Evolutionary Anthropology 25:253–266 (2016)

ARTICLE

Digestive Enzymes of Human and Nonhuman Primates

MAREIKE CORA JANIAK

All living organisms need to consume nutrients to grow, survive, and reproduce, making the successful acquisition of food resources a powerful selective pressure. However, acquiring food is only part of the challenge. While all animals spend much of their daily activity budget hunting, searching for, or otherwise procuring food, a large part of what is involved in overall nutrition occurs once the meal has been swallowed. Most nutritional components are too complex for immediate use and must be broken down into simpler compounds, which can then be absorbed by the body. This process, digestion, is catalyzed by enzymes that are either endogenous or produced by the host's microbial population.[1] Research shows that the nutritional value of food is partially constrained by the digestive abilities of the microbial community present in the host's gut and that these microbes rapidly adapt to changes in diet and other environmental pressures.[2] An accumulating body of evidence suggests that endogenously produced digestive enzymes also have been, and still are, common targets of natural selection, further cementing their crucial role in an organism's digestive system.[3–5]

In this paper, I focus on the endogenous digestive enzymes that are known to be important to primates. Primates exhibit a particularly diverse array of dietary ecologies. From exclusively insectivorous species to grass-eating monkeys, the primate digestive system, including the enzymes in it, has evolved in response to a multitude of pressures. Recently, many research efforts have focused on the gut microbiome, providing new insights into the interplay between diet and gut adaptation for a variety of animals, including human and nonhuman primates.[6–11] These are exciting new findings, but to achieve a full picture of an animal's digestive adaptations, the gut microbiome and endogenously produced digestive enzymes should be viewed as complementary parts of the system. While the genes coding for digestive enzymes do not change as quickly as those of the microbiome, the variety of endogenous digestive enzymes within primates nevertheless constitutes a major adaptive strategy and warrants special attention in this paper.

Changes in the expression of digestive enzymes are important dietary adaptations that may allow an organism to exploit food sources that were previously difficult or impossible to digest. These changes can occur quite rapidly[12] and thus could be an important adaptive response that allows animals to carve out separate dietary niches in environments where several species are competing for food resources. Both South America and Madagascar were populated by a small number of primates rafting from the African mainland.[13] Upon arrival, these primates rapidly diversified and filled the available dietary niches,[14] evolving a suite of physiological, morphological, and behavioral characteristics to process a range of different diets.[15–18] Since digestive enzyme adaptations are not just important for the ability to tolerate new food resources, but also to maximize the energy obtained from them, changes in digestive enzymes were likely part of this adaptive suite. Especially in human evolution, maximizing the energy extracted from foods may have been a crucial factor in fueling the growth of our large brains.[19] In nonhuman primates, many species depend on relatively low-quality foods, such as leaves, which can only be digested efficiently with specific gut adaptations, such as foregut fermentation and/or special digestive enzymes.

Recent work on primate nutritional ecology has highlighted the many challenges primates face to meet not just overall energy requirements, but also to balance micronutrients and protein intake,[20–23] all while dealing with fiber, tannins, and toxins contained in foods.[24,25] The ability to meet nutritional goals depends in part on foraging decisions and the

Mareike Janiak is a doctoral candidate in the Department of Anthropology at Rutgers University. She is interested in the diets and dietary adaptations of primates. Her doctoral dissertation research seeks to identify whether the digestive enzymes of New World monkeys exhibit adaptations for insectivorous diets.
Email: mareike.janiak@rutgers.edu

Key words: dietary adaptations; amylase; lactase; chitinase; pepsin

DOI: 10.1002/evan.21498
Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Endogenously Produced Digestive Enzymes of Primates

Enzyme	Gene symbol	Substrate
Alpha-Amylase	*AMY1*	Starch
Maltase	*MGAM*	Disaccharide maltose (product of starch digestion by α-amylase)
Chitinase	*CHIA*	Chitin (present in cell walls of fungi and exoskeletons)
Pepsin A	*PGA*	Protein
Chymosin	*CYM*	Protein
Ribonuclease	*RNASE1*	In ruminants and colobines: foregut bacteria
Lysozyme	*LYZ, LZM*	In ruminants and colobines: foregut bacteria
Trypsin	*PRSS1*	Protein
Gastricsin	*PGC*	Protein
Lipases	*PNLIP, CEL*	Lipids
Lactase	*LCT*	Lactose (main carbohydrate in milk)
Trehalase	*TREH*	Trehalose (disaccharide found in insects, fungi, and plants)
Sucrase	*SI*	Sucrose, maltose

nutritional composition of food items.[25–27] However, it is also constrained by the gut's capability to extract these nutrients, which is where digestive enzymes and variation in them undoubtedly play a key role.[1,3,4,28]

The enzymes discussed here include amylase, lactase, pepsin A, chymosin, chitinase, ribonuclease, and lysozyme. While this is not an exhaustive list of the enzymes at work in primate digestive tracts, they are the more important ones because they usually represent the first step in the digestion of their respective substrates,[1] and research in primates has largely been limited to them. Table 1 details both the enzymes discussed here and ones that have not been studied in depth in primates. I describe the specific role of each digestive enzyme, summarize what is known about its inter- and intraspecies variability, and discuss the adaptive implications of such variation. I include information on digestive enzymes in nonprimate mammals to build a comparative evolutionary framework.

AMYLASE

Starches are a staple in the diets of many contemporary human populations.[29] They are also present in the diets of some nonhuman primates and other mammals in the form of underground storage organs, unripe fruits, and seeds.[30,31] Alpha-amylase is the enzyme that catalyzes the breakdown of starch into sugar by cleaving the glycosidic bonds of the polysaccharide to produce the disaccharide maltose, which can then be hydrolyzed into glucose and absorbed into the bloodstream.[32] All vertebrates express this digestive enzyme in their pancreas, but only some mammals have evolved to additionally express α-amylase in their mouths, where it is secreted by parotid and/or submaxillary glands.[33] Species that express α-amylase in their saliva include some primates, rodents, lagomorphs, and bats.[34] Strikingly, the secretion of α-amylase by the salivary glands has evolved independently several times, suggesting that this phenotype provides a selective advantage for certain species.[32] Furthermore, there is considerable variation, both between and within species in which salivary amylase has evolved, in the amount of enzyme that is expressed.[3,31,35] Evidence that this may also be the case for pancreatic amylase comes from a study comparing copy number variation of pancreatic amylase genes in wolves and domestic dogs.[4]

Within primates, only Old World monkeys, apes, and humans express α-amylase in their saliva; New World monkeys do not (Fig. 1).[3,34] No investigation of salivary amylase activity in strepsirrhines has been published, suggesting a potential avenue for future research. However, given current understanding of the evolutionary pattern of this trait in primates, it is unlikely that strepsirrhines express α-amylase in their saliva.

A comparison of the amylase gene structures in New World and Old World monkeys, apes, and humans shows that the ability to express α-amylase in saliva evolved after several duplications of the pancreatic amylase gene *AMY2* within the primate lineage.[34] Two insertions occur in the promoter region of *AMY1*, resulting in the expression of α-amylase in saliva. Comparing the gene structures between different primates shows that the first insertion, a γ-actin pseudogene, arose after the divergence of the New World monkey lineage.[34] The second insertion, an endogenous retrovirus, occurred after the split from the Old World monkeys and is found only in hominoids (Fig. 1).[34] Studies with transgenic mice indicate the retroviral insertion is required to change the expression site of amylase from the pancreas to the parotid gland.[36] This is consistent with the lack of salivary amylase activity in New World monkeys. Old World monkeys, however, express salivary amylase despite lacking the retroviral insertion. More work is needed to tease apart whether the insertion of the γ-actin pseudogene plays a role in salivary amylase expression or if another mechanism is responsible for this phenotype in Old World monkeys. The latter would suggest an additional independent evolution event within the primate order.[34]

Rodents also express salivary amylase, but must have evolved the ability independently from primates.[36] However, similarities between pancreatic and salivary amylase genes in mice indicate that the latter resulted from duplication of the pancreatic amylase gene, as it did in the primate

