EVOLUTIONARY BIOLOGY

Ambush predation and the origin of euprimates

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Primates of modern aspect (euprimates) are characterized by a suite of characteristics (e.g., convergent orbits, grasping hands and feet, reduced claws, and leaping), but the selective pressures responsible for the evolution of these euprimate characteristics have long remained controversial. Here, we used a molecular phyloecological approach to determine the diet of the common ancestor of living primates (CALP), and the results showed that the CALP had increased carnivory. Given the carnivory of the CALP, along with the general observation that orbital convergence is largely restricted to ambush predators, our study suggests that the euprimate characteristics could have been more specifically adapted for ambush predation. In particular, our behavior experiment further shows that nonclaw climbing can significantly reduce noises, which could benefit the ancestral euprimates' stalking to ambush their prey in trees. Therefore, our study suggests that the distinctive euprimate characteristics may have evolved as their specialized adaptation for ambush predation in arboreal environments.

INTRODUCTION

Diet is the key to understanding the origin of euprimates. Among the theories explaining the origin of euprimates (*1, 2*), there has been a continuing debate between two commonly cited hypotheses, the vision predation hypothesis (VPH) (*3–7*) and the angiosperm-primate coevolution hypothesis (*8, 9*), as to whether insectivory or herbivory was the driving force of the evolution of distinctive euprimate morphological features (*10*). The VPH proposes that euprimate characteristics such as orbital convergence and grasping hands and feet may have evolved as the result of the predatory adaptation of the ancestral primates in the arboreal setting (*3–5*). The VPH is further modified as the nocturnal visual predation (NVP) hypothesis, considering that orbital convergence is particularly needed for nocturnal predators (e.g., ancestral euprimates) to improve image quality (*5, 11–15*). The angiosperm-primate coevolution hypothesis, on the other hand, proposes that euprimates may have developed grasping hands and feet so that they could explore plant food sources such as fruits and flowers besides insects in the fine-branch niche and may have developed convergent orbits as these are helpful for either the fine discrimination of small food items or "seeing through" leaf clutter (*8, 9*). Both hypotheses have supporting evidence, but which of them is correct has yet to be determined by reconstructing the diet of the ancestral euprimates.

Living primates have diverse diet preferences, as did their extinct euprimate relatives. Similar to the ancestral euprimates, such as omomyoids and adapoids (*16*), there are also frugivores, folivores, and insectivores (or faunivores) among the living primates (*1*). Most living primates (except obligate insectivores, e.g., tarsiers) are considered omnivores, incorporating both insects and plants in their diets to varying extents (*8, 17*). Similar to many living primates, plesiadapiforms, regarded as the fossil relatives of living primates,

showed anatomical features indicating that they ate diverse foods, such as fruits, insects, and leaves (*18–20*). The diverse diets of living primates and their fossil relatives (e.g., plesiadapiforms) make it difficult to infer the diet of the ancestral euprimates, and different possibilities, such as insectivory, herbivory, and omnivory, have been suggested on the basis of fossil and/or molecular evidence (*3–9, 21–23*), demonstrating the uncertainty of their diet.

The recent development of molecular phyloecology provides a new approach to reconstructing ancestral traits using molecular data (*24–28*). For diet reconstruction, it uses digestive system–related genes as the molecular markers indicative of diets, the adaptive evolution of these molecular markers is analyzed, and diets are then inferred along the branches in the context of the phylogeny (*27, 28*). Accordingly, carnivores are considered to show enhanced selection of protein and fat utilization, while herbivores are normally characterized by the enhanced selection of carbohydrates (*27, 29, 30*). This is because animal-based foods are high in proteins and fats, while plant-based foods are normally high in carbohydrates (*27, 29–33*). In the present study, we used the molecular phyloecological (MPE) approach to determine the diets of the ancestral euprimates and their progenitors (ancestral Euarchonta and ancestral Primatomorpha). Our study revealed the increased carnivory of the ancestral euprimates relative to their progenitors, which provides important insights into the ecological pressures responsible for the origin of euprimates.

RESULTS AND DISCUSSION

Carnivory (e.g., insectivory) of the ancestral euprimates

To determine the ancestral diets, we examined the positive selection signals of 117 digestive system–related genes in the context of the Euarchonta phylogeny, which includes living primates, colugos, and treeshrews (Fig. 1). These genes are known to play important roles in the digestion and absorption of carbohydrates, proteins, and fats (*27*) and are involved in three digestive system–related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways: carbohydrate digestion and absorption, protein digestion and absorption, and fat digestion and absorption (Fig. 2). Using these digestive system–related genes as the molecular markers of diets, following the MPE approach to reconstruct the ancestral diets (*27, 28*), we examined their adaptive evolution using the branch and branch-site models implemented in

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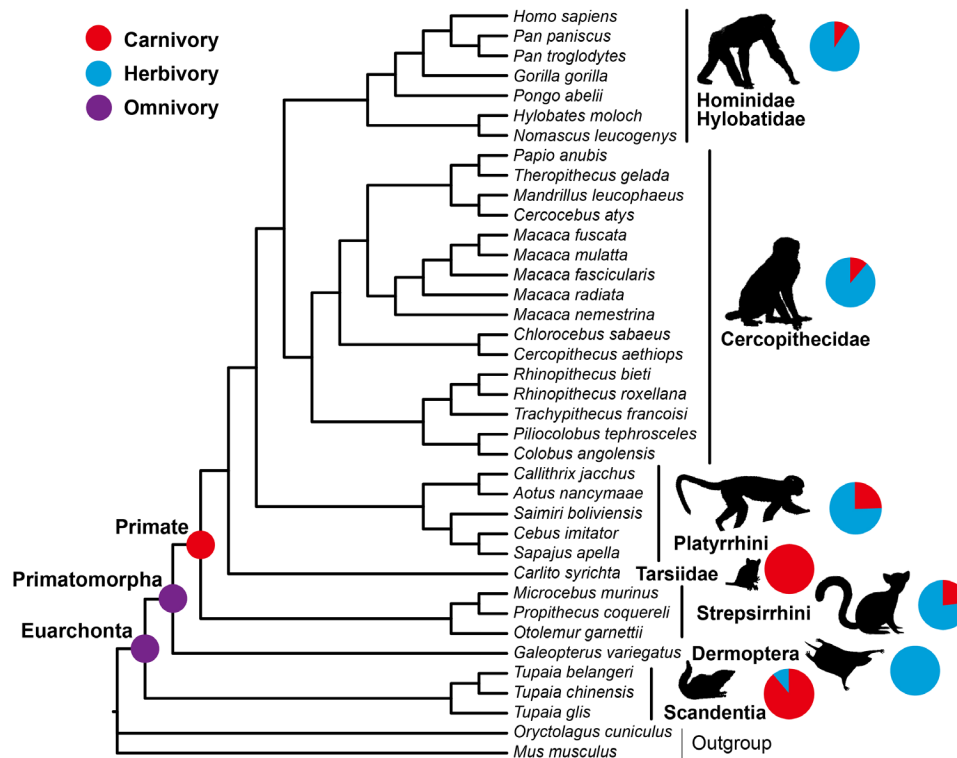*Corresponding author. Email: wuyh442@nenu.edu.cn (Y.W.); fengj@nenu.edu.cn (J.F.); jiangtl730@nenu.edu.cn (T.J.)

