



RESEARCH ARTICLE SUMMARY

PRIMATE GENOMES

Adaptations to a cold climate promoted social evolution in Asian colobine primates

Xiao-Guang Qi^{*,†}, Jinwei Wu[†], Lan Zhao[†], Lu Wang, Xuanmin Guang, Paul A. Garber, Christopher Opie, Yuan Yuan, Runjie Diao, Gang Li, Kun Wang, Ruliang Pan, Weihong Ji, Hailu Sun, Zhi-Pang Huang, Chunzhong Xu, Arief B. Witarto, Rui Jia, Chi Zhang, Cheng Deng, Qiang Qiu, Guojie Zhang, Cyril C. Grueter^{*}, Dongdong Wu^{*}, Baoguo Li^{*}

INTRODUCTION: Primates have evolved a diverse set of social systems, from solitary living to large multilevel societies. The traditional socioecological model explains this diversity as a response to changing environments, which shaped patterns of cooperation and competition for resources and predator defense. However, the socioecological model does not explain why sympatric species living in the same environment exhibit different social systems. There is a growing consensus that primate social organization shows a strong phylogenetic signal as a result of shared inheritance from a common ancestor and evolved stepwise along with species differentiation. This implies a genetic basis for the evolution of animal social

systems. However, the genomic mechanisms that underlie the expression of primate social systems remain poorly understood.

RATIONALE: Asian colobines, a subfamily of Old World monkeys, are represented by seven genera and 55 species that are distributed from tropical rainforests to snow-covered mountains. They exhibit four distinct types of social organization and provide a good model for examining the mechanisms that drive social evolution from a common ancestral state to the diverse systems present today. By integrating new genomic data across all seven colobine genera with paleoenvironmental information, the fossil record, social organization

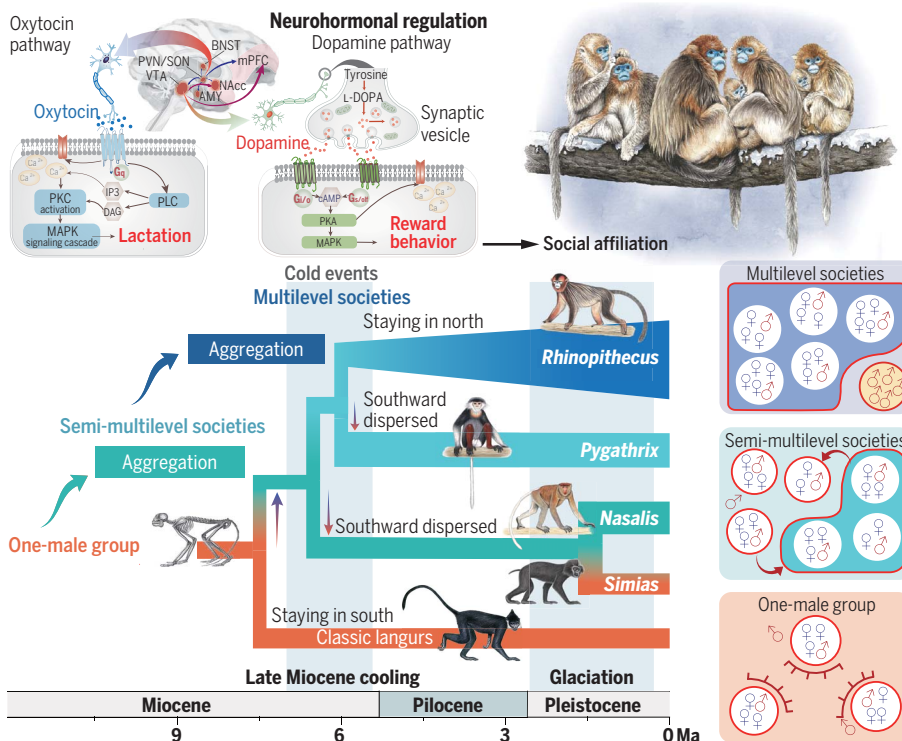
characteristics, social behavioral characteristics, and ecological niche modeling, we constructed a socioecological-genomic framework to identify selective pressures that form the genetic basis for social evolution in Asian colobines.

RESULTS: To understand the evolutionary process of social systems in Asian colobines, we first reconstructed their phylogenetic relationships using whole-genome data. In contrast to the previous hypothesis of three major clades, our study reveals that Asian colobines split into two clades: the odd-nosed monkeys and the classical langurs. Our phylogenetic analyses detected a strong signal in colobine social evolution, suggesting that these social systems evolved in a stepwise manner, with ancestral one-male, multifemale groups fusing into semi-multilevel societies characterized by fission-fusion and then merging into complex multilevel societies. Consistent with our ecological results indicating that extant colobine primates that inhabit colder environments tend to live in larger groups, we found that adaptations driven by ancient cold events, including the late Miocene cooling and Pleistocene glacial periods, played an important role in promoting these changes in social evolution. Furthermore, our genomic analyses revealed that these cold events promoted the selection of genes involved in energy metabolism and neurohormonal regulation. In particular, more-efficient dopamine and oxytocin pathways developed in odd-nosed monkeys, which might have resulted in the prolongation of maternal care and lactation, favoring infant survival in cold environments. These adaptive changes also appear to have strengthened interindividual affiliation, increased male-male tolerance, and facilitated the stepwise social aggregation from independent one-male, multifemale groups to large multilevel societies in Asian colobines.

CONCLUSION: Our results reveal a stepwise evolutionary scenario of social organization in Asian colobines. We show that ancient glacial events selected for neurohormonal regulation, including dopamine and oxytocin pathways that promoted aggregation from one-male, multifemale groups into large multilevel societies. Our study demonstrates a direct link between a genomically regulated adaptation and social evolution in primates and offers new insights into the mechanisms that underpin behavioral evolution across animal taxa. ■

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 Cite this article as X. G. Qi *et al.*, *Science* **380**, eabl8621 (2023). DOI: 10.1126/science.abl8621

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<https://doi.org/10.1126/science.abl8621>



Adaptation for survival in cold climates facilitated evolution of social behavior in colobine monkeys. Cold environments promoted the social evolution of Asian colobines in a stepwise manner. Genomic changes in neurohormonal regulation, including in the dopamine and oxytocin pathways, improved social affiliation in odd-nosed monkeys and thus promoted social aggregations from independent one-male, multifemale groups into large multilevel societies. Ma, million years ago.

RESEARCH ARTICLE

PRIMATE GENOMES

Adaptations to a cold climate promoted social evolution in Asian colobine primates

Xiao-Guang Qi^{1*}†, Jinwei Wu^{1†}, Lan Zhao^{1†}, Lu Wang¹, Xuanmin Guang², Paul A. Garber³, Christopher Opie⁴, Yuan Yuan⁵, Runjie Diao⁶, Gang Li⁷, Kun Wang⁵, Ruliang Pan¹, Weihong Ji⁸, Hailu Sun², Zhi-Pang Huang¹, Chunzhong Xu⁹, Arief B. Witarto¹⁰, Rui Jia⁷, Chi Zhang², Cheng Deng⁶, Qiang Qiu⁵, Guojie Zhang¹¹, Cyril C. Grueter^{12*}, Dongdong Wu^{11*}, Baoguo Li^{1*}

The biological mechanisms that underpin primate social evolution remain poorly understood. Asian colobines display a range of social organizations, which makes them good models for investigating social evolution. By integrating ecological, geological, fossil, behavioral, and genomic analyses, we found that colobine primates that inhabit colder environments tend to live in larger, more complex groups. Specifically, glacial periods during the past 6 million years promoted the selection of genes involved in cold-related energy metabolism and neurohormonal regulation. More-efficient dopamine and oxytocin pathways developed in odd-nosed monkeys, which may have favored the prolongation of maternal care and lactation, increasing infant survival in cold environments. These adaptive changes appear to have strengthened interindividual affiliation, increased male-male tolerance, and facilitated the stepwise aggregation from independent one-male groups to large multilevel societies.

Primates have evolved a diverse set of social systems (1–3). From solitary living and small families to large multilevel societies, evolution associated with varied behavioral tactics has allowed primates to successfully exploit a wide range of habitats (4–9). The socioecological model explains the diversity of primate social systems as a response to changing environments, which shaped patterns of cooperation and competition for resources and predator defense (10–12). However, the socioecological model does not explain why sympatric species can live in the same environment but exhibit different social systems (13, 14).

Evidence increasingly supports that the social system of different primate taxa is likely inherited from a recent common ancestor, rather than evolving as a direct adaptation to current environmental conditions (15, 16). For example, although they inhabit the same rain-

forest, white-handed gibbons form monogamous pairs, whereas Thomas's langurs live in a one-male, multifemale polygynous group; long-tailed macaques live in multimale, multifemale groups; and Bornean orangutans live solitarily with occasional social contact (17). Therefore, there is a growing consensus that certain components of social systems have a strong phylogenetic signal (5, 18) and evolved in a stepwise manner in conjunction with species differentiation (16, 19). However, the genomic mechanisms that constrain or promote the expression of primate social systems remain poorly understood (20, 21).

Asian colobines, a subfamily of Old World monkeys, are represented by seven genera and 55 species that are distributed from tropical rainforests to snow-covered mountains. They exhibit four distinct types of social organization and provide a good model for examining the multiple mechanisms that have driven their social evolution from a common ancestral state to the diverse systems that are present today (Fig. 1 and data S1). These Asian colobines are categorized into two clades (22). The classical langurs (genera *Presbytis*, *Semnopithecus*, and *Trachypithecus*) are each principally characterized by a one-male, multifemale unit; polygynous mating; and strict male territorial defense. In addition, a small number of species in this clade such as the Himalayan gray langur (*Semnopithecus schistaceus*) and the Indochinese langur (*Trachypithecus crepusculus*) exploit high-altitude forests and occasionally form cohesive larger multimale, multifemale groups (Fig. 1C). By contrast, species in the odd-nosed monkey clade exhibit a wide spectrum of social systems. Simakobus (genus *Simias*)

live in independent one-male, multifemale units, whereas doucs (genus *Pygathrix*) and proboscis monkeys (genus *Nasalis*) live in distinct nonterritorial one-male, multifemale units, which seasonally fuse into a single breeding band or aggregate together at nighttime sleeping sites (23) (data S1). We term these semi-multilevel societies because this social system is characterized by flexibility in switching between independent one-male, multifemale units and multilevel societies. The last group of odd-nosed monkeys are the snub-nosed monkeys (genus *Rhinopithecus*). They live in typical multilevel societies, which are composed of several core one-male, multifemale units embedded within a stable and larger social matrix and associated all-male bachelor bands (24).