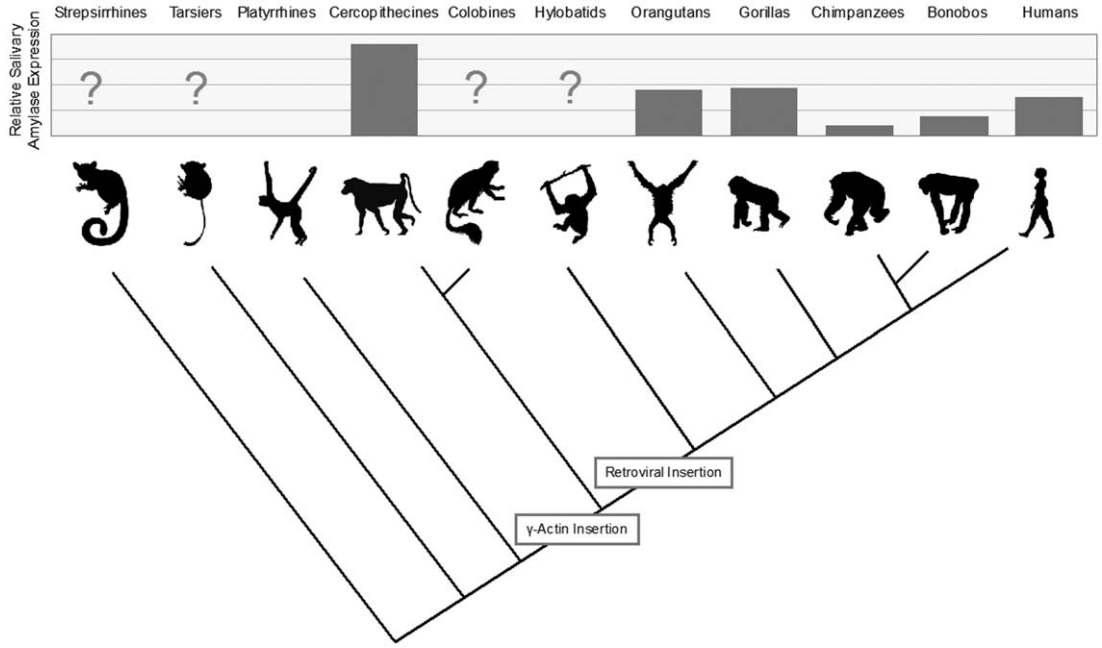

Figure 1. Salivary amylase expression in primates relative to that in humans and the proposed timing of the two insertions thought to cause amylase expression in saliva.[11] Cercopithecines express salivary amylase despite lacking the retroviral insertion, suggesting the possibility of an alternate mechanism.

lineage.[37] Within rodents, the salivary amylase gene appears to have undergone duplication early in the muroid lineage (including hamsters, gerbils, true mice, and rats), but only arvicoline rodents (voles, lemmings, and muskrat) and hamsters express two gene copies. It is unclear whether or not this pattern is actually homologous, as the two groups are only distantly related.[37]

Lagomorphs, pigs, and some bats also produce α-amylase in their salivary glands,[33] but no studies investigating the genetic bases of this phenotype have been conducted in these groups. Evidence for salivary amylase has been found in several species of bats, including *Eidolon helvum*,[38] *Epomophorus labiatus*,[39] and *Myotis grisescens*,[40] but a systematic survey comparing variation in digestive enzymes, including salivary amylase, across the order Chiroptera has not been done. Given that bats exhibit great dietary diversity, feeding on blood, insects, small vertebrates, nectar, fruit, and pollen,[41] knowledge of the digestive enzymes found in different species could provide a better understanding of the types of selective pressures under which dietary physiology evolves.

Independent evolution of the expression of α-amylase by salivary glands in several taxa strongly suggests that this digestive enzyme provides a selective advantage.[34,36] Several studies have provided evidence of the possible selective benefits for salivary amylase.[3,42,43] The primary role of α-amylase, in both the pancreas and in saliva, is starch digestion. There is strong evidence that a diet rich in starch acts as a selective pressure on the evolution of amylase genes.[3,4] Canines express only pancreatic, not salivary amylase, but an interesting variation was discovered in a recent study that compared pancreatic amylase gene copy numbers in wolves and domesticated dog breeds.[4] While wolves consistently had only two copies of the *AMY2B* gene, diploid copy numbers in dogs ranged from 4 to 30. This increase in copy number correlated with a significant increase in α-amylase activity in dogs, leading the authors to conclude that efficient starch digestion represented a selective benefit in the process of dog domestication, presumably because dogs that were able to digest the potentially high-starch food discarded or provided by humans would have had an advantage over dogs that could not.[4]

Copy numbers also vary widely for the *AMY1* gene within humans and this genetic variation is correlated with the level of salivary amylase expressed.[3] Individuals from populations whose diets have traditionally included large amounts of starch, such as Europeans, Japanese, and the Hadza, tend to have more copies of the salivary amylase gene than do members of populations that eat little starch, such as the Mbuti, Datoga, Yakut, and Biaka.[3] Individuals with higher salivary amylase levels have been shown to perceive starch as less viscous and, as compared to individuals with low salivary amylase levels, report that starch viscosity decreases more quickly during mastication.[42] Changes in viscosity, such as viscosity thinning, are considered desirable and an important factor in determining liking of foods.[42] These differences may lead to, in individuals that express high levels of amylase in their saliva, a stronger preference for starchy foods and result in the adoption of a high-starch diet in populations with a high frequency of such a phenotype.[42] Thus, it is possible that the variation in *AMY1* copy number and salivary amylase levels predates the observed dietary

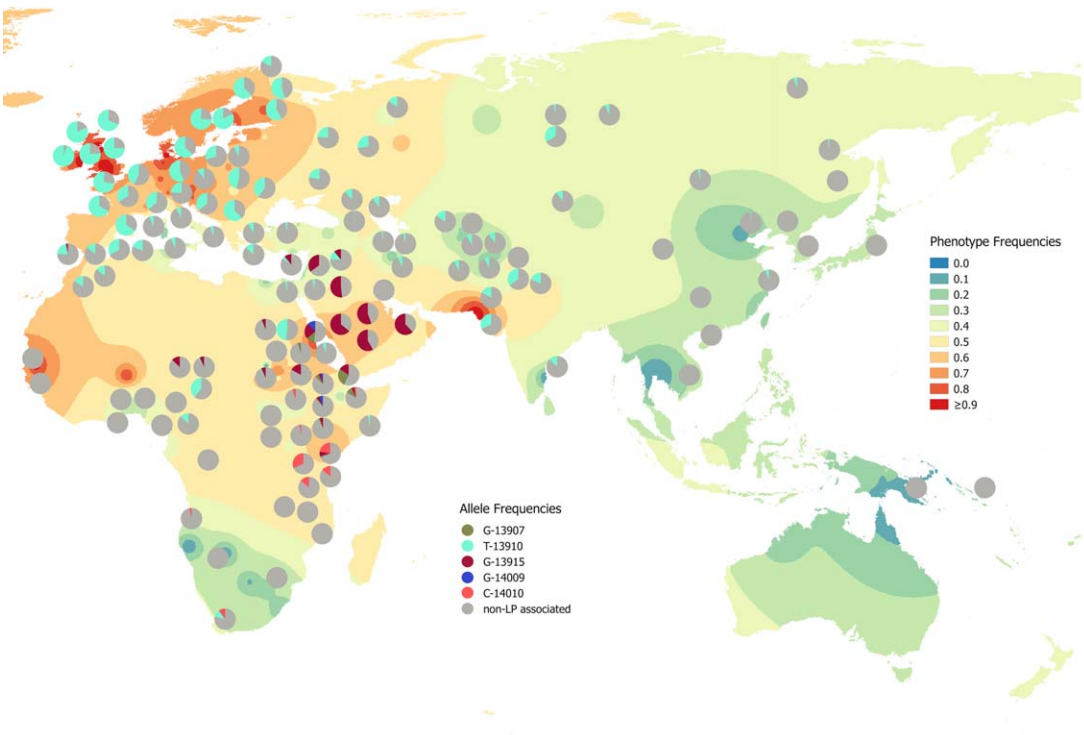

Figure 2. Frequency of the lactase persistence phenotype and frequencies of known associated alleles of the *LCT* gene. Phenotype frequencies predicted by surface interpolation are based on measurements at 235 locations.[57] Allele frequencies are based on data from the Global Lactase Persistence Association Database[57] and Jones and colleagues.[67]

ecologies and, in turn, drove the dietary choices of these populations rather than vice versa. However, geographic location is not a good predictor of copy-number variation,[3] providing evidence against this hypothesis and suggesting instead that diet acts as a selective pressure on digestive enzymes.[3]

One study showed that blood glucose levels are higher when high-starch foods are chewed before swallowing than they are when high-starch foods are swallowed whole.[44] This suggests that the absorption of glucose, and thus the amount of energy made available from starch, is increased by contact with saliva, likely due to salivary amylase. An improved ability to digest starch, increasing the amount of easily-absorbed glucose, may have conferred an important fitness advantage on individuals living in an environment where resources were limited.[45] Many modern human populations, however, especially those living in urban and industrialized settings, certainly are not limited by the amount of food resources available to them, resulting in rising levels of obesity and type 2 diabetes. Therefore, increased availability of glucose following starch consumption due to higher salivary amylase levels may now actually be maladaptive. A recent study found a link between lower *AMY1* copy number and increased obesity risk,[46] but these results could not be replicated.[47,48]

Variation in salivary amylase expression and salivary amylase gene copy numbers is also observed across other hominids. A recent study of ancient DNA showed that both Neanderthals and Denisovans had only a single copy of *AMY1* per chromosome, suggesting that the copy number variation observed in modern humans originated comparatively recently.[49] As opposed to humans, individual chimpanzees (*Pan troglodytes verus*) do not differ in *AMY1* copy number and, instead, uniformly have two copies of the gene.[3] Bonobos (*Pan paniscus*) consistently have four copies of the *AMY1* gene, although, a disruption of the coding sequence suggests that these copies may all be nonfunctional[3] (but see Behringer and coworkers[35]). For gorillas, only relative (not absolute) copy numbers of *AMY1* have been reported; these numbers are relatively higher than those of chimpanzees and relatively lower than those of humans.[50] Although no information on copy numbers in orangutans has been published, a recent article providing measurements of salivary amylase levels expressed in all hominoids suggests that they may be similar to those of gorillas.[35] Gorillas and orangutans have very similar levels of salivary α-amylase, which are significantly higher than those of both chimpanzees and bonobos (Fig. 1). Alpha-amylase levels are somewhat higher in bonobos than in chimpanzees, which not only is in accordance with the higher *AMY1* copy number reported for this species, but also indicates that the copies are not actually nonfunctional, as had been suggested.[3,35]