Fig. 1. Reconstructed diets based on molecular data. The phylogenetic relationships follow published studies (*92–95*). The averaged proportions of carnivory and herbivory of each species of each clade are shown in a pie chart based on the diet data of 398 species according to a previous study (*90*).

the Phylogenetic Analysis by Maximum Likelihood (PAML) software to detect positively selected genes (PSGs) along our focal branches. As in our previous studies (*24–27*), PSGs were found on the basis of the branch-site model (Table 1).

We initially examined the positive selection signals of the aforementioned digestive system–related genes involved in the digestion and absorption of carbohydrates, proteins, and fats along the common-ancestor branch of living primates. Among the 117 genes analyzed, only the fat utilization–related genes were found to be under positive selection (Table 1 and Fig. 2). One positively selected fat utilization–related gene, *LIPF*, encodes a gastric lipase and plays an important role in the digestion of dietary triglycerides in the gastrointestinal tract (*34, 35*). The positive selection signal of *LIPF* remained unchanged even after Bonferroni multiple testing correction ($\omega = 208.378$; df = 1; $P = 0.022$). Another fat utilization–related gene, *MOGAT3*, showed a positive selection signal with marginal significance ($\omega = 85.948$; df = 1; $P = 0.053$). *MOGAT3* catalyzes the synthesis of diacylglycerol from 2-monoacylglycerol and fatty acyl-CoA (coenzyme A) (*36*). The finding of the selection enhancement of the fat utilization–related genes may suggest that the ancestral euprimates had a fat-rich diet.

We subsequently examined the positive selection of the digestive system–related genes along the branches of the ancestral Euarchonta and ancestral Primatomorpha, respectively. Notably, we found 13 PSGs along the branch of the ancestral Euarchonta (Table 1 and Fig. 2) and no PSG along the branch of the ancestral Primatomorpha. For the ancestral Euarchonta, the 13 PSGs that we found were across all the three pathways. For the carbohydrate digestion and absorption pathway, four PSGs (*SI*, *LCT*, *SLC2A2*, and *ATP1B1*) were detected,
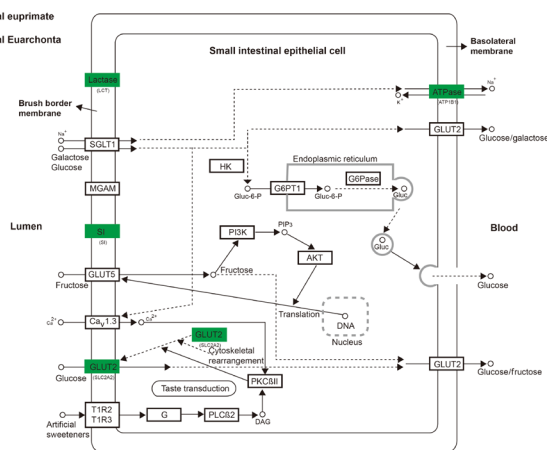
of which *SI* showed the most significant positive selection signal ($\omega = 36.197$; df = 1; $P = 0.001$) among the 13 PSGs found for the ancestral Euarchonta (Table 1). *SI* encodes sucrase-isomerase and is essential for the digestion of dietary carbohydrates (*37*). *LCT* encodes a molecule with both lactase activity and phlorizin hydrolase activity (*38*). *SLC2A2* encodes glucose transporter (*39*). *ATP1B1* encodes the beta subunits of Na^+- and K^+-dependent adenosine triphosphatase (Na^+, K^+-ATPase) involved in maintaining ionic homeostasis (*40*). For the fat digestion and absorption pathway, five PSGs (*PLA2G2A*, *DGAT1*, *PNLIPRP1*, *AGPAT2*, and *PLA2G12A*) were found. *PLA2G2A* encodes lipolytic enzymes and is involved in the digestion and absorption of lipids (*41*). *DGAT1* is involved in triacylglycerol synthesis (*42*). *PNLIPRP1* is a triglyceride digestion inhibitor and shows repeated loss in many herbivorous mammals (*32*). *AGPAT2* plays a role in converting lysophosphatidic acid into phosphatidic acid (*43*). *PLA2G12A* belongs to a family of Ca^{2+}-dependent lipolytic enzymes, and its function is less clear (*41*). In addition to the digestion and absorption of carbohydrates and fats, PSGs (*CPB1*, *CELA3B*, *ATP1B1*, and *XPNPEP2*) were also found in the protein digestion and absorption pathway. *CPB1* encodes pancreatic procarboxypeptidase (*44*). *CELA3B* is a pancreatic serine proteinase that digests dietary protein substrates (*45*). *ATP1B1* encodes the beta subunits of Na^+, K^+-ATPase involved in maintaining ionic homeostasis (*40*). *XPNPEP2*, which showed a positive selection signal with marginal significance ($\omega = 109.941$; df = 1; $P = 0.050$), presumably degrades both dietary and filtered peptides (*46*). Together, for the ancestral Euarchonta, we detected PSGs involved in the utilization of carbohydrates, proteins, and fats, which may suggest that the ancestral Euarchonta had a diet rich in carbohydrates, fats, and proteins.

Fig. 2. Positive selection gene mapping on three digestive system pathways. The digestion and absorption pathways of carbohydrates (**A**), proteins (**B**), and fats (**C**) are shown, which were modified on the basis of the KEGG pathways with accession numbers (map04973, map04974, and map04975). The positively selected molecules, with their corresponding genes in parentheses, are highlighted in blue (ancestral euprimate) and green (ancestral Euarchonta).