In this study, we integrated newly acquired de novo high-quality genome data representing all seven colobine genera with paleoenvironmental information, the fossil record, type of social organization, level of intrasexual tolerance, and ecological databases from 2189 habitat locations (data S2) of 48 extant Asian colobine species. This allowed us to construct a comparative dynamic socioecological-genomic framework that identifies the genetic basis of social evolution in primates.

Phylogeny reconstruction

To understand the social evolution of Asian colobines, we clarified their phylogenetic relationships and natural histories. To resolve previous inconsistencies concerning colobine phylogenetic relationships (25, 26), we sequenced and analyzed seven de novo genomes of species from all seven genera of Asian colobines [supplementary materials (SM) section 3.3.1]. Based on a combination of the concatenation method and the coalescent method, a new phylogenomic tree was reconstructed from a total of 4992 one-to-one orthologs (fig. S7). With calibrations from new fossil discoveries, we were able to develop greater precision in divergence time estimates (Fig. 2A). This new high-confidence topological structure enabled us to trace the evolutionary history of social systems in Asian colobines. The results revealed that Asian colobines split into two well-supported clades: the odd-nosed monkeys and the classical langurs. The genera *Presbytis*, *Semnopithecus*, and *Trachypithecus* are best described as a monophyly of the classical langurs (Fig. 2A). These results contrast with the hypothesis of three major clades, with *Presbytis* located at the basal position of an independent monophyly, which was proposed in previous studies (27, 28).

Phylogenetic signal of social evolution

To understand how the set of social organizations of extant Asian colobines was shaped by their phylogenetic lineage, we used phylogeny trait reconstruction modeling. Based on

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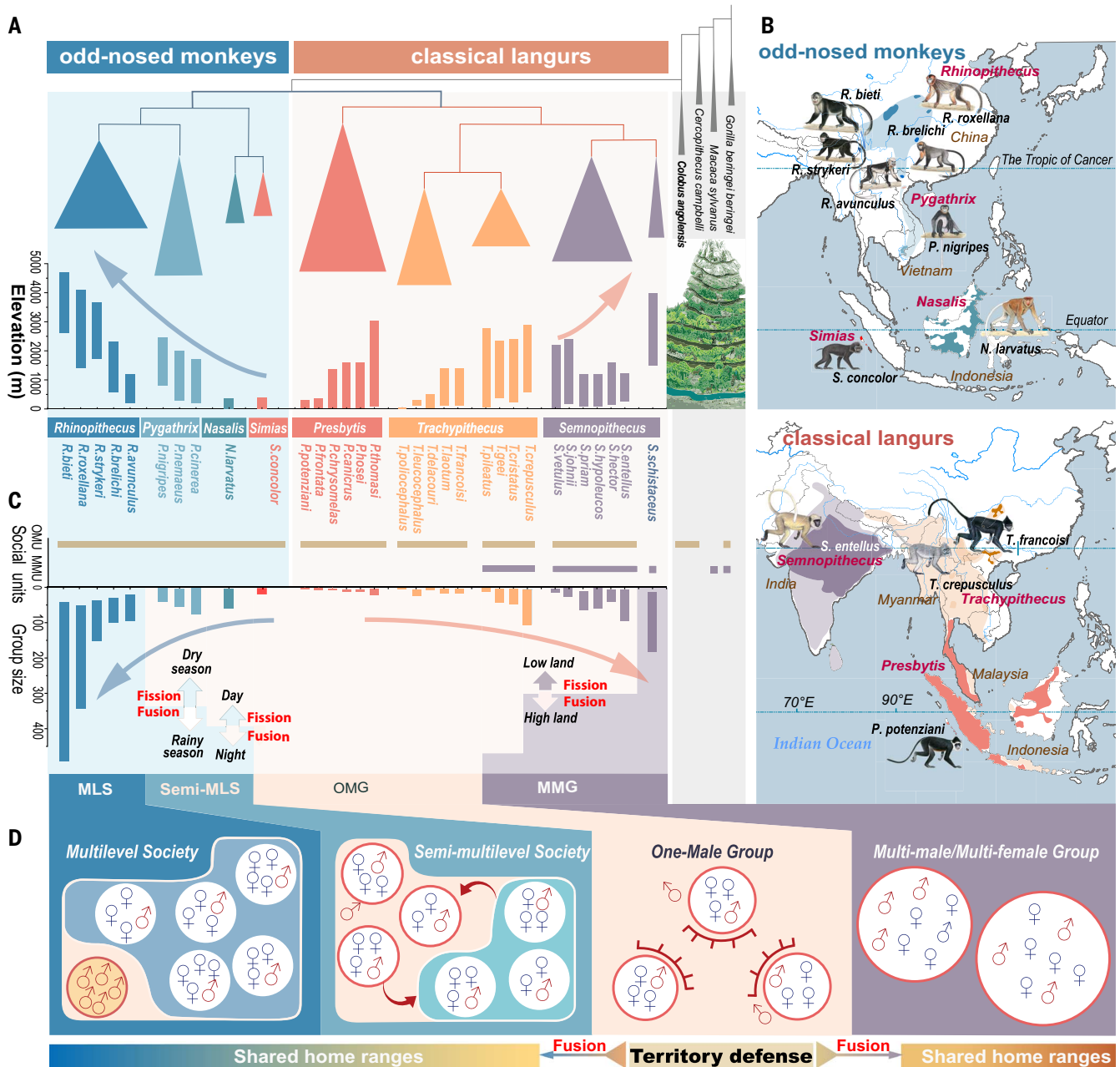


Fig. 1. Taxonomy and the social systems of Asian colobines. (A) Classification and vertical distribution of Asian colobines. (B) Geographical distribution of odd-nosed monkeys and classical langurs. (C) Group size increases with increasing elevation and latitude in both odd-nosed monkeys and classical langur species. MMU, multimale, multifemale unit; OMU, one-male, multifemale unit. (D) Stepwise evolution of social systems in Asian colobines. MLS, multilevel society; MMG, multimale, multifemale group; OMG, one-male group. [Credits: All monkey illustrations are copyrighted 2014 by Stephen D. Nash/IUCN/SSC Primate Specialist Group and used with permission]

the new phylogenomic tree (fig. S2, data S3, and SM section 3), we used Pagel's λ (29) and Phylo.D (30) to evaluate the strength of the phylogenetic signal in their social evolution. The results showed a strong signal [Pagel's $\lambda = 0.81$, log likelihood (LL) = 34.98, probability of λ resulting from Brownian model ($P_{\lambda, \text{Brownian}} = 1$; estimated Phylo.D (D) = -0.44, probability of D resulting from Brownian model ($P_{D, \text{Brownian}} = 0.87$] in colobine social evolution (table S6 and SM section 4.1). Next, we used a macroevolutionary

model fitting analysis to compare the fit factors of phylogenetically associated models [λ , κ , δ , early burst (EB)] with nonphylogenetic models (white-noise model) in Asian colobines. The results showed that the likelihood of each of the four phylogenetically associated models was significantly higher than that of the white-noise model (table S10). These results indicate that during their evolutionary history, phylogeny was a relevant driving factor rather than a random factor in colobine sociality (SM section 4.1.2).

To verify whether social evolution in Asian colobines was stepwise, we compared ordered (stepwise) models with an unordered evolution model using MultiState in BayesTraits. By comparing the marginal likelihoods among the three candidate stepwise models (SM section 4.1.3) and the unrestricted model (unordered model) (fig. S2), a strong Bayes factor ($\log \text{BF}_{\text{model_OMM or OSM}} > 10$; see SM section 4.1.3) suggested that Asian colobine social systems evolved in a stepwise manner (fig. S2A