The observed salivary amylase levels further correspond to the diets

Box 1. Cheek Pouches

The cercopithecines are a subgroup of Old World monkeys that are characterized by the evolution of cheek pouches, which they often use to store food.[3,119] Lambert[119] has suggested that cheek pouches may facilitate the digestion of unripe fruit, seeds, underground storage organs, or foods containing high levels of starch.[31,119] The high levels of salivary amylase expressed in these species[31] support this hypothesis. *Papio hamadryas* (hamadryas baboons) and *Theropithecus gelada* (gelada baboons) have salivary amylase levels that are twice as high as those of humans and about eight times higher than those *Pan troglodytes* (common chimpanzees). This is consistent with the diets of these species, which, in addition to being high in starch, are also likely to be high in tannins.[31] One study has provided evidence that cheek pouches in cercopithecine primates are important sites for the initial digestion of polysaccharides.[120] When a potato is inserted into the cheek pouch of a restrained bonnet macaque (*Macaca radiata*), more than 50% of its starch is converted into simpler sugars within five minutes, indicating that the high amylase levels in the primate cheek pouches are extremely effective at starch digestion.[120]

Box 1. Long-tailed macaques (*Macaca fascicularis*) on Sumatra with filled cheek pouches. Photo by Joram Berlowitz. (Color figure can be viewed at wileyonlinelibrary.com)

generally consumed by these species. Chimpanzees and bonobos feed on large amounts of ripe fruit and appear to have similarly low intake levels of starch.[51] Gorilla diets vary considerably across habitats, with some populations feeding on large amounts of fruit, while the diets of others are leaf-dominated.[52] However, compared to chimpanzee and bonobo diets, those of gorillas tend to include more structural carbohydrates, potentially including starch from roots and, presumably, higher tannin levels.[51,52] It is likely that high tannin levels are also included in the diet of orangutans, which, during times of fruit scarcity, may consume large amounts of seeds and cambium, both of which are rich in starch and tannins.[30,51,53]

Studies have indicated that tannins, which are characterized by an affinity to bind to proteins, inhibit salivary α-amylase in both humans and nonhumans.[43,54] In response to being fed a diet high in tannins, mice exhibited a significant increase in the expression of salivary α-amylase, presumably to counteract the inhibitory effects of the tannins. This suggests that having increased levels of this enzyme secreted in saliva may be an adaptive feature for species that consume large amounts of tannins.[43] An interesting avenue for future research would be to investigate salivary amylase expression in species with high-tannin diets. A good choice would be colobine monkeys, as the diet of these primates consists mostly of leaves and other herbaceous vegetation, as well as unripe fruit, all of which are presumed to contain high levels of tannins.[54] All cercopithecines that have been tested express high levels of amylase in their saliva. Indeed, the levels are often even higher than those in humans (see Box).[31]

To summarize, the expression of salivary α-amylase is found only in some mammalian taxa and appears to have evolved multiple times. The precise number and sequence of convergent evolutionary events is unclear at this time. Additional research is needed to delineate the full genetic basis of salivary α-amylase expression in all species. Further research should also include colobine monkeys and other nonprimate taxa with highly variable diets, such as the Chiroptera.

LACTASE

All mammals lactate and nurse their offspring with milk.[32] To digest their mothers' milk, young mammals produce the digestive enzyme lactase

in the small intestine. Lactase production is restricted to infancy; in most mammals, its activity begins to decline after the offspring is weaned. Some humans, however, continue to produce lactase throughout their lives and can successfully digest milk as adults.[55]

The main components of milk are water, lipids, carbohydrates, proteins, and salt, but the exact proportions of each varies from species to species.[32] Lactose, the principal carbohydrate in milk, is a disaccharide that is cleaved into the monosaccharides glucose and galactose by the enzyme lactase-phlorizin-hydrolase, also called lactase, found in the small intestine. While it is mainly known for its ability to digest the sugar lactose, lactase can also hydrolyze β-galactosides, phlorizin (found in the bark of some fruit trees), and several other β-glucosides.[56] Production of this enzyme is essential for young mammals because they need to be able to digest the lactose in their mother's milk, which they depend on for nutrition during the first part of their lives.[55] If mammals with small intestines that no longer produce lactase consume fresh milk, lactose passes to the colon undigested; there it may be fermented by bacteria, producing fatty acids and gases that cause flatulence and physical discomfort. Undigested lactose that passes into the colon can also cause diarrhea, which may be a much more serious problem, especially in environments lacking an adequate supply of safe drinking water.[55]

Like all other mammals, the majority of humans cease to produce lactase after infancy. The actual proportion of humans who are "lactase nonpersistent" is difficult to ascertain, but most studies suggest that it is around 65% worldwide.[57] The proportion of people who are lactase nonpersistent varies widely between different populations, from less than 10% in Northern Europe to more than 95% in Southern China (Fig. 2).[55] Some humans continue to produce lactase past infancy and throughout their lives, a condition referred to as "lactase persistence" (LP). Populations in which the majority of people exhibit this phenotype include Central and Northern Europeans, as well as various peoples in Africa and the Middle East. A common background for these individuals is that they descend from populations with a long history of pastoralism and fresh milk consumption.[5]

There are several problems with accurately determining the number of people who are lactase persistent rather than nonpersistent. First, difficulties and costs involved in accessing remote populations will lead to an overrepresentation of people from industrialized and Western countries, which may have a different frequency of the phenotype. Second, the noninvasive measures used to determine lactase activity in most studies may not be completely accurate. They involve administering a dose of lactose to a person, followed by measuring either the blood glucose response or the presence of hydrogen in the breath. However, the amount of lactose used in these tests is equivalent to that in 1-2 liters of cow's milk, which is significantly more than most people consume in one sitting and may lead to overdiagnosis of the lactase nonpersistent phenotype.[58] Furthermore, individuals who are genetically lactase persistent may lose the ability to produce the enzyme secondarily due to intestinal diseases.[58] These limitations should be kept in mind when discussing the relative proportions of each phenotype that have been measured in various populations.

The production of lactase is encoded by the gene *LCT*, which in humans is located on the long arm of chromosome 2.[58] To date, at least five independent single nucleotide polymorphisms (SNPs) have been identified that are associated with the LP trait: G-13907, T-13910, G-13915, G-14009, and C-14010 (Fig. 2).[58–62] The T-13910 allele is linked with LP in European, Indian, and Central Asian populations.[58,63,64] In African and Arabic populations, it is found only at very low frequencies, despite locally high prevalence of the LP phenotype.[58,65,66] Several alleles are associated with LP in Africa and the Arabian Peninsula. The complex history of human migrations and gene flow that have occurred in these regions is mirrored in the distribution of the LP alleles (Fig. 2).[5,67] The G-13915 variant likely originated on the Arabian peninsula and was spread to Africa by nomadic Arabian groups in the sixth or seventh century,[66,68] while an East African origin is most probable for the G-13907, G-14009 and C-14010 alleles (Table 2).[5,61,67]

Lactase persistence has evolved multiple times in humans, suggesting a strong selective pressure operating on this phenotype. In fact, several studies have shown that the *LCT* locus is under the strongest positive selection seen in humans so far[58,69] and it has become a textbook example of recent human adaptations. The evidence of both strong positive selection acting on the alleles associated with lactase persistence and multiple convergent evolution events show that there seems to be adaptive significance in the ability to digest fresh milk in adulthood.

Several authors have suggested that lactase persistence is an example of gene-culture coevolution: The cultural practice of dairying influenced the evolution and spread of the lactase persistence-causing allele and vice versa.[70] However, did dairying evolve in response to the evolution of lactase persistence or did lactase persistence evolve following the adoption of dairying practices? Most evidence suggests that pastoralism and the consumption of milk predates the emergence of lactase persistence. A recent study of a large sample of ancient Eurasian remains supports a relatively recent spread of the allele.[69] The earliest evidence of LP in this sample comes from an individual dated to 2450-2140 BCE.[69] Age estimates of the various lactase persistence alleles are also consistent with the hypothesis that the phenotype spread following the adoption of dairying practices.[5,12,58,66] These age estimates and archeological evidence for the advent of dairying are summarized in Table 2.