Considering that high amounts of proteins and fats are normally found in animal-based foods and that a high amount of carbohydrates typically characterizes plant-based foods (*27*, *29–33*), the fact that the ancestral Euarchonta could have had a diet high in carbohydrates, fats, and proteins suggests that they were more likely omnivorous (Fig. 1). The omnivory of the ancestral Euarchonta is also inferred by two previous studies using different methods (*47*, *48*). In addition, treeshrews, the basal lineage of the Euarchonta, are well known as being omnivorous animals feeding on insects, small vertebrates, fruits, and seeds (*49*). These lines of evidence suggest that the ancestral Euarchonta were more likely omnivorous. No PSG was found in the ancestral Primatomorpha. This may suggest that their diet could have been less changed relative to the ancestral Euarchonta and that they thus could also have been omnivorous (Fig. 1).
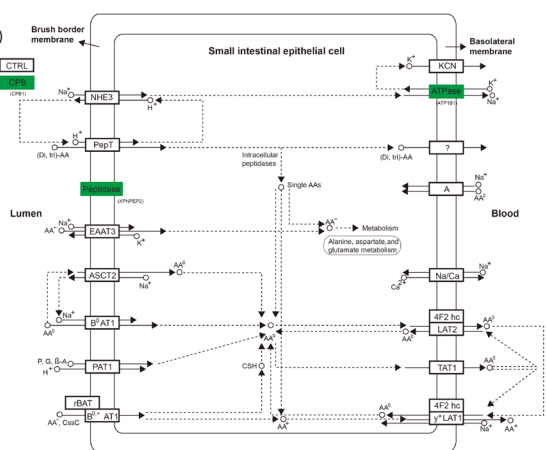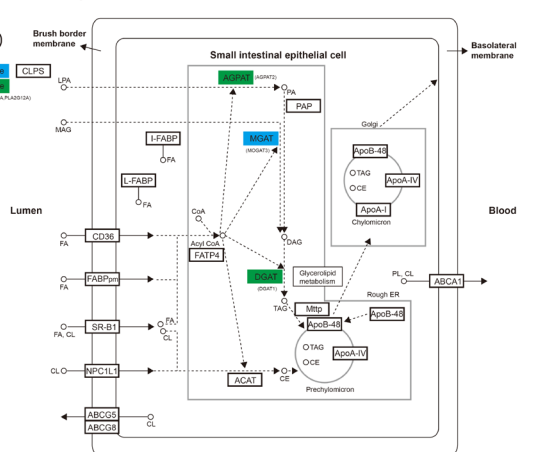
While the ancestral Euarchonta showed enhanced utilization of carbohydrates, fats, and proteins, indicating its omnivory, the common ancestor of living primates (CALP) exhibited an enhanced positive selection for fat utilization, suggesting that the ancestral euprimates had a fat-rich diet. Given that animal-based foods are rich in fat (*27*, *29–33*), this suggests that the ancestral euprimates were more likely carnivorous (Fig. 1). Alternatively, enhanced fat utilization may also occur in herbivores that mainly eat seeds or rely on microbial fermentation. Many seeds (e.g., nuts) are rich in lipids besides carbohydrates (*31*), and seed eaters may be expected to show enhanced utilization of fats in addition to carbohydrates. This possibility may be small for the ancestral euprimates, however, as no carbohydrate utilization–related PSGs were found in them (Fig. 2 and Table 1). Moreover, no living nonhuman primates are known to feed mainly on seeds (*1*). Similarly, the microbial fermentation (which converts abundant carbohydrates into volatile fatty acids or short-chain fatty acids) occurring in many herbivores (e.g., ruminants) may lead to enhanced utilization of fats such as short-chain fatty acids, but this possibility may also be small because the PSG *LIPF* found in CALP is known to be involved in the digestion of dietary triglycerides (*34*, *35*), which mainly contain long-chain fatty acids. Furthermore, previous studies have shown that the dietary lipids (e.g., triglycerides) of the herbivores (e.g., ruminants) that rely on microbial fermentation are predominantly hydrolyzed by the lipases of the rumen bacteria in their guts (*50–53*). These lines of evidence may suggest that the ancestral euprimates were less likely herbivores and were more likely carnivores.

Our molecular data suggest that the ancestral euprimates' evolution coincided with a diet shift from omnivory to carnivory. This is congruent with the increased evidence suggesting that the ancestral euprimates could have been primarily insectivorous (*3*, *4*, *21–23*, *54*). Fossil evidence shows that the earliest and most primitive euprimates (e.g., omomyiforms and adapiforms) had particularly small body sizes and were primarily insectivorous, although there is also evidence supporting herbivory (*10*, *54*). For instance, *Teilhardina asiatica*, which is phylogenetically near the root of the euprimate radiation, was reconstructed as a diurnal, visually oriented predator (*23*). In addition, a primitive haplorhine primate, *Archicebus achilles* from the early Eocene Epoch (about 55 million years ago), was considered probably diurnal, arboreal, and primarily insectivorous (*22*). In addition to fossil evidence, the results that we obtained are also consistent with those of more recent molecular studies on the *CHIA* gene in mammals (including primates) (*21*, *55*). The *CHIA* gene is a digestive enzyme capable of digesting insect exoskeletons, and its copy number

Table 1. Positively selected genes. PSGs are sorted on the basis of P values. For convenience, only the ω values of foreground branches are shown. $2\Delta L$, twice the difference of likelihood values between the modified model A and the corresponding null model with $\omega = 1$ fixed in the foreground branches; proportion of sites and their corresponding ω values in four site classes (p_0, p_1, p_{2a}, and p_{2b}) of the branch-site model are shown.

Taxa/genes	Parameter estimates	$2\Delta L$	df	P value	Positively selected sites
Ancestral euprimate					
LIPF	$p_0 = 0.719$; $p_1 = 0.269$; $p_{2a} = 0.008$; $p_{2b} = 0.003$	6.36	1	0.011	164E,244E,369P,380D
	$\omega_0 = 0.055$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{208.378}$; $\omega_{2b} = \mathbf{208.378}$				
MOGAT3	$p_0 = 0.709$; $p_1 = 0.279$; $p_{2a} = 0.008$; $p_{2b} = 0.003$	3.74	1	0.053	159E,200A,246D
	$\omega_0 = 0.077$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{85.948}$; $\omega_{2b} = \mathbf{85.948}$				
Ancestral Euarchonta					
SI	$p_0 = 0.751$; $p_1 = 0.239$; $p_{2a} = 0.006$; $p_{2b} = 0.002$	10.50	1	0.001	
	$\omega_0 = 0.057$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{36.197}$; $\omega_{2b} = \mathbf{36.197}$				3K,69E,344R,491N,544L,885E,934T,992P,1083K, 1442H,1561Q,1618D,1645T,1782I
CELA3B	$p_0 = 0.755$; $p_1 = 0.238$; $p_{2a} = 0.004$; $p_{2b} = 0.001$	9.74	1	0.001	111K,148E
	$\omega_0 = 0.060$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{998.999}$; $\omega_{2b} = \mathbf{998.999}$				
PNLIPRP1	$p_0 = 0.792$; $p_1 = 0.195$; $p_{2a} = 0.010$; $p_{2b} = 0.002$	8.88	1	0.002	334N,465L
	$\omega_0 = 0.083$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{69.500}$; $\omega_{2b} = \mathbf{69.500}$				
AGPAT2	$p_0 = 0.902$; $p_1 = 0.080$; $p_{2a} = 0.014$; $p_{2b} = 0.001$	8.10	1	0.004	85Q,194A,207S
	$\omega_0 = 0.055$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{998.992}$; $\omega_{2b} = \mathbf{998.992}$				
PLA2G12A	$p_0 = 0.000$; $p_1 = 0.000$; $p_{2a} = 0.927$; $p_{2b} = 0.072$	7.68	1	0.005	
	$\omega_0 = 0.041$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{19.257}$; $\omega_{2b} = \mathbf{19.257}$				
CPB1	$p_0 = 0.656$; $p_1 = 0.314$; $p_{2a} = 0.019$; $p_{2b} = 0.009$	7.52	1	0.006	14-,42Q,99H,142K,163R,171I
	$\omega_0 = 0.064$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{491.701}$; $\omega_{2b} = \mathbf{491.701}$				209E,248V,261V,277P,303V
LCT	$p_0 = 0.774$; $p_1 = 0.222$; $p_{2a} = 0.001$; $p_{2b} = 0.000$	7.00	1	0.008	942N,943G,1083N,1759D
	$\omega_0 = 0.079$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{50.556}$; $\omega_{2b} = \mathbf{50.556}$				
DGAT1	$p_0 = 0.861$; $p_1 = 0.133$; $p_{2a} = 0.003$; $p_{2b} = 0.000$	5.92	1	0.014	22D
	$\omega_0 = 0.042$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{999.000}$; $\omega_{2b} = \mathbf{999.000}$				
SLC2A2	$p_0 = 0.814$; $p_1 = 0.179$; $p_{2a} = 0.004$; $p_{2b} = 0.001$	5.64	1	0.017	44L,265I
	$\omega_0 = 0.068$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{999.000}$; $\omega_{2b} = \mathbf{999.000}$				
ATP1B1	$p_0 = 0.708$; $p_1 = 0.241$; $p_{2a} = 0.037$; $p_{2b} = 0.012$	3.94	1	0.047	231M
	$\omega_0 = 0.000$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{5.681}$; $\omega_{2b} = \mathbf{5.681}$				
PLA2G2A	$p_0 = 0.629$; $p_1 = 0.296$; $p_{2a} = 0.050$; $p_{2b} = 0.023$	3.94	1	0.047	3V,16I,36R,89S,140K
	$\omega_0 = 0.101$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{4.214}$; $\omega_{2b} = \mathbf{4.214}$				
XPNPEP2	$p_0 = 0.749$; $p_1 = 0.242$; $p_{2a} = 0.005$; $p_{2b} = 0.001$	3.82	1	0.050	21S,654H
	$\omega_0 = 0.079$; $\omega_1 = 1.000$; $\omega_{2a} = \mathbf{109.941}$; $\omega_{2b} = \mathbf{109.941}$				