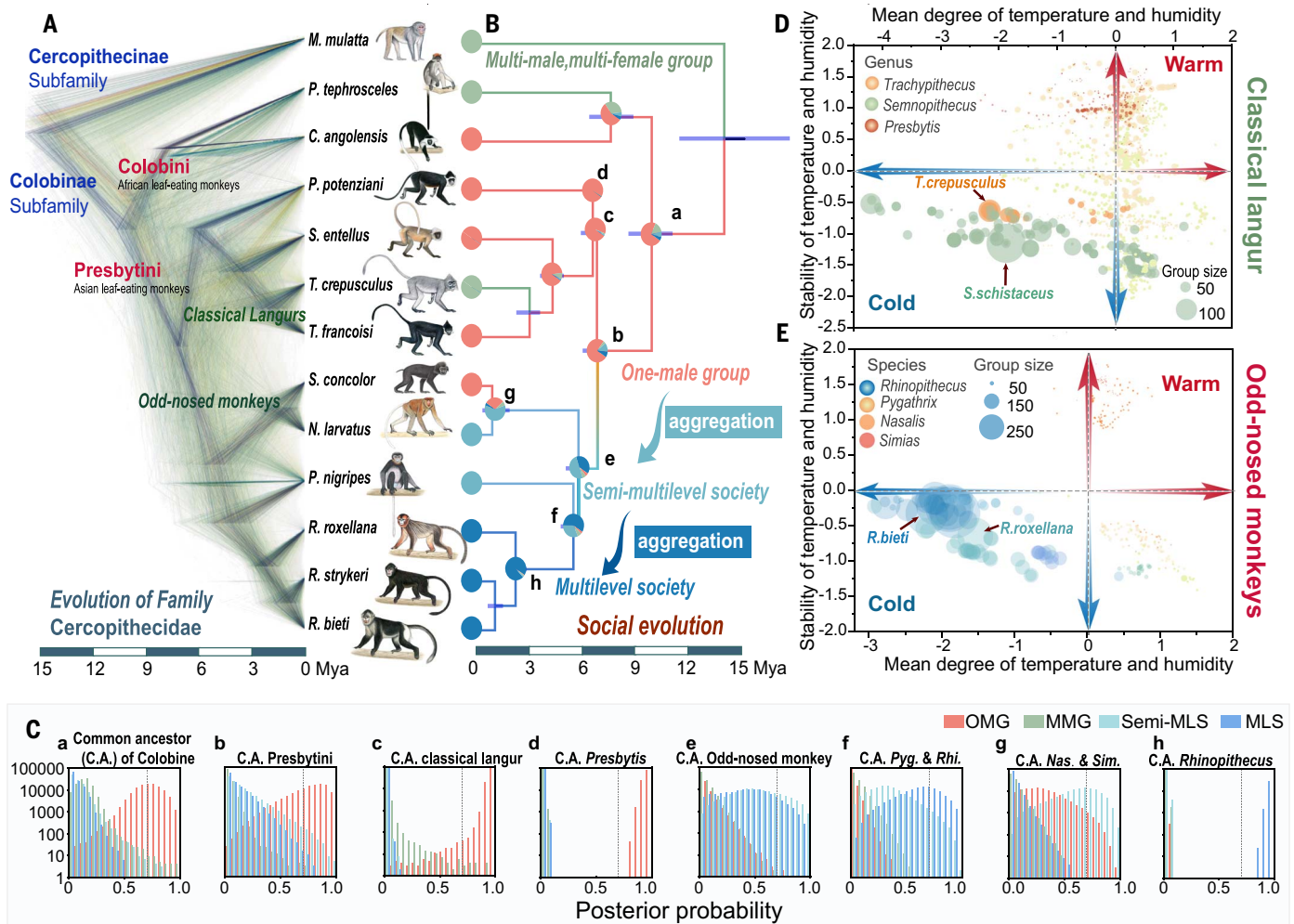


Fig. 2. Phylogenetic relationship and social system evolution in Asian colobines. (A) DensiTree presentation of phylogenetic trees of orthologous genes. Gene trees with a clade probability larger than 30% are shown. Mya, million years ago. (B) Social system evolution in Asian colobines. The pie chart at each ancestral node shows the reconstructed ancestral social state. Different colors correspond to the estimated social systems and are proportional to their posterior probability. (C) The posterior distributions

of the probabilities (x axis) of each of the ancestral nodes marked in (B). C.A., common ancestor. (D) High principal components PC1 scores indicate high temperature and humidity. (E) Low principal components PC2 scores indicate wide ranges of seasonality, annual temperature, and precipitation. The scatter size in a species is proportional to its group size. [Credits: All monkey illustrations are copyrighted 2014 by Stephen D. Nash/IUCN/SSC Primate Specialist Group and used with permission]

and table S8). Therefore, we investigated these lineage-specific evolutionary pathways in greater detail.

We traced the set of social conditions for each of the ancestral nodes using a Bayesian phylogenetic framework (SM section 4.1.4 and Fig. 2B). The results showed that the most likely ancestral social state of Asian colobines (Fig. 2B) was an independent one-male, multifemale unit [probability of ancestral state (ASP_{OMU}) = 0.76 ± 0.16]. Based on the Bayesian phylogenetic framework results, we identified three lineages of ancestral Asian colobines, each with a different social evolutionary history (Fig. 2B and fig. S2). The first lineage retained the ancestral one-male, multifemale unit system that is present in most of the classical langurs, such as *Presbytis* (Fig. 2, B and C). The second

lineage included a small number of classical langurs, such as the Indochinese langur (*T. crepusculus*) (Fig. 2B), that inhabit mountainous regions and tend to merge into larger multimale, multifemale groups. This contrasts with their sister species that live in warmer lowland regions and form single or independent one-male, multifemale units.

The third evolutionary pathway is related to the stepwise aggregation of core one-male, multifemale units into multilevel societies that characterize the odd-nosed monkey clade. The Bayesian phylogenetic framework results indicate that in this lineage, the ancestral independent one-male, multifemale units aggregated into semi-multilevel societies after splitting from the common ancestor of the odd-nosed monkey clade about 6.5 million (7.0 million to

5.7 million) years (Ma) ago (Figs. 2, B and C, and 3A). Subsequently, the lineage leading to the extant doucs (*Pygathrix*) and proboscis monkeys (*Nasalis*) inherited this social system, with multiple one-male, multifemale units sharing a home range through a process of fusion-fission (data S1 and S7). *Simias*, by contrast, independently reverted to an ancestral-like social system characterized by independent one-male, multifemale units. Our results indicate that the snub-nosed monkeys (*Rhinopithecus*) represent the second step of social aggregation from semi-multilevel societies to typical multilevel societies, with multiple one-male, multifemale units forming a large stable breeding band in which residents travel, rest, and feed together throughout the year. The breeding band, which may include more than 100 individuals,

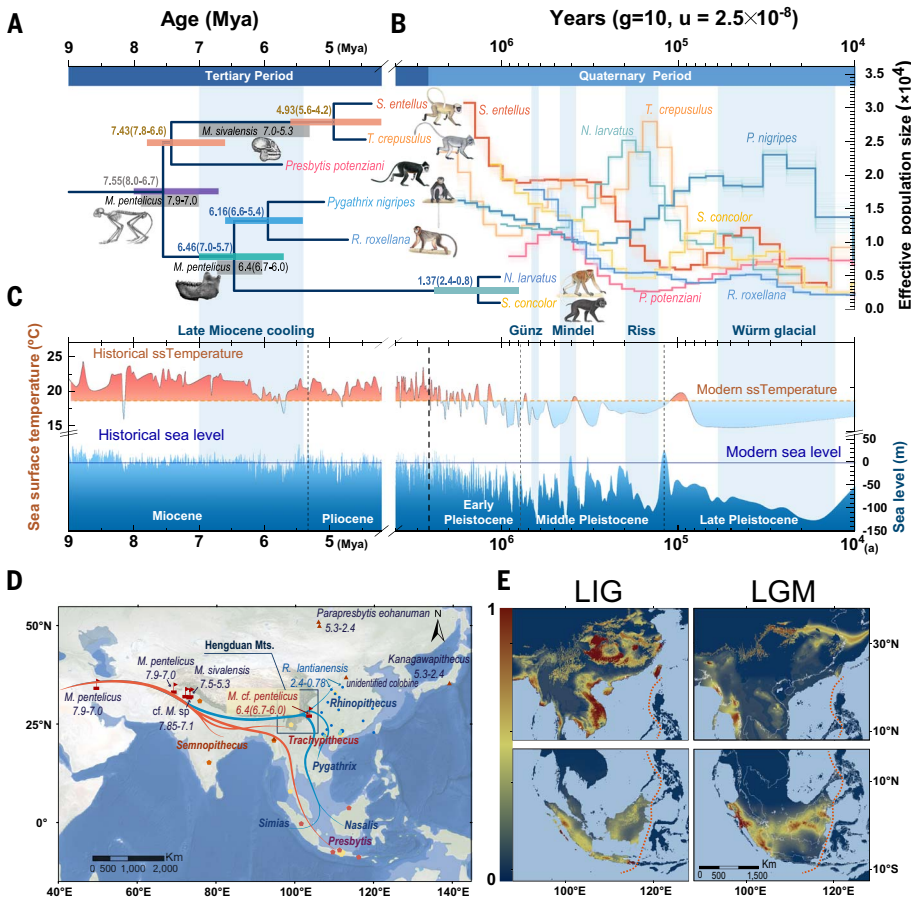


Fig. 3. Natural history of Asian colobines. (A) Reconstructed phylogenetic relationship of Asian colobines. The node bars indicate the 95% confidence interval for each branch. (B) Demographic history of seven Asian colobines estimated by PSMC. The regions marked with a vertical blue bar correspond to glacial periods. g , generation time; u , mutation rate. (C) Historical sea surface temperature and relative sea level over the past 9 Ma. (D) A new dispersal scenario proposed for Asian colobines. The orange line shows the proposed route of the odd-nosed clade (fossil records shown as dots), and the green line represents the classical langurs (fossil records shown as pentagons). (E) Ecological niche modeling for odd-nosed monkeys during the Last Interglacial (LIG; ~116 thousand to 130 thousand years before the present) and the Last Glacial Maximum (LGM; ~26.5 thousand to 19.0 thousand years before the present) period. [Credits: All monkey illustrations are copyrighted 2014 by Stephen D. Nash/IUCN/SSC Primate Specialist Group and used with permission]

is shadowed by all-male bachelor bands (Fig. 2B). These results demonstrate that social evolution in Asian colobines represents a newly discovered two-step pathway from ancestral independent one-male, multifemale units to large aggregated multilevel societies. This pathway is distinct from that of African papionins (e.g., gelada, hamadryas baboon), whose multilevel societies evolved through the internal fissioning of large multimale, multifemale groups (4, 31).

