hierarchy structures within captive colonies, as pig-tailed macaques are inherently social animals that live in multi-male, multi-female groups (Kemal *et al.* 2022; Anuar and Ullah 2011). Oi (1990a) classified the dominance hierarchy within pig-tailed macaque groups into high-ranking, middle-ranking, and low-ranking positions, determined through agonistic interactions among individuals.

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Temperament refers to an individual's characteristic pattern of behavioural expression rooted in emotional reactivity (Cloninger 1994). In primates, temperament is identifiable from a young age and develops into personality with maturation (Clarke and Boinski 1995; Sussman 2014). Understanding temperament and social hierarchy is essential for designing effective health management and training protocols for captive primate colonies (Anderson 2022). Previous positive reinforcement training (PRT) conducted on the same colony (Fitriani 2024) suggested that training outcomes were influenced by social hierarchy, but this relationship had not been systematically confirmed.

This study aimed to (1) assess the temperament of individual juvenile male pig-tailed macaques in colony cage GM 2/4 at the Primate Research Centre, IPB University (PRC-IPB), and (2) determine the social hierarchy structure of the colony based on aggressive–submissive interactions. The results are expected to inform refinement strategies for colony management and training that align with animal welfare principle

2. Materials and Methods

2.1 Study Site and Subjects

This study was conducted from January to March 2025 at the Research Animal Facility at Lodaya (RAF-L), PRC-IPB, Bogor, Indonesia. All procedures were approved by the Animal Care and Use Committee of the Primate Research Centre, IPB University (Approval No. IPB PRC-23-B002). The subjects were four juvenile male pig-tailed macaques, approximately 3 years old, housed together in colony cage GM 2/4 under social-housing conditions. Each individual was identifiable by distinct morphological features: Subject A had grayish-brown fur with a protuberance on the left elbow; Subject B had deeper eye sockets; Subject C had a broader and rounder face; and Subject D had a lighter facial complexion.

2.2 Behavioural Observation and Temperament Assessment

Temperament was assessed using focal sampling (modified from Altmann 1974) over 14 consecutive days. Before data collection, a six-day habituation period was conducted to acclimatise the subjects to the observer's presence and to determine optimal observation time points. Observations were carried out during four daily sessions: morning pre-feeding (approximately 07:30–09:00), morning post-feeding (approximately 09:30–10:00), afternoon pre-feeding (approximately 13:00–14:00), and afternoon post-feeding (approximately 14:00–14:30). Each session lasted 20 minutes, yielding a total of 56 sessions (1,120 minutes of observation). The observer stood at a safe distance of 1–2 m from the cage and recorded each individual's behaviours using a standardised ethogram.

The ethogram categorized behaviors into five temperament types: aggressive (biting, manual attack,

pursuit, and intimidation), neutral (relaxed posture), affiliative (approach, proximity, contact, coo call, grooming, lip-smacking, playing, rump presenting, and mounting), submissive (defensive aggression, fear grimace, locomotion in fear, gaze aversion, and crouching), and anxious (body shuddering, teeth grinding, scratching, yawning, and stereotypic behaviors including swinging, bouncing, pacing, flipping, shaking, self-grasping, self-biting, sucking, hair extraction, and hand clapping). The ethogram was adapted from Hinde and Rowell (1962), Kaufman and Rosenblum (1966), Grimm (1967), Pomerantz and Capitanio (2021), and Feng *et al.* (2023).

2.3 Social Hierarchy Determination

Social hierarchy was determined by recording aggressive–submissive interactions among individuals concurrently with temperament observations, following the method of Tokuda and Jensen (1969) with modifications. In each interaction, one individual was designated the initiator (exhibiting aggressive behaviour) and the other the recipient (responding with submissive behaviour). The paired aggressive–submissive categories recorded were: biting–defensive aggression, manual attack–defensive aggression, pursuit–locomotion in fear, biting–locomotion in fear, manual attack–locomotion in fear, intimidation–gaze aversion, and intimidation–fear grimace. Hierarchical positions were assigned based on the cumulative frequency of interactions as initiator versus recipient. An individual with a substantially higher frequency as initiator than recipient was classified as high rank; one with a relatively balanced frequency was classified as middle rank; and those with a higher frequency as recipient than initiator were classified as low rank.