variation has been found to be highly correlated with insectivory (*21*, *55*). In primates, most living primates have been found to have one functional *CHIA* gene, while the ancestral euprimates (e.g., CALP) have been inferred to likely have had three *CHIA* genes, suggesting that insects were an important component in their diets (*21*), as in the diets of the ancestral placental mammals (*55*). The inference that the ancestral euprimates were insectivores is consistent with the observation that many living primates are partly insectivores (*17*, *56*). Therefore, these lines of evidence suggest that the ancestral euprimates were more carnivorous, likely as insect eaters.

VPH and NVP hypothesis

The results of our study point to the possibility of carnivory in the CALP, which satisfies Cartmill's VPH, an important theory accounting for the origin of euprimates (*3–5*). According to VPH, the ancestral euprimates were insectivorous, and their predation on insects in the terminal branches of trees is believed to have been an important driving force of their early evolution (*3*). For instance, high orbit convergence, which is generally considered to enhance stereopsis or depth perception, is believed to improve the accuracy of judging the distance of one's prey. Moreover, grasping hands and feet may help in controlling movements when snatching insects in fine branches (*4*). Cartmill's VPH is an important theory for understanding the evolution of the distinctive morphological characteristics of euprimates. However, it has been questioned by some researchers (including Cartmill himself) (*2*, *5*, *8*, *9*, *57*). For instance, regarding the explanation of orbital convergence as a form of predation adaptation, Cartmill wondered why not all vision-directed predators (e.g., mongooses, tupaiine treeshrews, and robins) have such characteristic. He also reasoned that VPH per se cannot explain the evolution of orbital convergence in the ancestral euprimates, which has been regarded as such theory's major flaw (*2*, *5*, *57*).

To account for this, nocturnality, which needs not only depth perception but also scotopic acuity, has also been proposed in the NVP hypothesis to interpret the evolution of convergent orbits (*5*, *11*, *12*, *14*). However, this cannot explain why the orbital convergence of diurnal vision–directed predators such as forest leopards (*58*) persists. Moreover, several studies suggest that the ancestral euprimates are likely diurnal, although some controversies exist (*22*, *23*, *59*). Therefore, it is possible that NVP is still insufficient to account for the evolution of orbital convergence in euprimates.

Ambush predation: An alternative explanation for the origin of euprimates

Regarding the insufficiencies of the VPH and NVP hypothesis to account for the evolution of euprimates' characteristics, as mentioned above, additional factors may be involved. With respect to orbit convergence, as Cartmill has realized, although animals with high orbit convergence, such as cats and owls, tend to be vision-directed predators, not all vision-directed predators have such primate-like high orbit convergence (*5*). Considering this incongruence, it is possible that not all vision-directed predators are faced with the same strong selection pressures for high orbit convergence. The well-known vision-directed predators—such as owls, cats, hawks, eagles, falcons, and wolves—show variable predation adaptation in many aspects; they are largely different from each other in terms of hunting strategies, ranging from ambush predation to pursuit predation. Among these predators, it seems that those with high orbit convergence, such as owls and cats, are typically ambush predators, while the others

tend to be pursuit predators. This may suggest that high orbit convergence more likely occurs in typical ambush predators.

Ambush predators and pursuit predators are two different types of predators with distinct forms of predation adaptation. Ambush predators are typically characterized by stalking or sitting and waiting for their prey to come by and then launching a surprise attack on it, while pursuit predators generally actively search for and chase their prey over a short or long distance (*60–63*). Compared to pursuit predators, ambush predators have relatively lower prey encounter rates and smaller search areas, which consequently limit their prey availability (*63–65*). Their limited prey availability may subsequently lead to much stronger selection for their promoted hunting success rate, which relies on improved hunting skills, such as enhanced stereopsis, thereby facilitating the evolution of high orbit convergence. Previous studies have shown that reliable distance estimation is fundamentally important for ambush predators and that stereopsis is considered under strong selection pressure in ambush predators relative to pursuit predators and herbivores (*66*, *67*). Besides owls and cats, highly convergent orbits and/or eyes can also be found in many other typical ambush predators, such as flounders (flatfish), barreleye fish, crocodile fish, stargazer, and tasselled wobbegong, suggesting that highly convergent orbits and/or eyes are linked to typical ambush predators. Among the typical vision-directed ambush predators, chameleons particularly evolved highly mobile eyes; their eyes move independently of each other, but the moment they spot an insect, both their eyes are fixed on it before they extend their tongue to capture it (*68*). This suggests that binocular vision, and hence high orbit convergence, may be particularly critical for typical ambush predators to gauge the prey distance accurately so that they could successfully launch a surprise attack. The ambush predation–associated selection pressure may be an important ecological driver for the evolution of high orbit convergence in typical ambush predators.