As noted, all of this provides evidence that consuming milk and being able to digest lactose has significant adaptive benefits. Several hypotheses have been proposed to

TABLE 2. Ages and Origins of LP Alleles and Archeological Evidence of the Advent of Dairying

	Estimates of Allele Origin (years BP)	Likely Location/ Population of Origin	Archeological Evidence for Advent of Dairying in Region of Origin
T-13910	~8000-9000[58] ~5000-12000[65] ~6256-8683[12]	Central Balkans[12]	Slaughtering age profiles of sheep, goats, and cattle suggest milk use ~11,000 years BP[116] Organic residues on pottery provide evidence for milk use by 9000 years BP[117]
G-13915	~4000[5,66]	Arabian Peninsula[5,66]	Domestication of the camel occurred ~6000 years BP[66]
G-13907	~5000[5]	Cushitic Speakers/Eastern Ethiopia[5]	Stable isotope analyses of potsherds indicate dairying in Saharan Africa began by 7000 years BP[118]
G-14009		Ethiopia/East Africa (?)[62]	
C-14010	~2700-6800[58]	Cushitic Speakers/East Africa[5]	

explain the adaptive significance of digesting fresh milk. Flatz and Rotthauwe[71] proposed the calcium assimilation hypothesis, arguing that being able to digest lactose is particularly important in high latitudes, where levels of ultraviolet light are low. Exposure to UV light is necessary for mammals to synthesize vitamin D, a necessary step for the absorption of calcium, which is essential for bone health and growth. Because milk provides both vitamin D and calcium, these authors suggest that the ability to digest lactose and consume fresh milk without adverse effects was an important adaptation for humans living in northern Europe.[71] Since the hypothesis was proposed, it has become clear that the lactase persistence phenotype is widespread in additional populations not living in high latitudes, so there are likely other adaptive benefits to dairying in addition to calcium assimilation.[72] Milk and milk products are high-calorie foods and, as opposed to plants, which are only available seasonally, are often available year-round. Fresh milk is also a valuable source of clean and uncontaminated fluids, which may be particularly important in arid, semidesert environments.[12] Camels, for example, can survive in extremely arid conditions by metabolizing the fat stored in their humps. Their milk could be an important source of fluids for the humans keeping them.

There is limited evidence that lactase may have beneficial digestive properties in addition to the hydrolysis of lactose. The lactase persistence phenotype was present in 50% of Hadza hunter-gatherers, even though this population is not known to consume milk.[58] This may mean that the Hadza descended from a pastoralist population or that lactase aids in the digestion of another food resource.[58] Lactase does catalyze the hydrolysis of phlorizin, a bitter glycoside found in the roots and bark of plants in the *Rosaceae* family, which is native to Tanzania, where the Hadza live.[5,58]

The convergent evolution of lactase persistence in multiple human populations is a classic example of the strong selective pressure that diet of an organism can represent. Worldwide correlations of the lactase persistence phenotype and the known genotypes suggest that we still have not found all of the underlying genetic mechanisms of lactase persistence and that more research is needed (Fig. 2).[5]

CHITINASE

Chitin is one of the most common structural carbohydrates in nature, making up 58%-85% of the exoskeletons of arthropods and 8%-60% of the cell walls of fungi.[1] All primates include some insects in their diet, whether through accidental consumption or active insectivory (Fig. 3),[73] and fungi are a dietary staple of some New World monkeys, as well as humans.[74,75] It is unclear, however, whether any primates are actually able to digest chitin.

Chitinolytic enzymes have been isolated from the gastric mucosa of a capuchin monkey (*Cebus capucinus*)[76] and a potto (*Perodicticus potto*).[77] However, such experiments are extremely invasive and do not allow for differentiation between enzymes that are endogenously produced or of dietary origin, since chitinases are present in many plant resources.[1] Despite a lack of concrete evidence, it has been assumed that insectivorous animals, including primates, are able to synthesize chitinolytic enzymes to digest the exoskeletons of insects or, alternatively, have gut microbes that are able to do so.[74] Genetic research has identified a family of chitinase and chitinase-like proteins in mammals that are presumed to have arisen by gene duplication and evolved to fulfill a variety of functions,[78,79] including protection from pathogens and aiding in the hydrolysis of polysaccharides.[78]

Discovered in 2000, acidic mammalian chitinase (AMCase) is a chitinase found in mammals. It is structurally very similar to other chitinases, but functions optimally at a much lower pH and was named accordingly.[80] AMCase seems to play an important role as a digestive enzyme in mammals, in addition to being involved in pathogen defense.[78]

An Italian study found varying levels of AMCase activity in the gastric juices of 20 out of 25 human participants.[81] The AMCase purified from participants' gastric juice was able to hydrolyze fluorescein isothiocyanate-chitin in an experimental setting, leading the authors to hypothesize not only that it represents an

Figure 3. A cotton-top tamarin (*Saguinus oedipus*) feeds on an insect. Whether primates produce enzymes that can digest insects' chitinous exoskeletons has not yet been conclusively determined. Photo by Mickey Samuni-Blank via Wikimedia Commons. (Color figure can be viewed at wileyonlinelibrary.com)

adaptation for the digestion of arthropods, but that the varying levels, as well as absence in some participants, were due to decreased consumption of insects in the Western diet.[81] While this is an intriguing hypothesis, Muzzarelli and colleagues[61] have described an unpublished follow-up study, which found that human gastric juice containing high levels of AMCase was unable to digest the wings of the bluebottle fly *(Calliphora vomitoria)*.[82]

More conclusive evidence for a digestive function of AMCase comes from studies of mice and bats. Murine AMCase is optimally active at a low pH level and even remains functional in extremely acidic conditions, such as those found in the stomach, suggesting that it has been adapted for a digestive function.[80] Although it was previously thought that any chitinolytic activity in the digestive system of mammals was due to chitinases expressed by intestinal bacteria,[83] both mice and bats have recently been shown to secrete AMCase from the chief cells at the base of the gastric glands, where other digestive enzymes are also secreted.[84] The diets of wild mice often include significant amounts of insects and many bat species feed almost exclusively on insects.[84] While both bats and primates have been shown to harbor microorganisms capable of degrading chitin,[9,83] producing an endogenous digestive enzyme for the hydrolysis of chitin would allow faster and more efficient digestion of arthropod prey, providing an important adaptive benefit for insectivorous species.

In addition to rodents, bats, and humans, only macaques have so far been investigated for AMCase activity. Using the human AMCase gene, *CHIA* as a guide, researchers were able to identify homologous genes, named *mCHIA*, in the rhesus (*Macaca mulatta*) and long-tailed macaque (*M. fascicularis*) genomes.[85] A comparison of enzymatic activity of the proteins cloned from the macaque and human *CHIA* genes showed that both enzymes are most active at pH 5.0, but remain functional at pH 2.0. Unlike human AMCase, the enzyme cloned from the macaque sequence also remained active at pH 8.0, giving it a much broader pH range.[85] The most striking difference was that the macaque AMCase (MACase) was 50 times more efficient than human AMCase at hydrolyzing a chitin substrate.[85]

Like AMCase in humans, mice, and bats, MACase is expressed at high levels in the stomachs of macaques. The finding that MACase appears to be significantly more efficient at chitin digestion than is human AMCase raises the exciting possibility that MACase is an important digestive enzyme for nonhuman primates. Until recently, it was unclear whether primates or other mammals produced endogenous chitinases in their digestive systems, because an exogenous or microbial origin of any chitinolytic activity could not be excluded.[1,83] Furthermore, almost intact exoskeletons are reportedly found in the feces of primates,[86] which has led many to presume that they are indigestible.[87] The discovery of the MACase gene family has provided a new avenue for the study of dietary adaptations in insectivores.

Future research should investigate the *mCHIA* gene across the primate order to test whether *mCHIA* might exhibit genetic variation, such as higher copy numbers, in more insectivorous primate species. Such variation would provide evidence that MACase is important in the digestive system of primates that rely on chitin-containing food resources, such as arthropods and fungi.

PEPSINOGENS OR PEPSINS

Pepsins are enzymes that provide the first step in the digestion of proteins, an essential component of the diet of all animals. In order to avoid any unwanted digestion of the host tissue, all proteolytic digestive enzymes are secreted as inactive precursors known as zymogens. The zymogens are converted into their active form in the gastric lumen.

Pepsinogen or Pepsin A

Pepsinogen A is the zymogen of pepsin A, the most abundant gastric protease in most adult mammals, which appears to be highly polymorphic in primates.[88,89] This enzyme, secreted by the chief cells of the gastric mucosa, is maximally active at a pH of about 2, in accordance with its role in the acidic environment of the stomach.[90] Pepsin A hydrolyzes the peptide bonds of proteins,

creating smaller chains of amino acids that can then be further digested by the enzymes trypsin, chymotrypsin, and elastase.[1]

Pepsin A has been shown to be highly polymorphic at both the protein level and genetic level in various primates. Humans have a cluster of three genes that are known to be present in variable copy numbers.[91] The other great apes exhibit even greater variation in pepsinogen A isozymogens than do humans. Narita and colleagues[92] purified numerous forms of pepsinogen A from the gastric mucosa of a gibbon (*Hylobates lar*), an orangutan (*Pongo pygmaeus*), a gorilla (*Gorilla gorilla*), and a chimpanzee (*Pan troglodytes*). The number of pepsinogen A isozymogens found ranged from seven in the gorilla to eight in the gibbon, thirteen in the chimpanzee, and fourteen in the orangutan.[92] A follow-up study by the same group predicted that five and three genes respectively encode pepsinogen A1 and A2 in the orangutan.[89] These genes are also present in the chimpanzee, while the human pepsinogen genes are most like those for pepsinogen A1. This led the authors to conclude that pepsinogen A diverged into types A1 and A2 in the hominoid lineage, but that the latter was lost in humans.[89] It has been suggested that the extreme multiplicity of pepsinogen A in great apes is related to a dietary reliance on herbaceous material.[92] However, while gorillas and orangutans may consume relatively high amounts of foliage, chimpanzees and gibbons are considered to be more frugivorous.

