Digestive Enzymes of Human and Non-Human Primates

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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 is known as digestion and 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 been focused on the gut microbiome, providing new insights into the interplay between diet and gut adaptation for a variety of animals including human and non-human primates.\(^6-11\) These are exciting new findings, but to get a full picture of an animal’s digestive adaptations, the gut microbiome and endogenously produced digestive enzymes should be viewed as complementary parts of a system. While the genes coding for digestive enzymes will
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. There is evidence that these changes can occur quite rapidly 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. Upon arrival, these primates rapidly diversified and filled the available dietary niches, evolving a suite of physiological, morphological, and behavioral characteristics to process a range of different diets. Changes in digestive enzymes were likely part of this adaptive suite, as digestive enzyme adaptations are not just important for the ability to tolerate new food resources, but also to maximize the energy obtained from them. Especially in human evolution maximizing the energy extracted from foods may have been a crucial factor in fueling the growth of our large brains. In non-human primates, many species depend on relatively low-quality foods (e.g., leaves), which can only be digested efficiently with specific gut adaptations, such as foregut fermentation and/or special digestive enzymes, as I describe below.

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, all while dealing with fiber, tannins and toxins contained in foods. The ability to meet nutritional goals depends in part on foraging decisions and the nutritional composition of the food item. However, it is also constrained by the gut’s capability to extract these nutrients, which is where digestive enzymes and digestive enzyme variation undoubtedly play a
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, as they usually represent the first step in the digestion of their respective substrates, and research in primates has largely been limited to them. Table 1 details the enzymes discussed here and additional digestive enzymes that have not been studied in depth in primates. In this review, I describe the specific role of each digestive enzyme, summarize what is known about its inter- and intra-species variability, and discuss what the adaptive implications of such variation are. I include information on digestive enzymes in non-primate mammals to build a comparative, evolutionary framework.

**Amylase**

Starches are a staple in the diets of many contemporary human populations, and are present in the diets of some non-human primates and other mammals in the form of underground storage organs, unripe fruits, and seeds.

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. 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. Species that express α-amylase in their saliva include some primates, rodents, lagomorphs, and bats. 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. Furthermore, both between and within the species in which salivary amylase has evolved there is considerable variation in the amount of enzyme that is expressed. 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, while New World monkeys do not (Fig. 1).\(^3,3^4\) No work investigating salivary amylase activity in strepsirrhines has been published, suggesting a potential avenue for future research, although it is unlikely that strepsirrhines express α-amylase in their saliva given the current understanding of the evolutionary pattern of this trait in primates. A comparison of the amylase gene structures in New World monkeys, Old World monkeys, apes and humans shows that the ability to express α-amylase in saliva evolved following several duplications of the pancreatic amylase gene \(AMY2\) within the primate lineage. 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.\(^3^4\) The second insertion, an endogenous retrovirus, occurred after the split from the Old World monkeys and is only found in hominoids (Fig. 1).\(^3^4\) Studies with transgenic mice indicate that the retroviral insertion is required to change the expression site of amylase from the pancreas to the parotid gland.\(^3^6\) This is consistent with the lack of salivary amylase activity observed 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, which would suggest an additional independent evolution event within the primate order.\(^3^4\)

Rodents also express salivary amylase but must have evolved the ability independently from primates.\(^3^6\) However, the similarities between pancreatic and salivary amylase genes in
mice indicate that the latter resulted from a duplication of the pancreatic amylase gene, as it did in the primate 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 and it is unclear if this pattern is actually homologous, as the two groups are only distantly related.\(^{37}\)

Lagomorphs, pigs and some bats also produce α-amylase in 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 conducted. 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 the different species could provide a better understanding of the types of selective pressures under which dietary physiology evolves.

The 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 for the possible selective benefits of salivary amylase.\(^{3,42,43}\) The primary role of α-amylase, both in the pancreas and in saliva, is starch digestion and 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 between 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 those who 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 populations who 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 report starch viscosity to decrease faster during mastication than individuals with low salivary amylase levels.\(^42\) 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 a stronger preference for starchy foods in individuals that express high levels of amylase in their saliva 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 ecologies and in turn drove the dietary choices of these populations, rather than vice versa. However, geographic location is not a good predictor for the copy number variation observed\(^3\) 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 prior to swallowing than when high-starch foods are swallowed whole.\(^44\) This suggests that the absorption of glucose, and thereby 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. Many modern human populations, however, especially those living in urban and industrialized settings are certainly not limited by the amount of food resources available to them, resulting in rising levels of obesity and type 2 diabetes. Therefore, an 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, but these results could not be replicated.