2.4 Data Analysis

The predominant temperament of the colony was determined from the total behavioural frequency per temperament category across 14 days and the daily mean frequency per category across all four individuals. Data were analysed using a one-way ANOVA followed by Tukey's HSD post hoc test ($\alpha = 0.05$) using Minitab 18. Individual temperament was assigned based on the temperament category with the highest proportion, calculated as: Temperament (%) = (frequency of behaviours in a given temperament category / total behavioural frequency) \times 100.

3. Results

3.1 Colony Temperament Profile

A total of 2,838 behavioural events indicative of specific temperament categories were recorded across 56 observation sessions. One-way ANOVA revealed significant differences among temperament categories ($p < 0.05$). Affiliative behaviours were the most frequently observed (total frequency = 967; daily mean = 69.07 ± 25.75), followed by aggressive (729; 52.07 ± 28.67), neutral (669; 47.79 ± 10.55), submissive (352; 25.21 ± 13.75), and anxious (61; 8.57 ± 9.16) behaviours (Table 1). Tukey's

HSD test indicated that affiliative temperament differed significantly from submissive and anxious temperaments, while aggressive and neutral temperaments were intermediate.

Table 1. Frequency of temperament categories in colony GM 2/4

Temperament	Total frequency	Daily mean frequency
Aggressive	729	52.07 ± 28.67 ^{ab}
Neutral	669	47.79 ± 10.55 ^b
Affiliative	967	69.07 ± 25.75 ^a
Submissive	352	25.21 ± 13.75 ^c
Anxious	61	8.57 ± 9.16 ^c

Values followed by the same superscript letter are not significantly different (Tukey HSD, $p > 0.05$).

At the individual level, Subjects B, C, and D exhibited predominantly affiliative temperament profiles, whereas Subject A exhibited a predominantly aggressive temperament. Subject D displayed the highest proportion of anxious behaviours among all individuals, including stereotypic pacing.

3.2 Social Hierarchy

A total of 424 aggressive–submissive interactions were recorded. Subject B had the highest frequency as initiator (172), followed by Subject A (150), Subject D (55), and Subject C (47). Conversely, Subject C received the most aggression (157 events as recipient), followed by Subjects A (108), D (104), and B (55) (Table 2).

Table 2. Frequency of aggressive–submissive interactions among individuals

Initiator	Subject A	Subject B	Subject C	Subject D
Subject A	-	32	60	58
Subject B	63	-	79	30
Subject C	16	15	-	16
Subject D	29	8	18	-

Row values indicate the frequency of an individual acting as the initiator of aggressive behaviour toward the column individual (recipient).

Table 3. Hierarchy positions in colony GM 2/4

Individual	Initiator freq	Recipient freq	Position
Subject B	172	55	High rank
Subject A	150	108	Middle rank
Subject D	55	104	Low rank
Subject C	47	157	Low rank

Discussion

The present study characterised the temperament profiles and social hierarchy of a colony of juvenile male pig-tailed macaques at the PRC-IPB. The predominance of affiliative temperament across the colony is consistent with the characterisation of pig-tailed macaques as a relatively tolerant and egalitarian macaque species (Thierry 2000). Compared to long-tailed macaques (*M. fascicularis*) and rhesus macaques

(*M. mulatta*), pig-tailed macaques display lower levels of aggression and higher rates of affiliative behaviour, a pattern attributed to evolutionary responses to differing predation pressures (Sussman *et al.* 2013). Long-tailed macaques, which are smaller-bodied and frequently occupy forest edges and riparian habitats that are accessible to predators, experience higher predation pressure, which may have selected for heightened aggression and vigilance. Pig-tailed macaques, which are larger-bodied and typically inhabit primary highland rainforests, face comparatively lower predation risk.

Temperament in primates is a heritable trait identifiable from early life that influences social behaviour and stress responsivity (Clarke and Boinski 1995; Cloninger 1994; Dyussenbayev 2014). In juvenile primates, temperament provides the foundation for adult personality development through learning and experience (Cloninger *et al.* 1993; Sussman 2014). The individual-level variation in temperament observed in this study—three individuals displaying affiliative temperament and one displaying aggressive temperament—underscores the importance of assessing each animal individually rather than relying on species-level generalisations when designing colony management protocols.