Social systems under contrasting environments

To understand how ecological factors have shaped primate social evolution, we constructed an Asian colobine ecological dataset (data S2) based on 19 bioclimatic variables that were extracted from a total of 2189 current locations

across the ranges of 48 extant Asian colobine species (data S2). Based on principal components analyses, we found that species that are presently distributed in colder, drier, and more seasonal climates tend to live in larger groups, whereas species that inhabit warmer and moister environments tend to form smaller groups (Fig. 2, D and E). The mean and stability of temperature and humidity were identified as the main factors that affect group size in odd-nosed monkeys (which explained 84.8% of the variance) and classical langurs (which explained 85.7% of the variance) (table S9). Furthermore, the random-walk model for continuous traits in BayesTraits (32) showed that group size was negatively correlated with annual mean temperature [Pagel's $\lambda = 0.59$; correlation coefficient (R) = -0.69 ; log BF = 17.75,

which is greater than 10], indicating that cold conditions may have selected for increased group size in both clades of Asian colobines (SM section 4.3.2). This pattern of enhanced sociality in cold and dry environments has also been reported in Australian rodents (33) and cooperative breeding birds (34). In the case of Asian colobines, transitions from one social system to another appear to have occurred at ancient evolutionary nodes and have been retained over long periods of time. This suggests that colobine social systems may reflect adaptations to ancient environmental conditions rather than a direct response to current environmental conditions.

Evolutionary history and radiation

Assuming that ancient ecological factors played an important role in promoting stepwise social evolution (Figs. 1 and 2), we traced the natural and social evolutionary history of Asian colobines over the past 8 Ma. This was accomplished by integrating data from new discoveries in the fossil record (data S4), paleogeology, paleogeography, paleoclimate, and historical sea level dynamics (data S5), as well as the present geographical distribution of individual Asian-colobine taxa (data S2). Using BioGeography with Bayesian and likelihood evolutionary analysis, we reconstructed the ancestral distribution pattern of Asian colobines (SM section 4.4.3 and fig. S12). In comparing the likelihoods of the resulting candidate models, with results from geographic and multiple-state speciation and extinction analyses (SM section 4.4.2 and fig. S11), we found that ancient dispersal routes and geographic isolation appear to have played important roles in Asian colobine speciation (Fig. 3D).

In contrast to the previous hypothesis that ancestral colobines dispersed into Asia via a northern route through China (35), we combined data on newly reported *Mesopithecus* fossils (7.9 to 7.0 Ma ago) found in Pakistan, Iran, and Afghanistan (SM section 4.4.5 and fig. S3) that support an alternative scenario. The common ancestor of Asian colobines, *Mesopithecus*, first entered Eastern Asia via the Indian subcontinent during the late Miocene (10.8 to 7.8 Ma ago) (Fig. 3D and data S4). Integrating this scenario with divergence times estimated from our newly constructed phylogenetic tree, we suggest that *Mesopithecus* spread throughout India and then divided into two clades at about 7.6 (8.0 to 6.7) Ma ago (Fig. 3, A and D).

One clade likely gave rise to the common ancestor of classical langurs, including *Presbytis*, *Semnopithecus*, and *Trachypithecus*, within a monophyletic clustering (Fig. 3A). Because of the uplifting of the Himalayas, some elements of this radiation spread eastward through the Indo-China Peninsula into warmer tropical forests in Sundaland during the late Miocene, around 7.4 (7.8 to 6.6) Ma ago. This group

evolved into the genus *Presbytis* (Fig. 3, A and D). During the Pliocene, about 4.9 (5.6 to 4.2) Ma ago, other members of this clade divided into two populations. One remained in the Indian subcontinent and evolved into *Semnopithecus*, whereas the other migrated eastward, spreading into southwest China and the Indo-China Peninsula in the Pleistocene. This lineage evolved into the genus *Trachypithecus* (Fig. 3, A and D).

Cold events promoted social aggregation in odd-nosed monkeys

In contrast to the classical langurs, our results suggest that cold events played an important role in adaptation and social aggregation along with speciation in the common ancestor of odd-nosed monkeys (Fig. 3). Combined with the new fossil *Mesopithecus pentelicus*, which was found in Zhaotong, Yunnan Province, China (identified as the most recent common ancestor of the odd-nosed monkey clade) (36) and was dated to 6.4 (6.7 to 6.0) Ma ago during Late Miocene Cooling (7.0 to 5.4 Ma ago), we propose that the ancestor of odd-nosed monkeys dispersed eastward from the Indian subcontinent, along the uplifted Himalayas, and then dispersed into the southeastern margin of the Tibetan Plateau (Hengduan Mountains region) (7.6 to 6.5 Ma ago) (Fig. 3, A and C). Paleoenvironmental evidence shows that after their arrival, the common ancestor of odd-nosed monkeys encountered a cooler and drier climate caused by the rapid uplifting of the Hengduan Mountains (8.0 to 6.0 Ma ago) during a global cooling period in the late Miocene (Fig. 3D and data S5). An additional changing monsoon climate in the area has also enhanced the cooling effects (fig. S3 and data S5). These events coincided with the evolution from an ancestral one-male, multifemale unit to a semi-multilevel society in odd-nosed monkeys (Figs. 2B and 3B). The results indicate that adaptations related to these cold events appear to have resulted in larger and more aggregated social groups in the odd-nosed monkey clade (Fig. 3).

Subsequently, the ancestors of odd-nosed monkeys evolved into four genera (Fig. 2A). Along with these cold events, the common ancestor of proboscis monkeys (*Nasalis*) and simakobus (*Simias*) migrated southward, crossing the land bridge that connected isolated islands in Southeast Asia (Sundaland) at about 6.5 (7.0 to 5.7) Ma ago. This radiation dispersed into tropical forests as far as Sumatra and Borneo (Fig. 3, D and E), facilitated by a fall in sea level caused by expanding ice sheets in the polar regions during glacial events (Fig. 3 and fig. S3).

The ecological niche modeling and pairwise sequentially Markovian coalescent (PSMC) analyses suggest that alternating glacial and interglacial events during the Pleistocene re-

sulted in reconnection and disconnection of land bridges as well as the expansion and contraction of suitable habitats (Fig. 3, B and E). This led to the isolation and divergence of proboscis monkeys and simakobus about 1.4 (2.4 to 0.8) Ma ago (Fig. 3, A and D). This dispersal scenario is consistent with the semi-multilevel society social grouping pattern maintained by proboscis monkeys, even though they presently inhabit warmer environments. By contrast, simakobus, which today only inhabit the Mentawai Islands west of Sumatra, reverted to independent one-male groups, similar to the Asian colobine ancestral condition.

The remaining odd-nosed monkeys gave rise to the common ancestor of doucs (*Pygathrix*) and snub-nosed monkeys (*Rhinopithecus*), which adapted to the cold climate present in the northern region of East Asia during the Late Miocene Cooling (6.5 to 6.2 Ma ago). Later, a branch of this radiation migrated south into the Indo-China Peninsula and evolved into *Pygathrix* at 6.2 (6.6 to 5.4) Ma ago (Fig. 3A). The PSMC analysis also showed that an expansion in the effective population size of doucs was associated with an increase in cold temperatures during the middle and late Pleistocene glacial event (Fig. 3B). Compared with the semi-multilevel societies of proboscis monkeys, in which non-territorial one-male, multifemale units aggregate together only at night, the semi-multilevel societies of doucs are characterized by an extended aggregation period during the rainy season. The more cohesive semi-multilevel societies of doucs appear to be related to a longer period of inhabiting glacial environments in colder northern regions compared with proboscis monkeys.

By contrast, the snub-nosed monkeys (genus *Rhinopithecus*) evolved from an ancestral lineage that remained in the north and experienced all major Pleistocene glacial cold events in high-latitude forests (data S1 and S2). Today, four of the five *Rhinopithecus* species are constrained to high-altitude temperate mountain forests up to 4500 m. These habitats are characterized by relatively cool summers and extended cold winters. This includes the golden snub-nosed monkeys (*Rhinopithecus roxellana*), which occupy the northern-most distribution of all colobine species (Fig. 1B). Through stepwise social evolution, snub-nosed monkeys evolved a social system distinguished by larger group size, increased male intrasexual tolerance, and the stable social aggregation of one-male, multifemale units that characterize their typical multilevel societies (Fig. 2B).

Colobine genomic evolution

These phylogenetic-based and cold-driven evolutionary scenarios point to a potential genetic mechanism that promoted the stepwise process of social aggregation in Asian colobines. Ecological pressures may have selected for genomic

changes early in colobine evolution that promoted an expansion of prosocial behaviors. Therefore, to identify the genetic basis of primate social evolution, in addition to the reference genomes of two African colobines as outgroups, we provide 10 genomes that represent all seven genera of Asian colobines, including six genomes from all four genera of odd-nosed monkeys (table S2).

Given that the ancestor of the odd-nosed monkey clade was initially aggregated into semi-multilevel groups in response to glacial events, based on the genomes of four extant genera, we reconstructed the genome of the common ancestor of odd-nosed monkeys using likelihood-based and maximum parsimony methods. Based on the branch-site and branch model in phylogenetic analysis by maximum likelihood (PAML) (37) and the evolutionary rate model (38), we compared the adaptive divergence between the ancestral odd-nosed monkey and other primates in coding genes, as well as the conserved model generated by PhastCons (39) and the aov.phylo model in GEIGER (40) for comparison of the conserved noncoding elements (CNEs). For coding genes, we identified 78 candidate positively selected genes and 371 candidate rapidly evolving genes from a total of 17,191 one-to-one orthologous genes from whole-genome alignment. We then filtered these candidate genes to reduce false-positive results (SM section 5.1.6) and detected 30 positively selected genes and 228 rapidly evolving genes ($P < 0.05$) (tables S14 and S16). After obtaining the QQplot from all orthologous genes (fig. S15) and the false discovery rate corrections, we further noticed a set of genes with higher levels of significance (tables S14 and S16). These genes are associated with multiple functions, for example, cold-related energy metabolism as the positively selected gene *HMCN2*, which is involved in lipid metabolism (41) and may aid in energy maintenance in cold environments. We also identified *LTBP2* and *FLNC* as rapidly evolving genes, which are involved in adipocyte differentiation and fat degradation (42, 43) and may be associated with nonshivering thermogenesis to increase body heat during periods of low temperature (44). In addition, we found a set of rapidly evolving genes (table S16) related to neurohormonal regulation, such as *DLGAP3* and *AP2AI*, which are involved in neurotransmission systems, such as the neurotransmission system that involves 5-hydroxytryptamine, which regulates grooming and other social behaviors (45, 46).