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. As opposed to humans, individual chimpanzees (Pan troglodytes verus) do not differ in AMY1 copy number and uniformly have two copies of the gene. Bonobos (Pan paniscus) consistently have four copies of the AMY1 gene, however, a disruption of the coding sequence suggests that these copies may all be non-functional (but see Ref. 35 and below). For gorillas only relative (not absolute) copy numbers of AMY1 have been reported, which are relatively higher than those of chimpanzees and relatively lower than those of humans. No information on copy numbers in orangutans has been published, however, a recent article providing measurements of salivary amylase levels expressed in all hominoids suggests that they may be similar to gorillas. Gorillas and orangutans exhibit very similar levels of salivary α-amylase, which are significantly higher than those of both chimpanzees and bonobos (Fig. 2). Alpha-amylase levels are somewhat
higher in bonobos than in chimpanzees, which is in accordance with the higher AMY1 copy number reported for this species and indicates that the copies are not actually non-functional, as had been previously suggested. The observed salivary amylase levels further correspond to the diets 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. Gorilla diets vary considerably across habitats, with some populations feeding on large amounts of fruit, while the diets of others are leaf-dominated. However, compared to chimpanzees and bonobos, gorilla diets tend to include more structural carbohydrates, potentially including starch from roots, and presumably higher tannin levels. High tannin levels are also likely included in the diet of orangutans, who may consume large amounts of seeds and cambium during times of fruit scarcity, both of which are rich in starch and tannins.

Studies have indicated that tannins, which are characterized by an affinity to bind to proteins, inhibit salivary α-amylase in both humans and non-human animals. 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. 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 these primates have diets that include mostly leaves and other herbaceous vegetation, as well as unripe fruit, all of which are presumed to contain high levels of tannins. All cercopithecines that have been tested express high levels of amylase in their saliva, often even higher than the levels observed in humans (see Box 1).

To summarize, the expression of salivary α-amylase is a trait that is only found in some
mammalian taxa and appears to have evolved multiple times. The precise number and sequence of convergent evolution events is unclear at this time and further research is necessary to fully understand the genetic basis of salivary α-amylase expression in all species. Further research should also be conducted in the colobine monkeys and other non-primate taxa with highly variable diets, such as the Chiroptera.

Lactase
All mammals lactate and nurse their offspring with milk. In order to be able to digest their mothers’ milk young mammals produce the digestive enzyme lactase in the small intestine. Lactase production is restricted to infancy and its activity begins to decline after the offspring is weaned in most mammals. Some humans, however, continue to produce lactase throughout their lifetime and can successfully digest milk as adults.

The main components of milk are water, lipids, carbohydrates, proteins, and salt, but the exact proportions of each vary from species to species. Lactose is the principal carbohydrate in milk and 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. Production of this enzyme is essential for young mammals, because they need to be able to digest the lactose contained in their mother’s milk, on which they depend for nutrition during the first part of their lives. If individuals whose small intestines no longer produce lactase consume fresh milk, lactose passes to the colon undigested where it may then 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 that lack an adequate supply of safe drinking water.\textsuperscript{55} Like all other mammals, the majority of humans cease to produce lactase after infancy. The actual proportion of humans who are “lactase non-persistent” is difficult to ascertain, but most studies suggest that it is around 65\% worldwide.\textsuperscript{57} The proportion of people who are lactase non-persistent varies widely between different populations, from less than 10\% in Northern Europe to over 95\% in Southern China (Fig. 2).\textsuperscript{55} Some humans continue to produce lactase past infancy and throughout their lives, a condition that is 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.\textsuperscript{5}

There are several problems with accurately determining the number of people who are lactose persistent versus non-persistent. First, difficulties and costs involved with accessing remote populations will lead to an overrepresentation of individuals from industrialized and western countries, which may have a different frequency of the phenotype. Second, the non-invasive measures used to determine lactase activity in most studies may not be completely accurate. They involve administering a dose of lactose to the individual, which is 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 1-2 liters of cow’s milk, which is a significantly larger amount of lactose than most people consume in one sitting and may lead to an overdiagnosis of the lactase non-persistent phenotype.\textsuperscript{58} Furthermore, individuals who are genetically lactase persistent may lose the ability to produce the enzyme secondarily due to intestinal diseases.\textsuperscript{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 \textit{LCT}, located on the long arm of chromosome 2 in humans\textsuperscript{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).\textsuperscript{58-62} The T-13910 allele is linked with LP in European, Indian, and Central Asian populations.\textsuperscript{58,63,64} In African and Arabic populations it is only found at very low frequencies, despite locally high prevalence of the LP phenotype.\textsuperscript{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 here is mirrored in the distribution of the LP alleles (Fig. 2).\textsuperscript{5,67} The G-13915 variant likely originated on the Arabian peninsula and was spread to Africa by nomadic Arabian groups in the 6th or 7th century,\textsuperscript{66,68} while an East African origin is most probable for the G-13907, G-14009 and C-14010 alleles (Table 2).\textsuperscript{5,61,67}