Macaques express four closely related forms of pepsinogen A that are apparently encoded by four separate genes.[88,93] A study of Japanese macaques (*Macaca fuscata*) found that relative expression of the four pepsinogen A forms varied with age[88]; this was similar to what had previously been found in rabbits.[94] This increase in pepsinogen A production commonly occurs among mammals and is likely related to the changing digestive demands associated with weaning and the adoption of an adult diet.[95,96]

Platyrrhines differ from other primates in that multiple forms of pepsinogen A do not appear to be typical in this group. Of the four species of New World monkeys tested, only capuchin monkeys (*Cebus apella*) had two isozymogens of pepsinogen A; the common marmoset (*Callithrix jacchus*), squirrel monkey (*Saimiri sciureus*), and cotton-top tamarin (*Saguinus oedipus*) all had a single form of pepsinogen A.[97] Whether this pattern is related to the dietary ecologies of the platyrrhines is unclear. More research is needed to determine if these results are also found in the rest of the New World monkey species. As a group, the platyrrhines tend to rely more heavily on insects than foliage for protein, while the opposite is generally seen in the catarrhines.[30] More insectivorous species may not require multiple pepsinogens to digest protein, but rather express chitinolytic enzymes for the digestion of insect exoskeletons. Future research on both pepsinogens and chitinases in New World monkeys may provide additional insight into the relationship between these digestive enzymes and dietary ecology.

It is unclear, however, whether any primates are actually able to digest chitin.

Many primate species, including most of the cercopithecines and all of the strepsirrhines, have not been tested for genetic variation relating to pepsinogen A. However, as discussed, some interesting patterns have already emerged in the great apes and the macaques.[88,89,93]

PROCHYMOSIN OR CHYMOSIN

Prochymosin, the zymogen of chymosin, is secreted by the gastric mucosa of neonate mammals. Because of its use in the cheesemaking industry, chymosin is often better known as rennin. Chymosin was first discovered in neonate cattle and, as noted, its ability to clot milk has long been used by humans in the production of cheese.[1,98] Indeed, its importance to the dairy industry has led to the publication of many articles regarding the clotting activity of chymosin. Its physiological role in animal digestive systems is less well understood.[99]

As suggested by its function in the clotting of milk, chymosin is common to a variety of mammalian species, including, but most likely not limited to cows, zebras, horses, seals, cats, pigs, kangaroos, opossums, porcupines, and rats. In almost all of these species it is found only in the stomachs of fetuses and neonates.[99] With one exception, chymosin is absent from adult mammals. Experiments with rats and pigs have shown that there appears to be a switch in proteases around the time of weaning.[96] While pepsinogen A, in the pig, and progastricsin, in the rat, were almost undetectable in fetuses and neonates, chymosin was expressed at high levels in the first phase of development. When chymosin production began to cease in rats seven days after birth, progastricsin expression increased rapidly and continued to be expressed strongly throughout adulthood.[96] Similarly, porcine stomachs reduced production of chymosin 5-10 days after birth, while pepsinogen A production began to increase one week after birth and showed a rapid increase at three weeks of age.[95]

Newborn Japanese macaques (*Macaca fuscata*) were not shown to produce chymosin or any other neonate-specific digestive enzymes although, as noted, relative expression of different pepsinogens changes with age.[88] Human infants also lack chymosin and the human gene (*hPC*) for this enzyme appears to be nonfunctional.[99,100] It is plausible that the *hPC* pseudogene is a shared trait of the catarrhines. New World primates, on the other hand, differ from both other primates and other mammals in their expression of chymosin. Platyrrhines express chymosin and furthermore, express it in adulthood. Prochymosin was

purified from the gastric mucosa of a common marmoset (*Callithrix jacchus*), a cotton-top tamarin (*Saguinus oedipus*), a squirrel monkey (*Saimiri sciureus*), and a capuchin monkey (*Cebus apella*), suggesting that this trait is common to all platyrrhines. More research is needed to confirm this and to explain why this enzyme persists into adulthood.[97]

Compared to pepsin, chymosin tends to exhibit weaker general proteolytic activity and approximately similar milk clotting activity.[99] The latter finding suggests that chymosin is not essential for the digestion of milk in neonate mammals and begs the question of why chymosin is expressed in neonates and only gradually replaced by pepsin A.

It has been suggested that chymosin is advantageous during the postnatal transfer of immunoglobulins, which are contained in the colostrum.[95,101] Experiments with porcine pepsin have shown that this protease cleaves immunoglobulins into smaller fragments. It is likely that pepsin from other species has the same effect.[95] Considering that the general proteolytic activity of chymosin is weaker than that of other proteases, it presumably limits damage to the immunoglobulins while retaining the ability to clot milk. The postnatal transfer of immunoglobulins through the colostrum is critically important to many mammalian species in which immunoglobulins are not transferred through the placenta before birth. Placental transfer of immunity occurs in primates, rabbits, and possibly other mammals,[88] but species in which neonatal chymosin activity has been demonstrated tend to rely on postnatal transfer through the colostrum.[95] The presence of trypsin inhibitors in the colostrum provides further evidence not only that proteases are disadvantageous to the transfer of immunoglobulins, but that chymosin may have evolved as a neonate-specific digestive enzyme that effectively clots milk while, at the same time, having a low enough general proteolytic activity that it does not damage immunoglobulins.[95,99]

Kageyama[90] hypothesizes that most infant primates do not express chymosin because immunoglobulins are transferred via the placenta before birth rather than through their mothers' milk. The vast literature on immune markers in human breast milk (for a review, see Agarwal and colleagues[102]) and studies linking breastfeeding to improved infant health[103] cast doubts on the validity of this hypothesis.

Both humans and rabbits transfer some immunoglobulins through the placenta before birth, which may explain why the neonates of both taxa lack chymosin. Rabbits, however, do express a different neonate-specific protease, pepsinogen F.[94] Kageyama and colleagues[94] suggest that the presence of a neonate-specific pepsin might be due to the high protein content of rabbit milk. Because primate milk is comparatively low in protein and high in lactose, specific proteases may not be necessary to digest it efficiently.[88] While this certainly is a reasonable explanation for the lack of chymosin in humans and macaques, it makes the finding that adult New World monkeys express chymosin even more puzzling. More research is needed to elucidate the adaptive significance of this enzyme in platyrrhine digestion. A more thorough survey of chymosin expression throughout the platyrrhines may reveal patterns of variation that could provide clues to a potential adaptive benefit of this digestive enzyme.

The lysozymes of ruminants and colobines are an excellent example of convergent evolution: They have undergone extremely similar changes without sharing a common origin.

RIBONUCLEASE AND LYSOZYME

The leaf-eating primates, the colobines, are often compared to ruminants because they have evolved quite similar digestive systems. Both colobines and ruminants have sacculated forestomachs wherein bacteria break down foliage consumed. The animal, in turn, receives important nutrients from digesting these bacteria once they have entered the true stomach and small intestine.[30] Two enzymes that are involved in digesting the forestomach bacteria of colobines and ruminant artiodactyls are pancreatic ribonuclease and lysozyme. These enzymes perform other, nondigestive functions in most animals, but have been adapted for a digestive function in foregut-fermenters.[104,105]

Lysozyme is usually expressed in macrophages, where it digests the peptidoglycan cell walls of bacteria as part of the host animal's immune defense system.[106] In ruminant artiodactyls and colobines, however, lysozyme is expressed at high levels in the stomach and has evolved to be better suited for the task of digesting foregut bacteria. The lysozymes of ruminants and colobines are an excellent example of convergent evolution: They have undergone extremely similar changes without sharing a common origin.[107] As opposed to ruminant artiodactyls, which have up to ten different genes for lysozyme, colobines have retained the single gene that occurs in most mammals.[106,107] This gene has accumulated nine amino acid substitutions,[107] which appear to create functional differences that improve the resulting protein's performance as a digestive enzyme. In order to function in the acidic environment of the stomach, ruminant and colobine digestive lysozyme has an optimum pH of 5.0, as opposed to nondigestive lysozymes, which function best at a neutral pH.[108] In addition, digestive lysozyme is more resistant to digestion by pepsin. Stomach lysozymes of the cow (*Bos taurus*) and the langur monkey (*Presbytis entellus*) retained around 75% of their activity after an hour of exposure to pepsin, whereas the lysozymes of rats and humans retained less than 7.5% of their activity.[108]

Like lysozyme, ribonuclease has been exapted for a digestive function in ruminants and colobines. However, the digestive function of ribonuclease

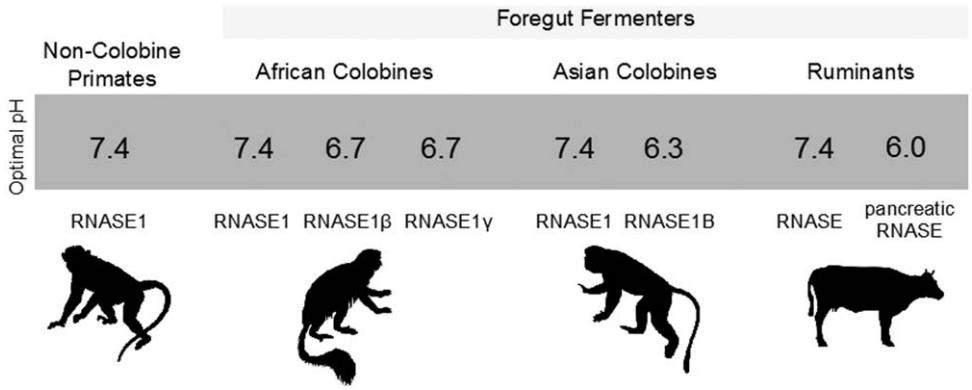

Figure 4. The independently duplicated ribonucleases in Asian and African colobines and ruminants exhibit parallel functional changes, including similar optimal pH.[105,109]

was not only acquired in parallel by ruminants and leaf-monkeys, but actually evolved independently at least twice within the colobine lineage, once in Asian and once in African colobines (Fig. 4).[105,109,110] Gene duplications were followed by parallel functional changes in the daughter genes, but these changes, unlike those in digestive lysozyme, are caused by different amino acid substitutions in the ruminants versus the colobines.