In addition, we obtained a total of 23,038 CNEs and 4351 ultraconserved noncoding elements (UCNEs) and identified 636 specific CNEs and 283 fast-evolving UCNEs ($P < 0.05$) in ancestral odd-nosed monkeys that distinguished them from the outgroups (SM section 5.1.2 and Fig. 4A). Focusing on the selected

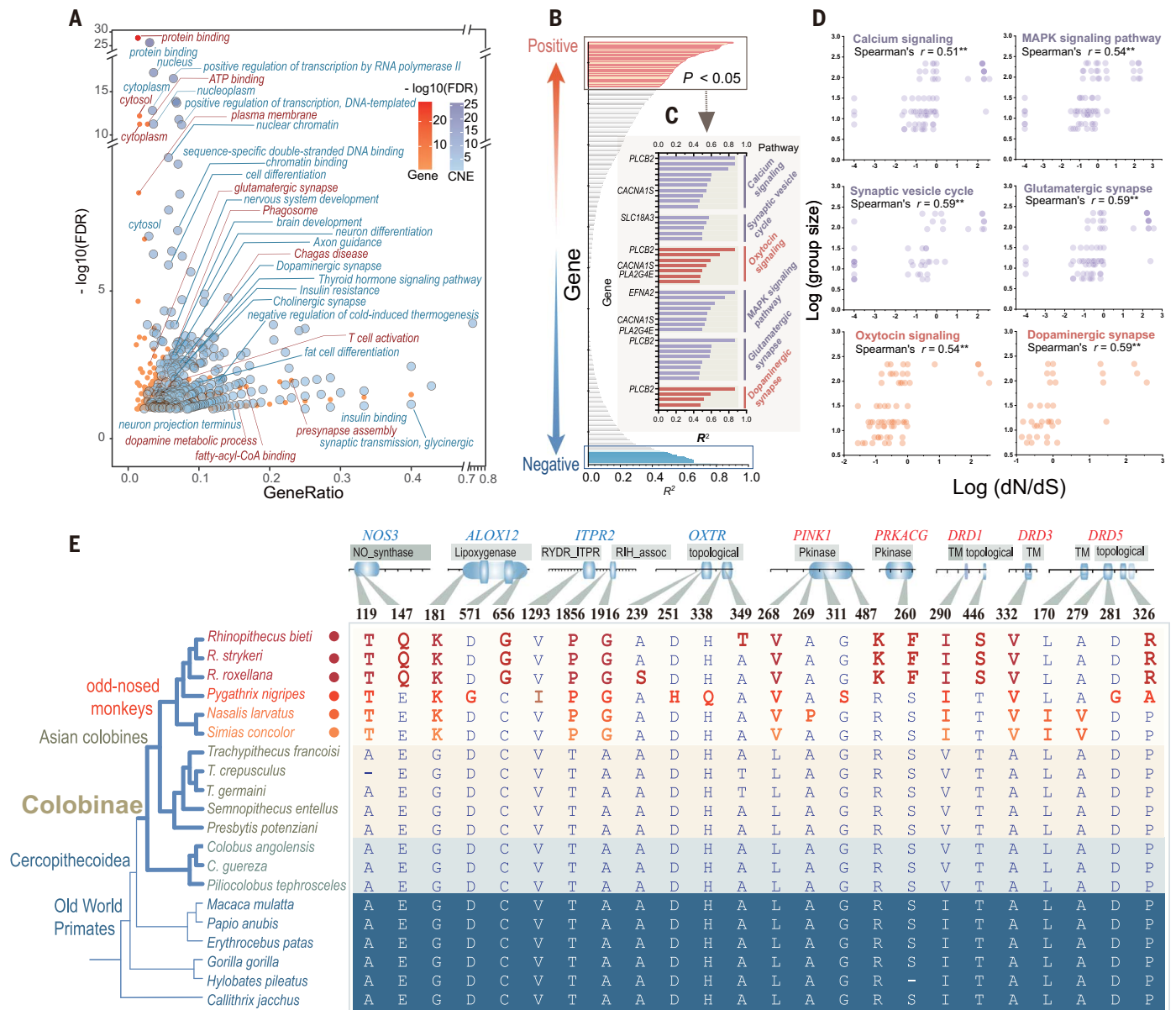


Fig. 4. Genome landscape of Asian colobines associated with social evolution.

(A) Enrichment analysis of specific CNEs (blue dots) and genes (red dots) in the common ancestor of odd-nosed monkeys. The full results are shown in tables S19 and S22. FDR, false discovery rate. (B) Genome-wide PGLS analyses between the evolutionary rate and group size across Asian colobines. Genes enclosed in rectangles indicate that their evolutionary rate (dN/dS , where N is the number of nonsynonymous

sites and S is the number of synonymous sites) is significantly correlated with group size. R^2 , coefficient of determination. (C) The correlation coefficients of each of the genes in each of the distinguished pathways. (D) Regression analysis between dN/dS and group size of the pathways. (E) Genes exhibit specific mutations in the odd-nosed monkeys. Genes involved in the oxytocin pathway are colored blue, whereas those involved in the dopamine pathway are colored red.

genes and UCNE- and CNE-associated genes, we annotated these genes to the Gene Ontology terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database and performed gene enrichment analyses using the KEGG Orthology Based Annotation System (KOBAS) (47) (SM section 5.2). The results showed that most of the high-ranking significant Gene Ontology terms and the pathways were involved in immunity, fat metabolism, and adaptations to a high-cellulose diet (Fig. 4A and fig. S17). These pathways are as-

sociated with energy- and heat-acquiring pathways that maintain body temperature to survive in the cold, such as the phagosome and Chagas pathways (SM section 5.2 and table S18). In addition, based on the evolutionary rate model, the analysis of rapidly evolving Gene Ontology terms also distinguished similar patterns as the enrichment analyses described earlier in this section, such as mammary gland development, fatty acid metabolism, and cellular glucose homeostasis (figs. S17 and S18). Importantly, both of these analyses revealed that

genes associated with neurohormonal regulation were significantly enriched (Fig. 4A). These results imply that cold-related energy metabolism and neurohormonal evolution appear to have jointly evolved in the common ancestor of the odd-nosed monkey clade.

Genome-wide association with social evolution

Based on these results, we investigated genomic changes in all extant Asian colobines that are relevant to social aggregation by exploring the potential genes and pathways that

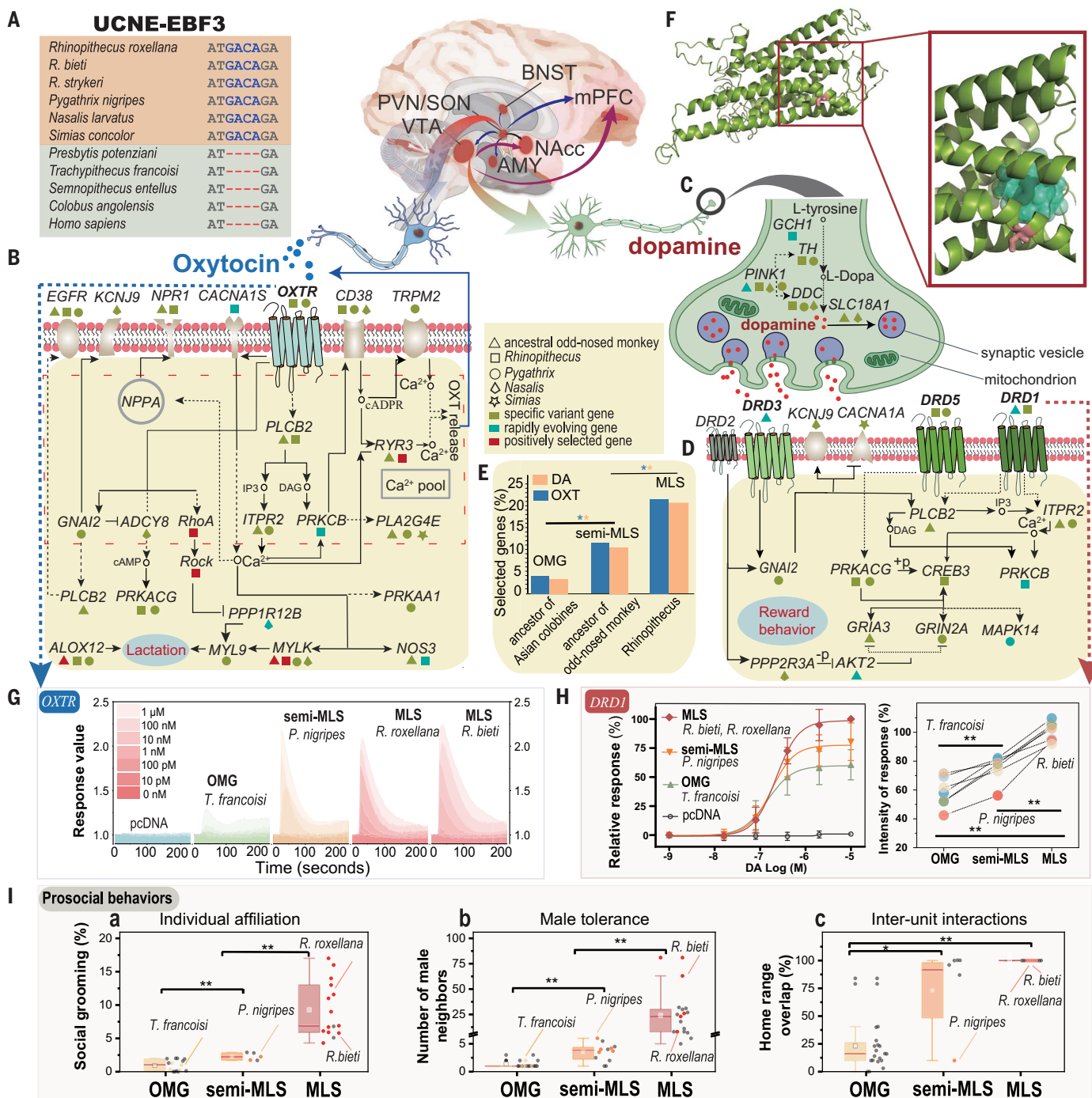


Fig. 5. Mutations in genes that encode proteins in the oxytocin and dopamine pathways and functional validation in odd-nosed monkeys.