Lactase persistence has evolved multiple times in humans, which is suggestive of a strong selective pressure operating on this phenotype. In fact, several studies have shown that the \textit{LCT} locus is under the strongest positive selection seen in humans so far\textsuperscript{58,69} and it has become a textbook example of recent human adaptations. Both the evidence for strong positive selection acting on the alleles associated with lactase persistence and the evidence for multiple convergent evolution events show that there seems to be an adaptive significance to 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.\textsuperscript{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 with a large sample of ancient Eurasian remains supports a relatively recent spread of the allele.\textsuperscript{69} The earliest evidence of LP in this sample comes from an individual dated to 2450-2140 BCE.\textsuperscript{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.\textsuperscript{5,12,58,66} These age estimates and archeological evidence for the advent of dairying are summarized in Table 2.

As mentioned above, all of this provides evidence that consuming milk and being able to digest lactose provide significant adaptive benefits. Several hypotheses have been proposed to explain the adaptive significance of digesting fresh milk. Flatz and Rotthauwe\textsuperscript{71} proposed the calcium assimilation hypothesis. They argue that being able to digest lactose is of particular importance 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. As milk provides both Vitamin D and calcium, the 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.\textsuperscript{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 to be other adaptive benefits to dairying in addition to calcium assimilation.\textsuperscript{72} Milk and milk products are high-calorie foods and, as opposed to plants, which are only available seasonally, this resource is often available year-round. Fresh milk further represents a valuable source of clean and uncontaminated fluids, which may be of particular importance in arid, semi-desert environments.\textsuperscript{12} Camels, for example, can survive in extremely arid conditions by metabolizing the fat stored in their humps and their milk
could be an important source of fluids for the humans keeping them.

Finally, 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.\textsuperscript{58} This may mean the Hadza descended from a pastoralist population or that lactase aids in the digestion of another food resource.\textsuperscript{58} Lactase does catalyze the hydrolysis of phlorizin, a bitter glycoside that is found in the roots and bark of plants in the \textit{Rosaceae} family that is native to Tanzania, where the Hadza live.\textsuperscript{5,58}

In conclusion, \textbf{the convergent evolution of lactase persistence in multiple populations of humans is a classic example of the strong selective pressure that the 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).\textsuperscript{5}

\textbf{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.\textsuperscript{1} All primates include some insects in their diet, whether through accidental consumption or through active insectivory (Fig. 3),\textsuperscript{73} and fungi are a dietary staple of some New World monkeys, as well as humans\textsuperscript{74,75} but it is still unclear if any primates are actually able to digest chitin.

Chitinolytic enzymes have been isolated from the gastric mucosa of a capuchin monkey (\textit{Cebus capucinus})\textsuperscript{76} and a potto (\textit{Perodicticus potto}).\textsuperscript{77} However, such experiments are extremely invasive and do not allow for differentiation between enzymes that are endogenously produced or of dietary origin, as chitinases are present in many plant resources.\textsuperscript{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. 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, including protection from pathogens and aiding in the hydrolysis of polysaccharides.

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

An Italian study found varying levels of AMCase activity in the gastric juices of 20 out of 25 human participants. Because the AMCase purified from the participants’ gastric juice was able to hydrolyze fluorescein isothiocyanate-chitin in an experimental setting, the authors hypothesized that it represents an adaptation for the digestion of arthropods and that the varying levels, as well as absence in some patients, were due to the decreased consumption of insects in the western diet. While this is an intriguing hypothesis, Muzzarelli and colleagues describe an unpublished follow-up study, which found that human gastric juice containing high levels of AMCase was unable to digest the wings of Calliphora vomitoria (bluebottle fly).

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. While it was previously thought that any chitinolytic activity in the digestive system of mammals was due to chitinases expressed by intestinal bacteria,
recently both mice and bats were shown to secrete AMCase from the chief cells found at the base of the gastric glands, where other digestive enzymes are also secreted. The diets of wild mice often include significant amounts of insects and many bat species feed on insects almost exclusively. While both bats and primates have been shown to harbor microorganisms capable of degrading chitin, producing an endogenous digestive enzyme for the hydrolysis of chitin would allow for faster and more efficient digestion of arthropod prey, representing 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 in the rhesus (Macaca mulatta) and long-tailed macaque (M. fascicularis) genomes, named mCHIA. 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. The most striking difference was that the macaque AMCase (MACase) was 50 times more efficient than human AMCase at hydrolyzing a chitin substrate.