The observation that high orbit convergence characterizes typical ambush predators, as discussed in the previous paragraphs, provides important insights into the evolution of orbital convergence in euprimates. As shown by this study and previous molecular and fossil evidence, the ancestral euprimates may have been vision-directed predators (*3*, *4*, *21–23*) with high orbit convergence (*23*). This may suggest that they were likely ambush predators (Fig. 3). Many living insectivorous primates—such as cheirogaleines, lorises, galagos, and tarsiers—show a hunting style featuring stalking and sudden striking with manual grasping (*3*, *69*), which characterizes ambush predation. In particular, tarsiers ambush their prey through a sudden leap. A similar ambush strategy can be observed even in more advanced euprimates, including chimpanzees and humans. This may suggest that ambush predation is common in living primates, and it is possible that the ancestral euprimates were also ambush predators. In the scenario of vision predation, Cartmill has already realized that the predation of insectivorous primates is characterized by a means (e.g., stalking) of ambush predation, although he does not use the phrase "ambush predation" (*4*, *5*). The ambush predation of ancestral euprimates may have led to their evolution of high orbit convergence, which is found in other typical ambush predators, such as owls and cats.

The possibility that the ancestral euprimates were ambush predators sheds light on the other characteristics of euprimates, besides their high orbit convergence. It is well known that ambush predators must avoid detection before launching a strike (*60*, *70*). Thus, many of them stalk their prey as unobtrusively as possible with their evolved adaptive silence (*60*). For instance, cats have soft pads to help
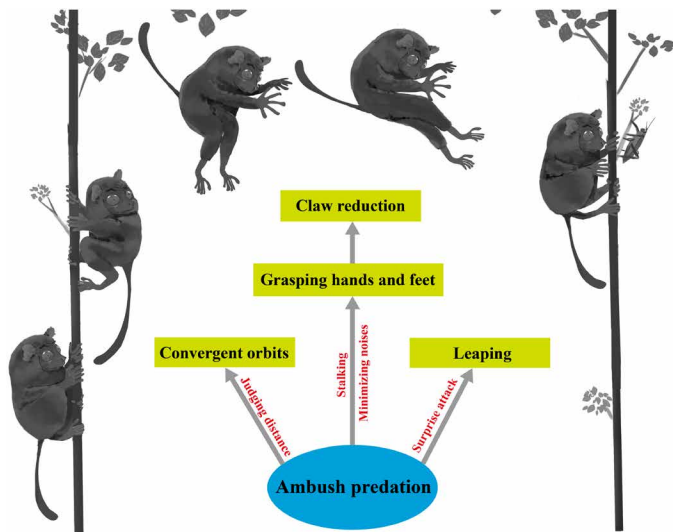
Fig. 3. The schematic of the ambush predation hypothesis underlying the origin of euprimates. In this ambush predation scenario, orbit convergence is considered helpful for judging the distance of the prey, the evolution of grasping hands and feet with claw reduction helps to minimize the noises made for stalking prey, and leaping is considered as an efficient means of launching a rapid attack (please see the text for details).

them walk silently, and owls have sound-absorbing velvety flight feathers and comb-like wing edges, which allow them to fly silently (*71*). Therefore, it seems that for ambush predators, minimizing the noise that they make while stalking prey is a strong selection for their hunting success. Thus, given the possible ambush predation of the ancestral euprimates, reducing the noise that they made so that they could move silently could also be particularly important for them.

Regarding movement, living primates are equipped with grasping hands and feet, and the type of locomotion suggested by these (nonclaw climbing) may have been derived from the claw climbing of their euprimate progenitors (e.g., plesiadapiforms) (*72*). The evolution of grasping hands and feet has long been considered specifically good for well-controlled movements in pursuit of prey on slender tree branches (*3*, *4*, *73*). This explanation seems plausible, but other explanations cannot be excluded. Given that the adaptive silence may be required for the ambush predation of the ancestral euprimates, we propose that the evolution of grasping hands and feet (nonclaw climbing) could have been selected to minimize the noise that they made while stalking their prey to avoid detection by it (Fig. 3). For many arboreal animals, including the euprimate ancestor, such as the plesiadapiforms (*72*), the claws are used for climbing trees. However, claw climbing is apparently noisy and thus not favorable for stalking prey. In comparison, nonclaw climbing can efficiently reduce noise as the finger and toe pads are soft. It is possible that grasping hands and feet evolved to minimize the noise that primates make while stalking their prey, as many of the insects (including winged insects) consumed by them (*17*, *69*) are capable of using sounds and substrate vibrations as cues to detect approaching predators (*74–78*). This is consistent with the observation that many living insectivorous primates catch insects by stalking them (*3*, *69*). Grasping hands and feet can also be found in other arboreal ambush predators, such as chameleons (*4*). This may suggest that the evolution of grasping hands and feet in these arboreal ambush predators could
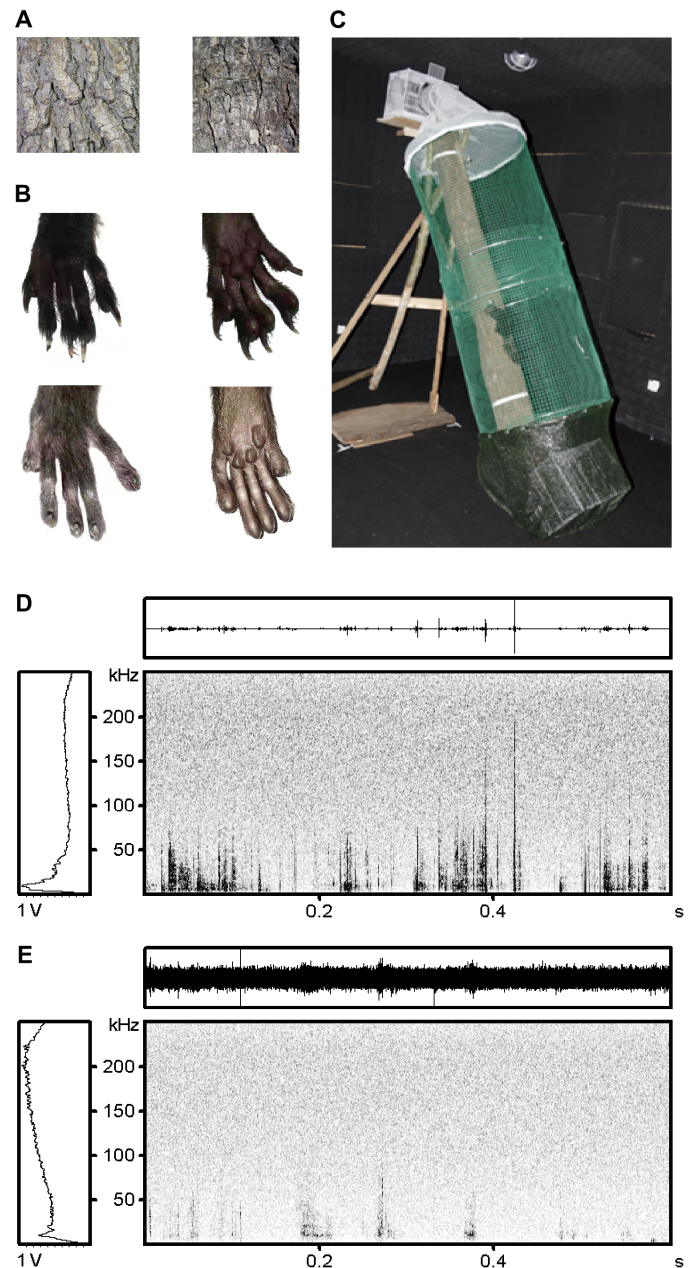