(A) Nucleotides inserted into the UCNEs in odd-nosed monkeys. (B) Genetic changes identified in the oxytocin pathway. (C and D) Genetic changes identified in the dopamine synthesis process as well as signal transduction in and cellular regulation of the dopamine pathway. (E) Comparison of the proportion

of selected genes in the oxytocin (OXT) and dopamine (DA) pathways. (F) Three-dimensional views of the DRD1 protein of douc monkeys. (G and H) The in vitro receptor activity tests for OXTR and DRD1. *R. roxellana* and *R. bieti* share the same DRD1 amino acid sequence. (I) Prosocial behavioral characteristics related to the oxytocin and dopamine pathways in Asian colobines. For (H) and (I), * $P < 0.05$ and ** $P < 0.01$.

correlated with the group-size spectrum from one-male, multifemale groups to multilevel societies. First, we constructed an orthologous gene set that focused on neurohormonal systems from nine genomes, including those

of extant odd-nosed monkeys and classical langurs. Following the a priori candidate genes method (48, 49), we obtained a total of 2103 orthologous genes that are defined as or exhibited annotations in neurohormonal regula-

tion and social behavior from Gene Ontology and the KEGG pathway database (table S25). Focusing on these 2103 genes, we next performed correlation analyses and used mean group size as a continuous variable to represent

different forms of social organization to compare the evolutionary rate of each gene across species. Based on a phylogenetic generalized least squares (PGLS) regression analysis (50) (SM section 5.4), we detected 213 genes that were positively correlated and 66 genes that were negatively correlated with group size (Fig. 4B and table S26).

Then, focusing on these correlated genes, we performed two independent analyses, the enrichment analyses and the pathway correlation analyses to distinguish the specific pathways that correlated with group size. The enrichment analyses from these 213 and 66 genes using KOBAS distinguished 349 pathways that exhibited significant *P* values after correction for false discovery rates. We then ranked these pathways based on the *P* values (table S28). For the pathway correlation analyses, we focused on the 213 positively correlated genes, which may serve multiple functions across pathways, and recategorized these genes into 105 corresponding pathways. By comparing the evolutionary rate for each gene of each species in a pathway with mean group size in the corresponding species, we estimated the Spearman's correlation coefficients for each pathway. We then ranked these pathways by their correlation coefficients (tables S29 and S30 and SM section 5.4).

The results of both analyses showed that high-ranking pathways were primarily associated with categories of energy metabolism, neural signal transmission regulation, and immunity that may relate to group living (tables S28 and S29). For example, the regulation of lipolysis in adipocytes is associated with glucose and lipid metabolism (51). These pathways are relevant to energy demands and utilization and help to maintain body temperature and compensate for heat loss in cold environments (52). These high-ranking pathways also include those involved during the bacterial invasion of epithelial cells, which are reported to facilitate infection avoidance (53, 54). These same pathways also appear to function in cellulose fermentation by the gut microbiome, which is related to the folivorous diet of colobine primates (55). In addition, both analyses indicated that the remaining high-ranking pathways are engaged in neural signal transmission and regulation, such as the sphingolipid signaling pathway, which is associated with brain development and neural system maintenance (56) (SM section 5.4), as well as the particular hormones such as glutamate, dopamine, oxytocin, and 5-hydroxytryptamine (tables S28 and S29).

Moreover, both of the analyses distinguished pathways related to materials that function in neuron structure and the neuronal connectivity system, including axon guidance, cholinergic synapse, and synaptic vesicles (tables S28 and S29). The enrichment analyses also

distinguished dendrite, dendritic spine, synapse, and neuron projection as high-ranking Gene Ontology terms (table S28). These findings lay the structural foundation for signal transduction in the neural interaction network (57). Importantly, based on the enrichment analyses, the axon guidance and cholinergic systems, which were the first- and the sixth-highest-ranking pathways estimated from the KEGG database, are reported to affect and control dopamine release (58). Moreover, these analyses also distinguished the mitogen-activated protein kinase signaling and glutamatergic synapse pathways, which mediate downstream calcium signaling for the oxytocin and dopamine pathways (Fig. 4, C and D, and tables S29 and S30). These neurotransmitter systems, and the particular hormone types that they serve, suggest that neurohormonal regulation, including the oxytocin and dopamine pathways, is significantly related to group size in extant Asian colobines.

Therefore, we explored how neurohormonal systems, including the dopamine and oxytocin pathways, function in social behavior and the evolution of social group size. Oxytocin and dopamine play essential roles in maternal reward attachment, strengthening the mother-infant bond and maintaining nursing (59–63). Mammals living in colder environments tend to increase maternal investment, such as prolonging lactation and huddling periods to avoid infant exposure during the cold season (64–66). Therefore, we hypothesized that in response to cold temperatures, more efficient oxytocin and dopamine pathways were selected for in the odd-nosed monkeys, resulting in enhanced maternal care and infant survival. Furthermore, higher levels of oxytocin and dopamine also promote interindividual affiliation, mitigate intergroup conflict, and increase social bonding (67, 68). This could have facilitated increased cooperation and neighbor-male tolerance (69, 70) and thus may have favored social aggregation from independent one-male, multifemale groups to multilevel societies.

Rapid evolution in the oxytocin and dopamine pathways is related to social aggregation

To understand the adaptive changes in the oxytocin and dopamine pathways, we compared all 104 oxytocin-related and 96 dopamine-related orthologous genes (table S31) among snub-nosed monkeys, which represent a multilevel society; ancestral odd-nosed monkeys, which represent a semi-multilevel society; and classical langurs, which form independent one-male, multifemale units. By using PAML (37), hypothesis testing using phylogenies (71), and specific amino acid change (72), our results show that 22 (21.2%) genes in the oxytocin pathway and 20 (20.8%) genes in the dopamine pathway were selected in species that form multilevel societies. This is significantly

higher than the 12 (11.5%) and 10 (10.4%) genes selected in the same pathways of species that form semi-multilevel societies (SM section 5.5 and tables S32 and S33), as well as significantly higher than the four (3.8%) and three (3.1%) genes selected in Asian classical langurs that form independent one-male, multifemale groups (chi-square test; Fig. 5E). This pattern of genome-wide change in neuron structures to signal transmission across different clades is consistent with differences in the level of social aggregation from one-male, multifemale units to multilevel societies.

In the case of the ancestral odd-nosed monkeys that initially formed semi-multilevel societies, a suite of gene changes was identified in the oxytocin pathway (Fig. 5B and table S32). These include *RYR3*, which showed specific mutations that affect oxytocin release, and *ALOX12*, which was positively selected and regulates downstream milk secretion (Fig. 4E and table S31). In the dopamine pathway, specific variations in genes and noncoding regulatory regions were identified (Fig. 5, A, C, and D), for example, genes that affect dopamine-regulation processes, such as *PINK1*, which is responsible for dopamine synthesis; *SLC18A1*, which influences dopamine transport; and *GRI43*, which functions in reward behavior (fig. S23). In particular, we found that dopamine receptor genes *DRD1* and *DRD3* were rapidly evolving genes in the ancestral odd-nosed monkey clade (table S32). These G protein-coupled receptors (GPCRs), which are precise targets located in the cell membrane, play an important role in binding extracellular dopamine and transmit signals for intracellular downstream responses (Fig. 5D). Taken together, these findings suggest that the oxytocin and dopamine pathways evolved rapidly in ancestral odd-nosed monkeys, presumably in response to the initial aggregation required to form a semi-multilevel society.

Based on these findings, we examined the specific amino acid changes in oxytocin and dopamine pathway genes in each of the extant species of odd-nosed monkeys after their radiation from their common ancestor. A total of 22, 20, 10, and 6 genes in the oxytocin pathway and 20, 15, 9, and 4 genes in the dopamine pathway were identified in snub-nosed monkeys, which represent multilevel societies; doucs and proboscis monkeys, which represent semi-multilevel societies; and pig-tailed simakobus, which represent one-male, multifemale units, respectively (SM section 5.5 and tables S31 to S35). For example, *DRD5*, which encodes a dopamine receptor, had specific mutations in extant multilevel societies and semi-multilevel societies species (Fig. 4D) that were not present in one-male, multifemale unit species. In particular, specific amino acid changes in genes *CD38* and *RYR1*, which are associated with oxytocin downstream regulation, and the coding

region of the gene *OXTR* were present in multilevel society and semi-multilevel society species but were absent in one-male, multifemale unit species (Fig. 5B and tables S31 to S35). By contrast, *GCHI* and *PRKCB*, which are associated with dopamine synthesis and downstream response regulation, were selected in multilevel society species but not in semi-multilevel society species or one-male, multifemale unit species (Figs. 4E and 5, C and D; and table S32). Furthermore, the multilevel society species exhibited a shared threonine-to-serine mutation in *DRDI*, which encodes a dopamine receptor, in contrast to semi-multilevel society species or one-male, multifemale unit species (Fig. 4E), which do not. These genetic changes in the oxytocin and dopamine pathways reveal changing patterns in the neurohormonal regulation system that appear related to different levels of affiliation behavior (Fig. 2B).