Dominance hierarchies in macaque groups emerge through repeated agonistic interactions and are influenced by age, body size, social alliances, and maternal rank (Holekamp and Strauss 2016; Bernstein 1969; Tokuda and Jensen 1969). Although the subjects in this study had not yet reached sexual maturity (approximately 4–4.5 years for male pig-tailed macaques; Saputro *et al.* 2023), clear hierarchical tendencies were already apparent. Subject B, who held a high-ranking position, exhibited the highest frequencies of both affiliative and aggressive behaviours, reflecting an active, socially dominant individual. This finding is consistent with Caine *et al.* (1983), who reported that high-ranking pig-tailed macaques are more confident, active, and aggressive. Individuals occupying higher-ranking positions generally enjoy greater access to resources such as food and mates (Arlet *et al.* 2024) and tend to employ active coping strategies when confronting stressors (LeClair and Russo 2021).

Subject A was classified as middle rank, displaying high frequencies of both aggressive initiation and submissive receipt, particularly in interactions with Subject B. This ambiguous position is consistent with descriptions by Capitanio *et al.* (1985), who noted that middle-ranking individuals dominate some group members while remaining subordinate to others. Anderson (2022) further observed that middle-ranking individuals often experience elevated social tension due to their potentially unstable position within the hierarchy.

Subjects C and D occupied the low-rank position, characterised by higher aggression frequencies as recipients than as initiators. Low-ranking individuals

tend to be more tense, fearful, and dependent (Caine *et al.* 1983) and adopt passive coping strategies in the face of stressors (LeClair and Russo 2021). Notably, both Subjects C and D were occasionally observed directing aggressive behaviours toward individuals in an adjacent cage (GM 2/5) housing younger macaques, suggesting that dominance expression is context-dependent and influenced by the relative age and familiarity of interactants (Tokuda and Jensen 1969).

Subject D, a hand-reared individual whose mother had rejected during infancy, displayed the highest proportion of anxious behaviours in the colony, including stereotypic pacing. Early maternal deprivation is a well-established risk factor for the development of anxiety-related and stereotypic behaviours in primates (Feng *et al.* 2023; Morin *et al.* 2025). Individuals deprived of maternal care in early life tend to develop suboptimal neural regulation of emotional responses, resulting in elevated anxiety and reduced social competence (Clarke and Boinski 1995; Worlein and Sackett 1997). Social housing, environmental enrichment, and positive reinforcement training have been recommended as interventions to mitigate anxiety in captive primates (Coleman and Pierre 2014; Worlein and Sackett 1997).

A previous PRT conducted on this colony by Fitriani (2024) found that Subject B required more time to complete training tasks than Subject C. Fitriani (2024) hypothesised that this difference was related to social dominance. Still, this relationship had not been systematically verified. The present study provides empirical support for this hypothesis by demonstrating that Subject B occupies the high-rank position and Subject C occupies a low-rank position, suggesting that dominant individuals may be less motivated by food rewards in training contexts because they already enjoy preferential access to resources.

Bernstein (1969) reported that pig-tailed macaque hierarchies are remarkably stable, changing primarily upon the death of the alpha male or female. However, given the subjects' juvenile age in this study, the hierarchy observed here should be considered a developing and potentially dynamic social structure. Longitudinal monitoring is recommended to track changes in hierarchy as these individuals mature, particularly following the introduction of new colony members or hormonal changes associated with puberty.

In conclusion, the juvenile male pig-tailed macaques in colony GM 2/4 displayed predominantly affiliative temperaments, consistent with the species' egalitarian social tendencies. A clear hierarchy comprising high-, middle-, and low-rank positions was evident in aggressive-submissive interactions. The identification of a hand-reared individual with elevated anxious behaviours highlights the need for targeted welfare interventions. These findings provide a behavioural baseline to inform the design of training programs and management protocols aligned with the principle of refinement within the 3Rs framework.

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