Fig. 4. Experimental setup and acoustic spectrum for the claw climbing and nonclaw climbing of tree squirrels. (**A**) The elm tree (left) and pine tree (right) used in this study. (**B**) The claws and nonclaws (claws trimmed) of the tree squirrels. (**C**) Experimental setup used for the climbing experiment. (**D** and **E**) Representative acoustic spectrum (black) against background noises (gray) for claw climbing (D) and nonclaw climbing (E) of the same tree squirrel on the pine tree. The relative amplitude (top) and power spectrum (left side) of the climbing sounds are also shown.

have been under convergent selection pressures to minimize the noises that they made in favor of stalking prey. This is consistent with VPH, which indicates that the evolution of the grasping extremities in primates and chameleons partly facilitates stealthy locomotion (*4*, *5*). Once grasping hands and feet are favored for ambush predation, claw climbing may then be selected against, which may subsequently lead to claw reduction (Fig. 3). A previous study showed that claw

reduction could have occurred in the ancestral euprimates (*79*), which is prevalently explained as a form of adaptation to the terminal-branch niche (*4, 80*). However, the terminal-branch niche hypothesis cannot explain the secondary evolution of claws in the Callitrichidae, which forage to a large extent among small terminal branches (*5, 79*). Rather, the results of our study suggest that the reduced claws may have evolved as a result of minimizing locomotion noise in favor of ambush predation.

To explore the possible role of the nonclaw climbing of the ancestral euprimates in reducing noise, we further used the tree squirrel (*Sciurus vulgaris*) as a model system for measuring sound-related parameter changes from claw climbing to nonclaw (claw-trimmed)

climbing, including minimum frequency, maximum frequency, and sound pressure level (Fig. 4 and movie S1). The sound pressure level is directly related to the sound intensity and was represented by the root mean square (RMS). For both pine and elm trees, the sound frequency and sound pressure level of nonclaw climbing were substantially reduced relative to those of claw climbing (Figs. 4 and 5, Table 2, and audios S1 to S6). Further statistical analyses using the Wilcoxon signed-rank test showed that the RMS values of nonclaw climbing were reduced 1.2 times (elm tree, $V = 14,852$, $P < 2.20 \times 10^{-16}$) to 1.5 times (pine tree, $V = 16,237$, $P < 2.20 \times 10^{-16}$), that the maximum frequency was reduced 1.84 times (pine tree, $V = 14,968$, $P < 2.20 \times 10^{-16}$) to 2.05 times (elm tree, $V = 14,405$, $P < 2.20 \times 10^{-16}$) on average,
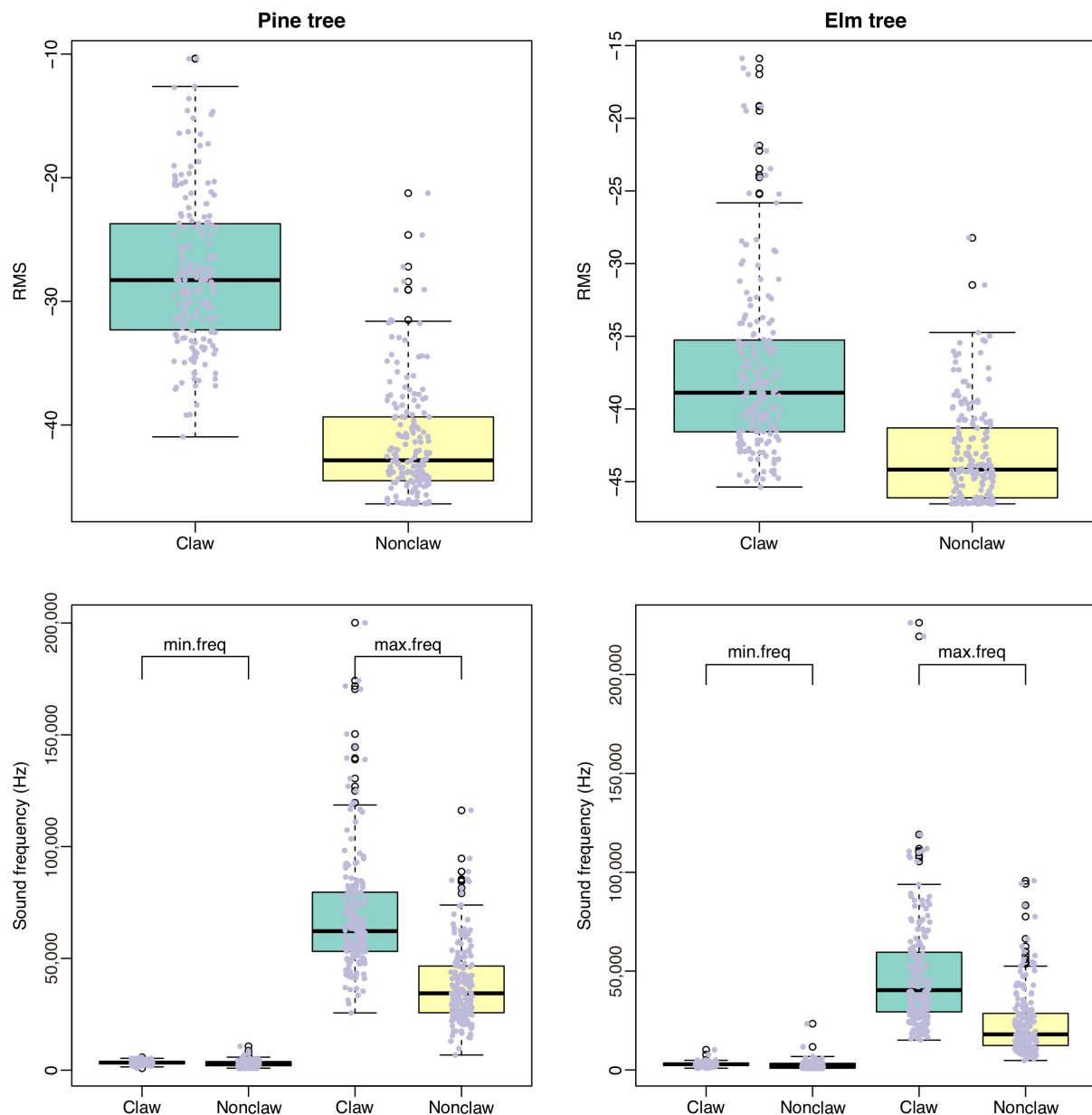

Fig. 5. Comparison of the acoustic parameters of the claw climbing and nonclaw climbing of the tree squirrels. The results based on both the pine tree (top left and bottom left) and the elm tree (top right and bottom right) are shown. RMS represents the sound pressure level, which is directly related to sound intensity. The min.freq (minimum frequency) and max.freq (maximum frequency) are also shown.