Considering the importance of receptors in intercellular signal transduction and intracellular downstream responses, we used GPCR-I-TASSER to construct three-dimensional models to simulate protein expression in four oxytocin and dopamine receptors in snub-nosed monkeys, doucs, and François' langurs, representing a multilevel society, a semi-multilevel society, and a one-male, multifemale unit species, respectively (73) (Fig. 5F and fig. S22). The results indicate that a specific amino acid change of valine to isoleucine, located in the sixth transmembrane region of *DRDI*, was present in the odd-nosed monkey clade, which represents the ancestral aggregation from one-male, multifemale units to semi-multilevel societies (Fig. 5F). This mutation site was simulated to lie close to the binding pocket and thus may affect dopamine binding activity in the odd-nosed monkey clade; this mutation is not present in *DRDI* in Asian classical langurs and African colobus monkeys of the subfamily Colobinae, which represent independent one-male, multifemale units (Fig. 5F). In addition, the specific amino acid change of threonine to serine in *DRDI* in snub-nosed monkey species that live in multilevel societies was modeled to locate the conserved topological domain. This domain, which is located in the C-terminal domain of the GPCR protein (fig. S22), plays an important function in G protein coupling and activation (74) and thus may enhance intracellular G protein binding in these species compared with other species of colobines (fig. S22).

To confirm the functional expression of these receptors, we conducted cellular experiments that synthesized each sequence of *DRDI* and *OXTR* of the corresponding species, and these were then transferred in vitro into human embryonic kidney 293 (HEK293) cells. The results showed that the expressed DRD1 had higher binding efficiency in multilevel society species than in semi-multilevel society species ($P < 0.05$; Fig. 5H). Furthermore, the binding efficiency of

the expressed DRD1 in species that exhibit either of these types of social organization was significantly higher than that in species with an independent one-male, multifemale unit social organization ($P < 0.05$; Fig. 5H). This finding is consistent with the pattern shown by three-dimensional modeling. In addition, *OXTR* had a significantly higher binding efficiency in multilevel society and semi-multilevel society species than in independent one-male, multifemale unit species ($P < 0.05$; Fig. 5G). These results demonstrate a correlation between species with increased social aggregation and increased binding efficiency of their dopamine and oxytocin receptors.

Overall, our results show integrated differences that involve multiple genetic changes across various biological processes genome wide, which are linked to neurohormonal regulation, including the oxytocin and dopamine pathways. These changes are consistent with differences in social organization and intermale tolerance in Asian colobine species and may underpin their ability to form large, stable, and cohesive groups.

Increased behavioral affiliation is related to oxytocin and dopamine regulation

To verify changes in social behavior in response to different levels of neurohormonal regulation, including the oxytocin and dopamine expression, we compared the strength of social affiliation among species represented by each of the three types of social organization. We constructed a behavioral dataset related to social affiliation that involved 17 behavioral categories collected from information reported in 45 extant species of Asian colobines (data S1, S2, S6, and S7). Analysis of variance (ANOVA) tests revealed that neighbor-male tolerance; interactions between one-male, multifemale units; and time spent in social grooming as a percentage of daily time budgets were significantly higher in multilevel society species than in semi-multilevel society species and independent one-male, multifemale unit species (Fig. 5I). This is consistent with the expression results of in vitro experiments, which support our contention that genomic changes in the regulation of neurohormonal systems, including the oxytocin and dopamine pathways, may promote affiliative behaviors that are more pronounced in cold-adapted species.

Conclusion

In this study, we found that Asian colobines that inhabit colder environments tend to live in larger, more complex groups. By constructing a socioecological-genomic framework, we found that instead of evidence of direct adaptation to current environmental conditions, historical patterns of dispersal, phylogenetic species radiations, and adaptations to ancient environmental conditions played a more crit-

ical role in the social evolution of Asian colobines. Cold adaptations during ancient glacial events in ancestral odd-nosed monkeys appear to have favored the selection of the neurohormonal regulation system, from neuron structure to signal transmission, which includes the dopamine and oxytocin pathways. These changes in the dopamine and oxytocin pathways appear to function in strengthening social bonds, in facilitating male-male tolerance, and in shaping social affiliation. This process played an important role in promoting social aggregation from small, independent one-male groups into larger multilevel societies. Our study identifies, for the first time, a genomically regulated adaptation that is linked to stepwise social evolution in primates and offers new insights into the mechanisms that underpin diverse behavioral evolution across a range of animal taxa.

Materials and methods summary

Sequencing and assembly

We sequenced seven Asian colobine genomes by using four technologies, including long-read sequencing of Oxford Nanopore or PacBio SMART, paired-end sequencing, and high-throughput chromosome conformation capture (Hi-C). Different de novo assemblies were performed using FALCON v.0.4.0 (75), wtdbg2 v.2.4.1 (76), and SOAPdenovo2 v. 1.0 (77) according to the sequencing strategy used. Genomes with Hi-C reads were further scaffolded to chromosome based on LACHESIS (78) or 3D-DNA (79).

Dataset resources

We compiled the datasets of social, behavioral, and ecological traits of Asian colobines using published information (SM section 2), which include (i) social organization, such as group size and composition (data S1); (ii) mating system (data S1); (iii) social structure, which is defined as social interactions and communication, including the proportion of the activity budget devoted to social grooming (data S6); (iv) ecological (bioclimatic) variables based on occurrence location coordinates (data S2); and (v) paleoecological data based on the fossil record, paleoclimate, and paleogeography across Asia (SM section 2.2).

Ecological analyses

Ecological niche modeling was conducted using Maxent to reconstruct species distribution in the present climate and under paleoclimates. Principal components analysis was used to extract two main characters from 19 climate variables for 2189 species occurrences in the R package Multivariate Exploratory Data Analysis and Data Mining with FactoMineR v.3.6.1 (80). Geographic information was processed in ArcGIS (ArcGIS version 10.6, Environmental Systems Research Institutes, Inc., Redlands, CA, USA).

Reconstruction of phylogenomic relationships

One-to-one orthologs for phylogenomic relationship reconstruction were generated with OrthoFinder v.2.0.9 (81). Then, these orthologous genes were used to generate two dependent datasets, including a concatenated coding sequence alignment and the fourfold degenerate sites. For each dataset, a tree was constructed with the concatenation method of IQ-TREE v.1.6.12 (82) and coalescent method of Astral v.2.0 (83), respectively. The divergence time was estimated using MCMCTree v.4.5 (37).

Phylogenetic analyses

Pagel's λ was estimated using the R package GEIGER v.2.0.6 (40). The Phylo.D was estimated using R package CAPER v.1.0.1 (50), and the probability of the estimated D resulting from the Brownian phylogenetic structure was marked as $P_{D_Brownian}$. BayesTraits v.3.0.2 (32) was used to infer the social system state for each ancestral node, which was determined by calculating the ancestral state posterior probability. A random-walk Markov chain Monte Carlo procedure in BayesTraits v.3.0.2 was used to infer the correlated evolution between bioclimatic variables and group size.

Reconstruction of ancestral geographic ranges

We reconstructed the ancestral range through multiple biogeographical models (e.g., DIVA, DEC, or BayAreaLike) using Reconstruct Ancestral State in Phylogenies 4.2 (84). The best model generated was used to reconstruct the range in each ancestral node.

Demographic history reconstruction

Demographic history was inferred using PSMC v.0.6.5 (85) under a hidden Markov model. Paired-end Illumina sequences were aligned to the repeat-masked genome assembly of each species using the Burrows-Wheeler Alignment tool v.0.7.17-r1188 (86). Then, consensus sequences were generated using Sequence Alignment/Map format tools v.1.3.1 (87). Each PSMC test was examined with 100 bootstrap replicates.

Comparative genomics analyses

Divergent (fast-evolving) UCNEs were identified by using the R package GEIGER v.2.0.6 (40). PhyloFit v1.4 (88) and phastCons v1.4 (39) were used to infer CNEs. Orthologous genes were constructed by using LAST v.last982 (89). The pairwise synteny alignment analysis was conducted for Asian colobine species as well as outgroups, with the human genome serving as the reference. Then, the corresponding orthologous sequences were extracted based on the gff file of the human genome. The Gene Ontology and KEGG pathway enrichment analyses were conducted using KOBAS v.3.0 (47). Selection pressure tests were implemented by both branch-site models and branch models

using PAML v.4.9 (37) through a likelihood ratio test and strict filter criterion. An episodic positive selection signal was detected using the mixed effects model of evolution (90) implemented in Hypothesis Testing using Phylogenies v.2.5.25 (71). Rapidly evolving Gene Ontology terms were identified following the evolutionary model and method proposed by Wang *et al.* (38). Specific mutations were identified following the specific amino acid change pipeline from Chen *et al.* (72) and were further examined if they were located in functional regions using the protein families database Pfam v.1.6 (91). Genome-wide associations with social evolution were explored using PGLS regression analyses in the R package Comparative Analysis of Phylogenetics and Evolution in R (CAPER) v.1.0.1 (50).

Protein structure modeling

The 3D protein structure of the functional region was simulated by GPCR-I-TASSER (73) and then visualized using PyMOL (the PyMOL molecular graphics system, version 2.0, Schrödinger, LLC). The binding cavity was explored with the docking simulations in Dock v.ina (92).

In vitro expression assay

For in vitro experiments, orthologous sequences were synthesized by General Biosystems Corporation Limited (Anhui, China). All genes were cloned into pcDNA3.1-V5-His vector separately and expressed in HEK293 cells. After 48 hours, the supernatant was removed, the cells were rinsed twice with phosphate-buffered saline (PBS), and then multiple solutions were added for an enzyme-linked immunosorbent assay experiment. Absorbance measurements were conducted at 370 nm within 30 min. Results were analyzed using GraphPad Prism. Statistical significance was set at <0.05 , mean \pm SD.

Measurement of receptor activity

For DRD1, luciferase activities were determined using luciferase assay kits (Beyotime, Shanghai, China). In the case of OXTR, fluorescence was measured using microplate reader SYNERGY HI (BioTek Instruments). HEK293 cells transfected with pcDNA were used as a control in all luciferase experiments.