All primates share one gene for pancreatic ribonuclease called *RNASE1*. In numerous Asian colobines, a second pancreatic ribonuclease gene, *RNASE1B*, has been found.[110,111] It is presumed to be common to all Asian colobines, since its origin around 3.5 million years ago coincides with the timing of the radiation of this taxon.[111] In the African colobines, *RNASE1* was duplicated twice, resulting in two additional pancreatic ribonuclease genes, *RNASE1β* and *RNASE1γ*.[109,110] *RNASE1B* and the common ancestor of *RNASE1β* and *RNASE1γ* share three amino acid substitutions that are not found in the parent gene and have been shown to lower the optimal pH of the enzyme. The original ribonuclease, RNASE1 is optimally active in all primates at a pH of 7.4, while the Asian colobine enzyme, RNASE1B, and the African colobine enzymes, RNASE1β and RNASE1γ, respectively have optimal pH levels of 6.3 and 6.7 (Fig. 4).[109,111] The convergence of this functional change is strong evidence that it represents an adaptation for bacteriolytic activity in the small intestine of colobines, which has a pH between 6 and 7.[109]

As opposed to the colobine-specific ribonucleases, ribonuclease 1 is found in other tissues besides the pancreas. There it degrades double-stranded RNA and is hypothesized to have an antiviral function, although its role is not completely understood.[112] The enzymes RNASE1B, RNASE1β and RNASE1γ have lost the ability to degrade double-stranded RNA, suggesting that they have also lost the original physiological role of the parent enzyme in favor of specialization for a function in the colobine digestive system.[109]

Interestingly, *RNASE1* duplications have also been found in carnivores and bats, animals that are not foregut fermenters.[113,114] In the superfamily Musteloidea (order Carnivora), *RNASE1* was independently duplicated in four families: the Procyonidae (raccoons), Ailuridae (red pandas), Mephitidae (skunks), and Mustelidae (weasels).[115] In bats (order Chiroptera), *RNASE1* duplications are present in five species of the Vespertilionidae (*Myotis lucifugus*, *M. altarium*, *M. ricketti*, *Ia io*, and *Murina leucogaster*) and two species of the Molossidae (*Tadarida brasiliensis* and *T. insignis*).[114] All of these bat species are insectivorous, suggesting the possibility of a dietary adaptation. At this point, however, there is no conclusive evidence to support this hypothesis.[114] Likewise, more research is needed to understand the functional significance of *RNASE1* duplications in carnivores.[115] Nevertheless, the parallel evolution of a digestive system and the convergent adaptation of digestive enzymes in ruminants and colobines illustrate the power that common selective pressures can have, especially when related to diet.

CONCLUSION AND DIRECTIONS FOR FUTURE RESEARCH

Digestive enzymes are important in the primate digestive system and provide significant adaptive benefits. This is demonstrated by the fact that lactase persistence, salivary amylase, digestive ribonuclease, and lysozyme have all evolved independently in response to convergent selective pressures and have done so not just twice, but often multiple times.

While the research reviewed here has shown the powerful impact that digestive enzyme variation can have, many gaps remain in our knowledge of primate and, more broadly, mammalian digestive enzymes. Although primates are naturally the focus of biological anthropology, the evolutionary and ecological significance of a trait is best understood when it can be placed in a comparative context. Digestive enzyme variation in other mammals presents the relevant evolutionary context within which primate variation can be evaluated.

Among primates, strepsirrhines (especially lemurs) and platyrrhines are the two groups that have been the most neglected when it comes to digestive enzyme research. This gap

needs to be corrected in future studies. Lemurs and New World monkeys evolved following two dispersal events in which species rapidly diverged and filled the available dietary niches. The various dietary ecologies that are represented in these two primate radiations certainly posed a series of unique selective pressures that is likely reflected in their digestive enzymes. Furthermore, the larger phylogenetic context of digestive enzyme variation is essential to understand what traits are ancestral or derived, as well as to our ability to evaluate evolutionary hypotheses.

For example, additional research on strepsirrhines and platyrrhines may explain why the neonate-specific enzyme chymosin persists throughout life in New World monkeys. The hypotheses put forward thus far are limited. Without a more complete picture of which primate taxa express chymosin in adulthood, it is impossible to propose and test any alternatives.

The presence or absence of salivary amylase has yet to be confirmed in both colobines and strepsirrhines. Again, knowing whether species in these groups express amylase in their saliva may elucidate the evolutionary history of this trait. Based on our current understanding, it is unlikely that strepsirrhines have salivary amylase, but the genetic mechanism that causes amylase expression is only partially resolved in primates. Cercopithecines have higher levels of salivary amylase than do humans, even though their gene lacks the retroviral insertion that presumably is necessary. Additional research on Old World monkeys and strepsirrhines could show whether an alternative mechanism can confer salivary amylase expression.

Despite all of the work that has been done on human lactase persistence and its obvious adaptive benefits, open questions remain. As discussed here, it is highly likely that not all alleles conferring lactase persistence have been identified. For instance, as can be seen in Figure 1, levels of the lactase persistence phenotype are high in West Africa, although none of the known LP-alleles appear to be found there. This implies that an alternative haplotype is present that allows adults in this population to digest milk.

More research is also needed to elucidate the evolution of chitinases. Because most previous research on chitinase has been done on bats, a comparative approach will be fruitful here. Dietary specializations are exceptionally diverse across the Chiroptera and the adaptations that have accompanied the evolution of these different diets are relatively well studied compared to those among primates. Comparing the chitinases of insectivorous bats and insectivorous primates, for instance, may uncover shared adaptations for the digestion of insect exoskeletons.

Once it is more fully understood what genetic variation is present in the digestive enzymes of primates and other mammals and how this variation is phylogenetically distributed, the next step will be to study the functional effects of any polymorphisms. While such studies can be invasive and thus difficult to do with living primates, *in-vitro* experiments may initially be a useful alternative.

A more complete understanding of the enzymes we produce, how they evolved, and what their limits are may help us treat and prevent diseases or obesity. Moreover, knowledge of inter- and intraspecific variation in primate digestive enzymes can provide insight into the evolution of dietary ecologies and adaptations of primates past and present, as well as give us a better grasp of the true digestive capabilities of different species.

ACKNOWLEDGMENTS

Thanks to Frederick Foster, Alexander Pritchard, Rob Scott and Erin Vogel for comments on earlier drafts of this paper. I am grateful for the constructive comments offered by two anonymous reviewers and Dr. John Fleagle.

REFERENCES

1 Stevens CE, Hume ID. 1995. Comparative physiology of the vertebrate digestive system, 2nd ed. Cambridge: Cambridge University Press.

2 Graf D, Di Cagno R, Fåk F, et al. 2015. Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis 26:26164.

3 Perry GH, Dominy NJ, Claw KG, et al. 2007. Diet and the evolution of human amylase gene copy number variation. Nat Genet 39:1256–1260.

4 Axelsson E, Ratnakumar A, Arendt M-L, et al. 2013. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature 495:360–364.

5 Ranciaro A, Campbell MC, Hirbo JB, et al. 2014. Genetic origins of lactase persistence and the spread of pastoralism in Africa. Am J Hum Genet 94:496–510.

6 Yatsunenko T, Rey FE, Manary MJ, et al. 2012. Human gut microbiome viewed across age and geography. Nature 486:222–227.

7 Amato KR, Yeoman CJ, Kent A, et al. 2013. Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. ISME J 7:1344–1353.

8 O'Sullivan A, He X, McNiven EMS, et al. 2013. Early diet impacts infant rhesus gut microbiome, immunity, and metabolism. J Proteome Res 12:2833–2845.

9 Macdonald C, Barden S, Foley S. 2013. Isolation and characterization of chitin-degrading micro-organisms from the faeces of Goeldi's monkey, *Callimico goeldii*. J Appl Microbiol 116:52–59.

10 Albenberg LG, Wu GD. 2014. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 146:1564–1572.

11 Muegge BD, Kuczynski J, Knights D, et al. 2011. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332:970–974.

12 Itan Y, Powell A, Beaumont MA, et al. 2009. The origins of lactase persistence in Europe. PLoS Comput Biol 5:e1000491.

13 Fleagle JG, Gilbert CC. 2006. The biogeography of primate evolution: the role of plate tectonics, climate and chance. In: Lehman SM, Fleagle JG, editors. Primate biogeography. New York, NY: Springer. p 375–418.

14 Rosenberger AL. 1992. Evolution of feeding niches in New World monkeys. Am J Phys Anthropol 88:525–562.

15 Norconk MA, Wright BW, Conklin-Brittain NL, et al. 2009. Mechanical and nutritional properties of food as factors in platyrrhine dietary adaptations. In: Garber PA, Estrada A, Bicca-Marques JC, et al., editors. South American primates. New York, NY: Springer. p 279–319.