Table 2. Descriptive statistics and results of the Wilcoxon signed-rank test for the acoustic parameters. The RMS, min.freq (minimum frequency), and max.freq (maximum frequency) for both claw climbing and nonclaw climbing are shown.

	Variables	Mean (SD)		Median		V	P value
		Claw	Nonclaw	Claw	Nonclaw		
Pine tree	RMS	−27.69 (6.15)	−41.46 (4.59)	−28.28	−42.85	16,237	$<2.20 \times 10^{-16}$
	Max.freq	69,927.00 (28,343.22)	38,082.00 (17,582.61)	62,190.00	34,388.00	14,986	$<2.20 \times 10^{-16}$
	Min.freq	3,455.00 (820.76)	2,995.00 (1,452.59)	3,395.00	2,900.00	10,759	$=4.97 \times 10^{-5}$
Elm tree	RMS	−37.34 (6.16)	−43.20 (6.16)	−38.88	−44.17	14,852	$<2.20 \times 10^{-16}$
	Max.freq	48,566.00 (29,812.80)	23,687.00 (16,432.52)	40,375.00	18,016.00	14,405	$<2.20 \times 10^{-16}$
	Min.freq	2,937.00 (1,037.10)	2,492.00 (2,162.44)	2,883.00	2,160.00	10,842	$=1.22 \times 10^{-6}$

and that the minimum frequency was also decreased (elm tree, $V = 10,842$, $P = 1.22 \times 10^{-6}$; pine tree, $V = 10,795$, $P = 4.97 \times 10^{-5}$). These results indicate that there is a low-intensity and low-frequency sound shift in nonclaw climbing relative to claw climbing. Our results suggest that nonclaw climbing can largely reduce noise in terms of sound intensity and maximum frequency, which could have benefited the ancestral euprimates in stalking and ambushing insects in trees. Another possibility is that the reduced noise of nonclaw climbing may partly be due to the possible slower locomotion of squirrels because of their clawlessness and the possible soreness of their digits. This possibility should be small given that (i) no apparent slowness was observed during the climbing experiment, and (ii) the claw wounds experienced 7 days of recovery before the nonclaw climbing experiment, longer than the veterinarians' recommendation (3 to 5 days). Hence, we reasoned that the reduced noise of nonclaw climbing may be mainly due to the lack of claws.

Besides orbit convergence and adaptive silence, surprise attack is also important for the hunting success of ambush predators (*62, 70*). Many ambush predators—such as cats, owls, and chameleons—are capable of launching a rapid attack at their prey within striking range from them. This can also be observed in living primates; for instance, tarsiers leap to pounce on their prey. Leaping is common among living primates, and fossil evidence shows that it could have evolved in the ancestral euprimates (*72, 81–86*). This is consistent with the latest fossil evidence showing that a primitive haplorhine primate, *A. achilles* from the early Eocene Epoch in China (about 55 million years ago), exhibited many hindlimb features associated with frequent leaping (*22*). While leaping has been regarded as an important means to bridge gaps (*1*) or to avoid predators (*84*), it is also considered important for prey capture (*57, 81, 82*). Boyer *et al.* (*81, 82*) suggested that leaping may have evolved primarily for predatory adaptation. Considering the significance of leaping for prey capture, particularly its importance for ambush predation, as observed in tarsiers, it is possible that leaping as a means of launching a rapid attack evolved at least partly in favor of ambush predation during the early evolution of euprimates (Fig. 3).

Our results suggest that euprimate characteristics—including convergent orbits, grasping hands and feet, reduced claws, and leaping—may be, at least partly, adapted for arboreal ambush predation. If this is true, then we may expect that these ambush predation–related characteristics may evolve in transition fossils leading to euprimates. Previous studies have suggested that euprimate characteristics may evolve in a step-like fashion, showing that euprimate-like grasping hands and feet evolve first, before the euprimate node, and then

orbital convergence and leaping (*87–89*). This evolutionary sequence of euprimate characteristics seems to be consistent with the behavioral action sequence observed in an ambush predator, which usually first stalks its prey, then gauges prey distance, and lastly launches a surprise attack. If this is true for euprimates, grasping hands and feet could have been the first to evolve, as avoiding detection from one's prey is the first step toward ambush success. Future fossil studies will be helpful in determining the evolutionary sequence of euprimate characteristics.

Therefore, the molecular and behavior results in the present study suggest that the ancestral euprimates could have been ambush predators. This ambush predation scenario can provide alternative explanations into the evolution of distinctive euprimate characteristics. For example, the evolution of orbit convergence may help promote the accurate judging of prey distances, grasping hands and feet with claw reduction may be adapted for reducing the noise made in favor of stalking, and leaping may be used as a means to launch a rapid attack (Fig. 3). The ambush predation of the ancestral euprimates could have opened up a new niche for them that was not available to their relatives, although the selection pressures responsible for such evolution of their ambush predation remain unknown. The results of our study support that the shift of feeding habits to ambush predation could be a critical ecological selection pressure explaining the origin of euprimates.

MATERIALS AND METHODS
Taxa used for molecular study
A total of 36 Euarchonta species were used in this study. These included 3 Scandentia species, 1 Dermoptera species, and 32 primate species, representing three living groups of Euarchonta (Fig. 1). For the primates, 3 species from the suborder Strepsirrhini and 29 species from diverse taxa (including Hominidae, Hylobatidae, Cercopithecidae, Platyrrhini, and Tarsiidae) of the suborder Haplorrhini were used. In addition to the Euarchonta species, two species of Glires, the sister taxon of Euarchonta, were used as outgroups. For the two outgroup species, *Mus musculus* and *Oryctolagus cuniculus* were primarily used, and when their sequences were unavailable, the sequences of their relatives were used.

Dietary data used
The dietary data that were used in the present study were based on the previously published dataset EltonTraits 1.0 (*90*). For this dietary dataset, the dietary information of 398 Euarchonta species (living

primates, colugos, and treeshrews) is provided, and the dietary composition of each species is recorded in 10 percentage dietary categories. To determine the averaged proportion of carnivory and herbivory of each species of taxa in Fig. 1, we converted EltonTraits' 10 percentage dietary categories of each species into 2 dietary categories: carnivory and herbivory (carnivory = Diet-Inv + Diet-Vend + Diet-Vect + Diet-Vfish + Diet-Vunk + Diet-Scav; herbivory = Diet-Fruit + Diet-Nect + Diet-Seed + Diet-PlantO).