REFERENCES AND NOTES

1. T. Clutton-Brock, Sexual selection in males and females. *Science* **318**, 1882–1885 (2007). doi: [10.1126/science.1133311](https://doi.org/10.1126/science.1133311); pmid: [18096798](https://pubmed.ncbi.nlm.nih.gov/18096798/)
2. J. C. Mitani, J. Call, P. M. Kappeler, R. A. Palombit, J. B. Silk, *The Evolution of Primate Societies* (Univ. Chicago Press, 2012).
3. P. M. Kappeler, C. P. van Schaik, Evolution of primate social systems. *Int. J. Primatol.* **23**, 707–740 (2002). doi: [10.1023/A:1015520830318](https://doi.org/10.1023/A:1015520830318)
4. C. C. Grueter *et al.*, Multilevel organisation of animal sociality. *Trends Ecol. Evol.* **35**, 834–847 (2020). doi: [10.1016/j.tree.2020.05.003](https://doi.org/10.1016/j.tree.2020.05.003); pmid: [32473744](https://pubmed.ncbi.nlm.nih.gov/32473744/)
5. D. Lukas, T. H. Clutton-Brock, The evolution of social monogamy in mammals. *Science* **341**, 526–530 (2013). doi: [10.1126/science.1238677](https://doi.org/10.1126/science.1238677); pmid: [23896459](https://pubmed.ncbi.nlm.nih.gov/23896459/)

6. T. Clutton-Brock, Cooperation between non-kin in animal societies. *Nature* **462**, 51–57 (2009). doi: [10.1038/nature08366](https://doi.org/10.1038/nature08366); pmid: [19890322](https://pubmed.ncbi.nlm.nih.gov/19890322/)
7. J. B. Silk, S. C. Alberts, J. Altmann, Social bonds of female baboons enhance infant survival. *Science* **302**, 1231–1234 (2003). doi: [10.1126/science.1088580](https://doi.org/10.1126/science.1088580); pmid: [14615543](https://pubmed.ncbi.nlm.nih.gov/14615543/)
8. J. B. Silk, Social components of fitness in primate groups. *Science* **317**, 1347–1351 (2007). doi: [10.1126/science.1140734](https://doi.org/10.1126/science.1140734); pmid: [17823344](https://pubmed.ncbi.nlm.nih.gov/17823344/)
9. X.-G. Qi *et al.*, Satellite telemetry and social modeling offer new insights into the origin of primate multilevel societies. *Nat. Commun.* **5**, 5296 (2014). doi: [10.1038/ncomms6296](https://doi.org/10.1038/ncomms6296); pmid: [25335993](https://pubmed.ncbi.nlm.nih.gov/25335993/)
10. A. Koenig, C. J. Scarry, B. C. Wheeler, C. Borries, Variation in grouping patterns, mating systems and social structure: What socio-ecological models attempt to explain. *Philos. Trans. R. Soc. London Ser. B* **368**, 20120348 (2013). doi: [10.1098/rstb.2012.0348](https://doi.org/10.1098/rstb.2012.0348); pmid: [23569296](https://pubmed.ncbi.nlm.nih.gov/23569296/)
11. J. F. Eisenberg, N. A. Muckenhirn, R. Rundran, The relation between ecology and social structure in Primates. *Science* **176**, 863–874 (1972). doi: [10.1126/science.176.4037.863](https://doi.org/10.1126/science.176.4037.863); pmid: [17829291](https://pubmed.ncbi.nlm.nih.gov/17829291/)
12. P. M. Kappeler, A framework for studying social complexity. *Behav. Ecol. Sociobiol.* **73**, 13 (2019). doi: [10.1007/s00265-018-2601-8](https://doi.org/10.1007/s00265-018-2601-8)
13. C. H. Janson, Primate socio-ecology: The end of a golden age. *Evol. Anthropol.* **9**, 73–86 (2000). doi: [10.1002/\(SICI\)1520-6505\(2000\)9:2<73::AID-EVAN2>3.0.CO;2-X](https://doi.org/10.1002/(SICI)1520-6505(2000)9:2<73::AID-EVAN2>3.0.CO;2-X)
14. T. Clutton-Brock, C. Janson, Primate socioecology at the crossroads: Past, present, and future. *Evol. Anthropol.* **21**, 136–150 (2012). doi: [10.1002/evan.21316](https://doi.org/10.1002/evan.21316); pmid: [22907867](https://pubmed.ncbi.nlm.nih.gov/22907867/)
15. P. M. Kappeler, Evolution. Why male mammals are monogamous. *Science* **341**, 469–470 (2013). doi: [10.1126/science.1242001](https://doi.org/10.1126/science.1242001); pmid: [23908214](https://pubmed.ncbi.nlm.nih.gov/23908214/)
16. S. Shultz, C. Opie, Q. D. Atkinson, Stepwise evolution of stable sociality in primates. *Nature* **479**, 219–222 (2011). doi: [10.1038/nature10601](https://doi.org/10.1038/nature10601); pmid: [22071768](https://pubmed.ncbi.nlm.nih.gov/22071768/)
17. R. A. Mittermeier, A. B. Rylands, D. E. Wilson, Eds., *Primates*, vol. 3 of *Handbook of the Mammals of the World* (Lynx Edicions, 2013).
18. C. Opie, Q. D. Atkinson, R. I. Dunbar, S. Shultz, Male infanticide leads to social monogamy in primates. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 13328–13332 (2013). doi: [10.1073/pnas.1307903110](https://doi.org/10.1073/pnas.1307903110); pmid: [23898180](https://pubmed.ncbi.nlm.nih.gov/23898180/)
19. P. M. Kappeler, L. Pozzi, Evolutionary transitions toward pair living in nonhuman primates as stepping stones toward more complex societies. *Sci. Adv.* **5**, eaay1276 (2019). doi: [10.1126/sciadv.aay1276](https://doi.org/10.1126/sciadv.aay1276); pmid: [32064318](https://pubmed.ncbi.nlm.nih.gov/32064318/)
20. G. E. Robinson, R. D. Fernald, D. F. Clayton, Genes and social behavior. *Science* **322**, 896–900 (2008). doi: [10.1126/science.1159277](https://doi.org/10.1126/science.1159277); pmid: [18988841](https://pubmed.ncbi.nlm.nih.gov/18988841/)
21. R. I. M. Dunbar, S. Shultz, Evolution in the social brain. *Science* **317**, 1344–1347 (2007). doi: [10.1126/science.1145463](https://doi.org/10.1126/science.1145463); pmid: [17823343](https://pubmed.ncbi.nlm.nih.gov/17823343/)
22. E. H. M. Sterck, "The behavioral ecology of colobine monkeys" in *The Evolution of Primate Societies*, J. C. Mitani, J. Call, P. M. Kappeler, R. A. Palombit, J. B. Silk (Univ. Chicago Press, 2012), pp. 66–87.
23. L. R. Ullbarri, K. N. Gartland, Group composition and social structure of red-shanked doucs (*Pygathrix nemaeus*) at Son Tra Nature Reserve, Vietnam. *Folia Primatol.* **92**, 191–202 (2021). doi: [10.1159/000518594](https://doi.org/10.1159/000518594); pmid: [34535600](https://pubmed.ncbi.nlm.nih.gov/34535600/)
24. X.-G. Qi *et al.*, Male cooperation for breeding opportunities contributes to the evolution of multilevel societies. *Proc. Biol. Sci.* **284**, 20171480 (2017). doi: [10.1098/rspb.2017.1480](https://doi.org/10.1098/rspb.2017.1480); pmid: [28954911](https://pubmed.ncbi.nlm.nih.gov/28954911/)
25. X. Wang, Y. Zhang, L. Yu, Summary of phylogeny in subfamily Colobinae (Primate: Cercopithecoidea). *Chin. Sci. Bull.* **58**, 2097–2103 (2013). doi: [10.1007/s11434-012-5624-y](https://doi.org/10.1007/s11434-012-5624-y)
26. X. P. Wang *et al.*, Phylogenetic relationships among the colobine monkeys revisited: New insights from analyses of complete mt genomes and 44 nuclear non-coding markers. *PLOS ONE* **7**, e36274 (2012). doi: [10.1371/journal.pone.0036274](https://doi.org/10.1371/journal.pone.0036274); pmid: [22558416](https://pubmed.ncbi.nlm.nih.gov/22558416/)
27. K. N. Sterner, R. L. Raamun, Y.-P. Zhang, C.-B. Stewart, T. R. Disotell, Mitochondrial data support an odd-nosed colobine clade. *Mol. Phylogenet. Evol.* **40**, 1–7 (2006). doi: [10.1016/j.ympev.2006.01.017](https://doi.org/10.1016/j.ympev.2006.01.017); pmid: [16500120](https://pubmed.ncbi.nlm.nih.gov/16500120/)
28. A. Antonelli *et al.*, Toward a self-updating platform for estimating rates of speciation and migration, ages, and relationships of taxa. *Syst. Biol.* **66**, 152–166 (2017). pmid: [27616324](https://pubmed.ncbi.nlm.nih.gov/27616324/)
29. M. Pagel, Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884 (1999). doi: [10.1038/44766](https://doi.org/10.1038/44766); pmid: [10553904](https://pubmed.ncbi.nlm.nih.gov/10553904/)

authors declare no competing financial interests. **Data and materials availability:** Genome assemblies and DNA sequencing data have been deposited into the National Center for Biotechnology Information (NCBI) database under reference nos. PRJNA658634, PRJNA658635, PRJNA658636, PRJNA752402, and PRJNA752403. Other resources and data are available in the supplementary materials. **License information:** Copyright © 2023 the authors, some rights reserved; exclusive licensee American

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SUPPLEMENTARY MATERIALS

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Submitted 11 August 2021; accepted 6 July 2022
10.1126/science.abl8621



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Science, **380** (6648), eabl8621.
DOI: 10.1126/science.abl8621

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