16 Nash LT. 1986. Dietary, behavioral, and morphological aspects of gummivory in primates. Am J Phys Anthropol 29:113–137.

17 Janson CH, Boinski S. 1992. Morphological and behavioral adaptations for foraging in generalist primates: the case of the cebines. Am J Phys Anthropol 88:483–498.

18 Kinzey WG. 1992. Dietary and dental adaptations in the Pitheciinae. Am J Phys Anthropol 88:499–514.

19 Aiello LC, Wheeler P. 1995. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. Curr Anthropol 36:199–221.

20 Rothman JM, Raubenheimer D, Chapman CA. 2011. Nutritional geometry: gorillas prioritize non-protein energy while consuming surplus protein. Biol Lett 7:847–849.

21 Felton AM, Felton A, Raubenheimer D, et al. 2009. Protein content of diets dictates the

daily energy intake of a free-ranging primate. Behav Ecol 20:685–690.

22 Johnson CA, Raubenheimer D, Rothman JM, et al. 2013. 30 days in the life: daily nutrient balancing in a wild chacma baboon. PLoS ONE 8:e70383–e70387.

23 Lambert JE, Rothman JM. 2015. Fallback foods, optimal diets, and nutritional targets: primate responses to varying food availability and quality. Annu Rev Anthropol 44:493–512.

24 Simmen B, Tarnaud L, Hladik A. 2012. Leaf nutritional quality as a predictor of primate biomass: further evidence of an ecological anomaly within prosimian communities in Madagascar. J Trop Ecol 28:141–151.

25 Garber PA, Righini N, Kowalewski MM. 2014. Evidence of alternative dietary syndromes and nutritional goals in the genus Alouatta. In: Kowalewski MM, Garber PA, Cortés-Ortiz L, et al., editors. Howler monkeys. New York, NY: Springer. p 85–109.

26 Mertl-Millhollen AS, Moret ES, Felantsoa D, et al. 2003. Ring-tailed lemur home ranges correlate with food abundance and nutritional content at a time of environmental stress. Int J Primatol 24:969–985.

27 Amato KR, Garber PA. 2014. Nutrition and foraging strategies of the black howler monkey (*Alouatta pigra*) in Palenque National Park, Mexico. Am J Primatol 76:774–787.

28 Karasov WH, Douglas AE. 2013. Comparative digestive physiology. Compr Physiol 3:741–783.

29 Stephen AM. 1991. Starch in human nutrition. Can J Physiol Pharmacol 69:54–55.

30 Lambert JE. 1998. Primate digestion: interactions among anatomy, physiology, and feeding ecology. Evol Anthropol 7:8–20.

31 Mau M, Südekum KH, Johann A, et al. 2010. Indication of higher salivary alpha-amylase expression in hamadryas baboons and geladas compared to chimpanzees and humans. J Med Primatol 39:187–190.

32 Meisler MH, Ting CN. 1993. The remarkable evolutionary history of the human amylase genes. Crit Rev Oral Biol Med 4:503–509.

33 Chauncey HH, Henrigues BL, Tanzer JM. 1963. Comparative enzyme activity of saliva from the sheep, hog, dog, rabbit, rat, and human. Arch Oral Biol 8:615–627.

34 Samuelson LC, Phillips RS, Swanberg LJ. 1996. Amylase gene structures in primates: retroposon insertions and promoter evolution. Mol Biol Evol 13:767–779.

35 Behringer V, Borchers C, Deschner T, et al. 2013. Measurements of salivary alpha amylase and salivary cortisol in hominoid primates reveal within-species consistency and between-species differences. PLoS ONE 8:e60773.

36 Ting CN, Rosenberg MP, Snow CM, et al. 1992. Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene. Gene Dev 6: 1457–1465.

37 Kilpatrick CW. 1996. An ancient duplication of the salivary amylase locus in Arvicoline rodents. Biochem Syst Ecol 24:309–318.

38 Ogunbiyi OA, Okon EE. 1976. Studies on the digestive enzymes of the African fruit bat *Eidolon helvum* (Kerr). Comp Biochem Physiol A Comp Physiol 55:359–361.

39 Dumont ER, Etzel K, Hempel D. 1999. Bat salivary proteins segregate according to diet. Mammalia 63:159–166.

40 Buchholz RH, Wells PH, Conaway CH. 1958. Digestive enzymes of mole, bat and rat. J Mammal 39:452–454.

41 Schondube JE, Herrera-M LG, Martinez del Rio C. 2001. Diet and the evolution of digestion and renal function in phyllostomid bats. Zoology (Jena) 104:59–73.

42 Mandel AL, Peyrot des Gachons C, Plank KL, et al. 2010. Individual differences in AMY1 gene copy number, salivary α-amylase levels, and the perception of oral starch. PLoS ONE 5: e13352.

43 da Costa G, Lamy E, Capela e Silva F, et al. 2008. Salivary amylase induction by tannin-enriched diets as a possible countermeasure against tannins. J Chem Ecol 34:376–387.

44 Read NW, Welch IM, Austen CJ, et al. 1986. Swallowing food without chewing; a simple way to reduce postprandial glycaemia. Br J Nutr 55:43–47.

45 Mandel AL, Breslin PAS. 2012. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. J Nutr 142:853–858.

46 Falchi M, El-Sayed Moustafa JS, Takousis P, et al. 2014. Low copy number of the salivary amylase gene predisposes to obesity. Nat Genet 46:492–497.

47 Carpenter D, Dhar S, Mitchell LM, et al. 2015. Obesity, starch digestion and amylase: association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. Hum Mol Gen 24:3472–3480.

48 Usher CL, Handsaker RE, Esko T, et al. 2015. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. Nat Genet 47:921–925.

49 Perry GH, Kistler L, Kelaita MA, et al. 2015. Insights into hominin phenotypic and dietary evolution from ancient DNA sequence data. J Hum Evol 79:55–63.

50 Wilson GM. 2005. Identification by full-coverage array CGH of human DNA copy number increases relative to chimpanzee and gorilla. Genome Res 16:173–181.

51 Hohmann G. 2009. The diets of non-human primates: frugivory, food processing, and food sharing. In: Hublin JJ, Richards MP, editors. The evolution of hominin diets. Dordrecht, The Netherlands: Springer. p 1–14.

52 Rothman JM, Plumptre AJ, Dierenfeld ES, et al. 2007. Nutritional composition of the diet of the gorilla (*Gorilla beringei*): a comparison between two montane habitats. J Trop Ecol 23: 673–682.

53 Knott CD. 1998. Changes in orangutan caloric intake, energy balance, and ketones in response to fluctuating fruit availability. Int J Primatol 19:1061–1079.

54 Kandra L, Gyémánt G, Zajácz Á, et al. 2004. Inhibitory effects of tannin on human salivary alpha-amylase. Biochem Biophys Res Commun 319:1265–1271.

55 Ingram CJE, Mulcare CA, Itan Y, et al. 2009. Lactose digestion and the evolutionary genetics of lactase persistence. Hum Genet 124: 579–591.

56 Ehrenkranz JRL, Lewis NG, Kahn CR, et al. 2005. Phlorizin: a review. Diabetes Metab Res Rev 21:31–38.

57 Itan Y, Jones BL, Ingram CJ, et al. 2010. A worldwide correlation of lactase persistence phenotype and genotypes. BMC Evol Biol 10:36.

58 Tishkoff SA, Reed FA, Ranciaro A, et al. 2007. Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 39:31–40.

59 Enattah NS, Sahi T, Savilahti E, et al. 2002. Identification of a variant associated with adult-type hypolactasia. Nat Genet 30:233–237.

60 Ingram CJE, Elamin MF, Mulcare CA, et al. 2007. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? Hum Genet 120:779–788.

61 Ingram CJE, Raga TO, Tarekegn A, et al. 2009. Multiple rare variants as a cause of a common phenotype: several different lactase persistence associated alleles in a single ethnic group. J Mol Evol 69:579–588.

62 Jones BL, Raga TO, Liebert A, et al. 2013. Diversity of lactase persistence alleles in Ethiopia: signature of a soft selective sweep. Am J Hum Genet 93:538–544.

63 Gallego Romero I, Basu Mallick C, Liebert A, et al. 2012. Herders of Indian and European cattle share their predominant allele for lactase persistence. Mol Biol Evol 29:249–260.

64 Heyer E, Brazier L, Ségurel L, et al. 2011. Lactase persistence in central Asia: phenotype, genotype, and evolution. Hum Biol 83:379–392.

65 Enattah NS, Trudeau A, Pimenoff V, et al. 2007. Evidence of still-ongoing convergence evolution of the lactase persistence T-13910 alleles in humans. Am J Hum Genet 81:615–625.

66 Enattah NS, Jensen TGK, Nielsen M, et al. 2008. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. Am J Hum Genet 82:57–72.

67 Jones BL, Oljira T, Liebert A, et al. 2015. Diversity of lactase persistence in African milk drinkers. Hum Genet 134:917–925.

68 Priehodová E, Abdelsawy A, Heyer E, et al. 2014. Lactase persistence variants in Arabia and in the African Arabs. Hum Biol 86:7–18.

69 Mathieson I, Lazaridis I, Rohland N, et al. 2015. Genome-wide patterns of selection in 230 ancient Eurasians. Nature 528:499–503.