Genes used and sequence alignment

Following the MPE approach used for diet reconstruction (27), in this study, we used the genes annotated in three KEGG digestive system pathways, including carbohydrate digestion and absorption (map04973), protein digestion and absorption (map04974), and fat digestion and absorption (map04975) (Fig. 2). The gene sequences of these digestive system–related genes were downloaded from the GenBank database (table S1). Upon analyses, the sequences of some genes (e.g., amylase genes) were unavailable or were available for only a few species of our focal taxa and were thus excluded from our subsequent positive selection analyses. Eventually, 117 genes were used in this study. The gene sequences were aligned using webPRANK with default parameters (www.ebi.ac.uk/goldman-srv/webprank/). Individual species sequences with long indels and/or were too short were removed or replaced by other relevant transcript variants. To confirm the correctness of the sequence cutting, the sequence alignments were checked by eye, and the translated protein sequences of the genes were blasted against the nonredundant protein sequence database.

Positive selection analyses

Positive selection analyses were conducted using the branch and branch-site models implemented in the Codeml program of PAML (91). Through such analyses, we constructed the Euarchonta phylogeny mainly following a previous study (92), while for the species relationships within *Tupaia*, Cebidae, and *Macaca*, other relevant studies were followed (93–95). Given the Euarchonta phylogeny, the positive selection of genes was analyzed along our focal branches. For this, the ratio of nonsynonymous to synonymous substitutions per site (dN/dS or ω) was estimated, and likelihood ratio tests (LRTs) were used to determine statistical significance. Bonferroni multiple testing correction was used to adjust the P values.

Branch model

A two-rate branch model was used to detect the positive selection of genes along the branches. For this, our focal branches were respectively labeled foreground branches, and the rest were treated as background branches. For the two-rate branch model, the ω values of the foreground and background branches were assumed to be different, and the goodness of fit of the two-rate branch model relative to the one-rate branch model, which assumes a single ω value across all the branches, was analyzed using the LRT. If a statistically significant $\omega > 1$ value was found in a foreground branch, then we further compared the two-ratio branch model with the two-ratio branch model with a constraint of $\omega = 1$ in the foreground branch to determine whether the $\omega > 1$ value of the foreground branch was supported with statistical significance.

Branch-site model

The branch-site model (test 2) was used to detect the positively selected sites of genes along a particular branch. The branch-site model assumes four classes of sites, and site class 0 and site class 1, respectively, represent the evolutionarily conserved ($0 < \omega_0 < 1$) and neutral ($\omega_1 = 1$) codons across the branches, and site classes 2a and 2b, respectively, represent the evolutionarily conserved ($0 < \omega_0 < 1$) and neutral ($\omega_1 = 1$) codons for the background branches but were allowed to be under positive selection ($\omega_2 > 1$) for the foreground branches. Statistical significance was determined by comparing a modified model A with a null model with $\omega = 1$ constrained. The empirical Bayes method was used to identify the positively selected sites.

Behavior experiments

Animals

Six tree squirrels (*S. vulgaris*) were obtained with permission from an artificial breeding company (Longjiang, Heilongjiang). The six squirrels (four males and two females) were approximately 13 months old, with an average body weight of 335.5 g and an average body length of 22.9 cm. Each squirrel was kept in its own cage under natural light:dark conditions with food (walnuts, sunflower seeds, cakes, and apples) and water provided.

Experimental setup

An experimental setup was constructed for the acoustic recording of squirrel climbing (Fig. 4). Briefly, one 2.35-m-long trunk with a diameter of approximately 17.91 to 19.98 cm from a pine tree (*Larix olgensis*) and an elm tree (*Ulmus campestris*), representing a gymnosperm and an angiosperm, respectively, was used for the climbing experiment. The trunk was at a 60° angle to the horizontal direction, and to prevent the squirrels from escaping, we used a cylindrical grid with a diameter of 70.50 cm to enclose the trunk and enclosed both ends with gauze. We also built a wooden platform at the top of the trunk to catch the squirrel after each climbing. After catching the squirrel, we moved it from the platform and then released it from the bottom. For acoustic recording, one microphone was placed parallel to the upper side of the trunk, with a distance of 10 cm. The lower end of the microphone was 178 cm from the ground.

Claw trimming

Squirrels normally climb trees using their claws (claw climbing), and for nonclaw climbing, we trimmed their claws after the claw-climbing experiment. The squirrels' claws were trimmed in a pet clinic (Chenghong, Changchun) under anesthesia with isoflurane (inhalation anesthetic). After the trimming of each claw, we petted the wound dry with a clean tissue and applied an ointment with allantoin and homosulfamine as the main ingredients to the wound to prevent infection. When all the claws of a squirrel had been trimmed, it was moved to its cage. For nonclaw climbing, the claws were largely trimmed, and only a tiny part of each claw above its base was left (Fig. 4B). According to medical experience (Chenghong), claw wounds recover 3 to 5 days after they occur. In addition, to minimize the possible effects of wound pain on the climbing experiment, for conservation, our nonclaw climbing experiment was started 7 days after the operation. During the 7-day recovery period, the activity status of each squirrel was recorded every day. The common situation was that the squirrels were not active and ate very little food in the first 3 days, but after day 3, they became active and started eating normally.

Acoustic recordings

Acoustic recordings were conducted at an acoustic laboratory located in the Teaching and Scientific Research Base, Jilin Agricultural University, Changchun, P.R. China. Acoustic recording trials were conducted from 0800 to 1700 from 16 June to 18 July 2021, except on rainy days. The sounds made by the squirrels, which climbed

over the back of a 1.5-m-long tree trunk, between the two white strips thereon (Fig. 4C), were recorded using an ultrasonic sound acquisition system (UltraSoundGate 116, Avisoft Bioacoustics Recorder, version 4.2.25, Glienicke, Germany) with a microphone (CM16/CMPA, Avisoft Bioacoustics, Glienicke, Germany) connected to a laptop computer. The sampling rate was set to 250 kHz at a 16-bit resolution. We adjusted the gain to ensure that the sounds would not be overloaded. The beginning and end of each climb were manually recorded. For each squirrel, 30 efficient instances of claw climbing and nonclaw climbing on both the pine tree and the elm tree were recorded, respectively. We excluded the climbs in which there were audible vocalizations of the squirrels and the climbs that involved backward movement or the squirrels' hitting of the cylindrical grid.

Acoustic analysis

Sounds were visualized using Avisoft-SASLab Pro (version 5.2.10, Avisoft Bioacoustics, Glienicke, Germany). The measurement of the acoustic parameters was based on 512-point fast Fourier transformation, Hamming window, 75% frame size, and 93.75% temporal overlap. The frequency resolution was 977 Hz, and the temporal resolution was 0.064 ms. To quantify the acoustic features, we measured three spectral parameters (sound pressure level, minimum frequency, and maximum frequency) at a threshold of −15 dB below the peak spectral amplitude using an automatic parameter measurement setup. The sound pressure level, which is directly related to sound intensity, was represented by the RMS parameter.

Statistical analyses

Statistical analyses were performed with R software (version 4.0.3 GUI 1.73, Catalina Build). The boxplot function, summary function, and SD function were used to produce a boxplot and to calculate the mean with SD and median. The Wilcoxon signed-rank test with continuity correction was used to determine the statistical significance of the spectral parameters between claw climbing and nonclaw climbing.

SUPPLEMENTARY MATERIALS

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