70 Feldman MW, Laland KN. 1996. Gene-culture coevolutionary theory. Trends Ecol Evol 11:453–457.

71 Flatz G, Rotthauwe H. 1973. Lactose nutrition and natural selection. Lancet 302:76–77.

72 Sverrisdóttir OÓ, Timpson A, Toombs J, et al. 2014. Direct estimates of natural selection in Iberia indicate calcium absorption was not the only driver of lactase persistence in Europe. Mol Biol Evol 31:975–983.

73 Raubenheimer D, Rothman JM. 2013. Nutritional ecology of entomophagy in humans and other primates. Annu Rev Entomol 58:141–160.

74 Hanson AM, Hall MB, Porter LM, et al. 2006. Composition and nutritional characteristics of fungi consumed by *Callimico goeldii* in Pando, Bolivia. Int J Primatol 27:323–346.

75 Hilário RR, Ferrari SF. 2011. Why feed on fungi? The nutritional content of sporocarps consumed by buffy-headed marmosets, *Callithrix flaviceps* (Primates: Callitrichidae), in southeastern Brazil. J Chem Ecol 37:145–149.

76 Jeuniaux C, Cornelius C. 1978. Distribution and activity of chitinolytic enzymes in the digestive tract of birds and mammals. In: Muzzarelli RAA, Pariser ER, editors. Proceedings of the First International Conference on Chitin/Chitosan. p 542–549.

77 Cornelius C, Dandrifosse G, Jeuniaux C. 1976. Chitinolytic enzymes of the gastric mucosa of *Perodicticus potto* (primate prosimian): purification and enzyme specificity. Int J Biochem 7:445–448.

78 Boot RG, Bussink AP, Verhoek M, et al. 2005. Marked differences in tissue-specific expression of chitinases in mouse and man. J Histochem Cytochem 53:1283–1292.

79 Seibold MA, Reese TA, Choudhry S, et al. 2009. Differential enzymatic activity of common haplotypic versions of the human acidic Mammalian chitinase protein. J Biol Chem 284: 19650–19658.

80 Boot RG, Blommaart EF, Swart E, et al. 2001. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem 276:6770–6778.

81 Paoletti MG, Norberto L, Damini R, et al. 2007. Human gastric juice contains chitinase that can degrade chitin. Ann Nutr Metab 51: 244–251.

82 Muzzarelli RAA, Boudrant J, Meyer D, et al. 2012. Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: a tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. Carbohydr Polym 87:995–1012.

83 Whitaker JO, Dannelly HK, Prentice DA. 2004. Chitinase in insectivorous bats. J Mammal 85:15–18.

84 Strobel S, Roswag A, Becker NI, et al. 2013. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. PLoS ONE 8:e72770.

85 Krykbaev R, Fitz LJ, Reddy PS, et al. 2010. Evolutionary and biochemical differences between human and monkey acidic mammalian chitinases. Gene 452:63–71.

86 Galetti M, Pedroni F. 1994. Seasonal diet of capuchin monkeys (*Cebus apella*) in a semideciduous forest in south-east Brazil. J Trop Ecol 10:27–39.

87 Isbell LA, Rothman JM, Young PJ, et al. 2013. Nutritional benefits of *Crematogaster mimosae* ants and *Acacia drepanolobium* gum for patas monkeys and vervets in Laikipia, Kenya. Am J Phys Anthropol 150:286–300.

88 Kageyama T, Tanabe K, Koiwai O. 1991. Development-dependent expression of isozymogens of monkey pepsinogens and structural differences between them. Eur J Biochem 202: 205–215.

89 Narita Y, Oda S-I, Takenaka O, et al. 2010. Lineage-specific duplication and loss of pepsinogen genes in hominoid evolution. J Mol Evol 70:313–324.

90 Kageyama T. 2002. Pepsinogens, progastricsins, and prochymosins: structure, function, evolution, and development. Cell Mol Life Sci 59:288–306.

91 Taggart RT, Mohandas TK, Shows TB, et al. 1985. Variable numbers of pepsinogen genes are located in the centromeric region of human chromosome 11 and determine the high-frequency electrophoretic polymorphism. Proc Natl Acad Sci USA 82:6240–6244.

92 Narita Y, Oda S, Takenaka O, et al. 2000. Multiplicities and some enzymatic characteristics of ape pepsinogens and pepsins. J Med Primatol 29: 402–410.

93 Kageyama T. 1994. Asian macaque pepsinogens and pepsins. J Med Primatol 23:375–381.

94 Kageyama T, Tanabe K, Koiwai O. 1990. Structure and development of rabbit pepsinogens. Stage-specific zymogens, nucleotide sequences of cDNAs, molecular evolution, and gene expression during development. J Biol Chem 265:17031–17038.

95 Foltmann B, Jensen AL, Lønblad P, et al. 1981. A developmental analysis of the production of chymosin and pepsin in pigs. Comp Biochem Physiol B: Comp Biochem 68:9–13.

96 Kageyama T, Ichinose M, Tsukada-Kato S, et al. 2000. Molecular cloning of neonate/infant-specific pepsinogens from rat stomach mucosa and their expressional change during development. Biochem Biophys Res Commun 267:806–812.

97 Kageyama T. 2000. New World monkey pepsinogens A and C, and prochymosins. Purification, characterization of enzymatic properties, cDNA cloning, and molecular evolution. J Biochem 127:761–770.

98 Foltmann B. 1970. Prochymosin and chymosin (prorennin and rennin). Methods Enzymol 19:421–436.

99 Foltmann B. 1992. Chymosin: a short review on foetal and neonatal gastric proteases. Scand J Clin Lab Invest Suppl 210:65–79.

100 Ord T, Kolmer M, Villems R, et al. 1990. Structure of the human genomic region homologous to the bovine prochymosin-encoding gene. Gene 91:241–246.

101 Foltmann B. 1981. Gastric proteinases: structure, function, evolution and mechanism of action. Essays Biochem 17:52–84.

102 Agarwal S, Karmaus W, Davis S, et al. 2011. Review: immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. J Hum Lact 27:171–186.

103 Khan J, Vesel L, Bahl R, et al. 2015. Timing of breastfeeding initiation and exclusivity of breastfeeding during the first month of life: effects on neonatal mortality and morbidity: a systematic review and meta-analysis. Matern Child Health J 19:468–479.

104 Beintema JJ. 1990. The primary structure of langur (*Presbytis entellus*) pancreatic ribonuclease: adaptive features in digestive enzymes in mammals. Mol Biol Evol 7:470–477.

105 Zhang J. 2003. Parallel functional changes in the digestive RNases of ruminants and colobines by divergent amino acid substitutions. Mol Biol Evol 20:1310–1317.

106 Irwin DM, Prager EM, Wilson AC. 1992. Evolutionary genetics of ruminant lysozymes. Anim Genet 23:193–202.

107 Swanson KW, Irwin DM, Wilson AC. 1991. Stomach lysozyme gene of the langur monkey: tests for convergence and positive selection. J Mol Evol 33:418–425.

108 Dobson DE, Prager EM, Wilson AC. 1984. Stomach lysozymes of ruminants. I. Distribution and catalytic properties. J Biol Chem 259: 11607–11616.

109 Zhang J. 2006. Parallel adaptive origins of digestive RNases in Asian and African leaf monkeys. Nat Genet 38:819–823.

110 Yu L, Wang X-Y, Jin W, et al. 2010. Adaptive evolution of digestive RNASE1 genes in leaf-eating monkeys revisited: new insights from ten additional colobines. Mol Biol Evol 27:121–131.

111 Zhang J, Zhang Y-P, Rosenberg HF. 2002. Adaptive evolution of a duplicated pancreatic ribonuclease gene in a leaf-eating monkey. Nat Genet 30:411–415.

112 Sorrentino S, Libonati M. 1994. Human pancreatic-type and nonpancreatic-type ribonucleases: a direct side-by-side comparison of their catalytic properties. Arch Biochem Biophys 312:340–348.

113 Yu L, Zhang Y-P. 2006. The unusual adaptive expansion of pancreatic ribonuclease gene in carnivora. Mol Biol Evol 23:2326–2335.

114 Xu H, Liu Y, Meng F, et al. 2013. Multiple bursts of pancreatic ribonuclease gene duplication in insect-eating bats. Gene 526:112–117.

115 Liu J, Wang X-P, Cho S, et al. 2014. Evolutionary and functional novelty of pancreatic ribonuclease: a study of Musteloidea (order Carnivora). Sci Rep 4:5070.

116 Vigne J-D, Helmer D. 2007. Was milk a "secondary product" in the Old World Neolithisation process? Its role in the domestication of cattle, sheep and goats. Anthropozoologica 42: 9–40.

117 Evershed RP, Payne S, Sherratt AG, et al. 2008. Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. Nature 455:528–531.

118 Dunne J, Evershed RP, Salque M, et al. 2012. First dairying in green Saharan Africa in the fifth millennium BC. Nature 486:390–394.

119 Lambert JE. 2005. Competition, predation, and the evolutionary significance of the cercopithecine cheek pouch: The case of *Cercopithecus* and *Lophocebus*. Am J Phys Anthropol 126: 183–192.

120 Rahaman H, Srihari K, Krishnamoorthy RV. 1975. Polysaccharide digestion in cheek pouches of the bonnet macaque. Primates 16: 175–180.