Like AMCase in humans, mice, and bats, MACase is expressed at high levels in the stomachs of macaques. As MACase additionally appears to be significantly more efficient at chitin digestion than human AMCase, it is an exciting possibility that MACase is an important digestive enzyme for non-human primates. Until recently it was unclear whether primates or other mammals produced endogenous chitinases in their digestive systems, as it could not be excluded that any chitinolytic activity found was of exogenous or microbial origin. Furthermore, almost intact exoskeletons are reportedly found in the feces of primates, which
has led many to presume that they are indigestible.\textsuperscript{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 \textit{mCHIA} gene across the primate order to test if \textit{mCHIA} might exhibit genetic variation, such as higher copy numbers in more insectivorous primate species. Such variation would provide evidence that MACase plays an important role in the digestive system of primates that rely on chitin-containing food resources, such as arthropods and fungi.

\textbf{Pepsinogens/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.

\textbf{Pepsinogen/Pepsin A}

Pepsinogen A is the zymogen of pepsin A, which is the most abundant gastric protease in most adult mammals and appears to be highly polymorphic in primates.\textsuperscript{88,89} This enzyme is secreted by the chief cells of the gastric mucosa and is maximally active at a pH of about 2, in accordance with its role in the acidic environment of the stomach.\textsuperscript{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.\textsuperscript{1}

Pepsin A has been shown to be highly polymorphic at both the protein level and the genetic level in various primates. Humans have a cluster of three genes that are known to be present in variable copy numbers.\textsuperscript{91} The other great apes exhibit even greater variation in pepsinogen A isozymogens than humans. Narita and colleagues\textsuperscript{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. A follow-up study by the same group predicted that five and three genes respectively encode pepsinogen A1 and A2 in the orangutan. These genes are also present in the chimpanzee, while the human pepsinogen genes are most like those for pepsinogen A1, leading the authors to conclude that pepsinogen A diverged into types A1 and A2 in the hominoid lineage but the latter was lost in humans. The extreme multiplicity of pepsinogen A seen in the great apes is suggested to be related to a dietary reliance on herbaceous material. 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. A study of Japanese macaques (*Macaca fuscata*) found that the relative expression of the four pepsinogen A forms varied with age, similar to what had previously been found in rabbits. This increase in pepsinogen A production is found commonly among mammals and is likely related to the changing digestive demands that occur with weaning and the adoption of an adult diet.

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

Many primate species, including most of the cercopithecines and all of the strepsirrhines, have not been tested for genetic variation relating to pepsinogen A, although some interesting patterns have already emerged in the great apes and the macaques, as discussed at the beginning of this section.

Prochymosin/Chymosin

Prochymosin is the zymogen of chymosin and is secreted by the gastric mucosa of neonate mammals. Due to its use in the cheesemaking industry, chymosin is often better known as rennin. Chymosin was first discovered in neonate cattle and its ability to effectively clot milk has long been utilized by humans in the production of cheese. Due to its importance to the dairy industry many articles regarding the clotting activity of chymosin have been published, whereas its physiological role in animal digestive systems is less well understood. As suggested by its function in the clotting of milk, chymosin is an enzyme that 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, but it is exclusively found in the stomachs of fetuses and neonates in almost all of these species. With one exception (that is discussed
below), chymosin is absent from adult mammals and experiments with rats and pigs have shown that there appears to be a switch in proteases around the time of weaning. While pepsinogen A, in the pig, and progastricsin, in the rat, were close to undetectable in fetal and neonate animals, 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. 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.

Newborn Japanese macaques (*Macaca fuscata*) were not shown to produce chymosin or any other neonate-specific digestive enzymes, although relative expression of different pepsinogens changed with age (see above). Human infants also lack chymosin and the human gene (*hPC*) for this enzyme appears to be non-functional. Because Japanese macaque infants also lacked chymosin, it is plausible that the *hPC* pseudogene is a shared trait of the catarrhines. New World primates, on the other hand, differ in their expression of chymosin both from other primates and other mammals. As opposed to Japanese macaques and humans, 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 is a trait common to all platyrrhines, although more research is needed to confirm this and to explain why this enzyme persists into adulthood.

Compared to pepsin, chymosin tends to exhibit weaker general proteolytic activity and approximately similar milk clotting activity. The finding that pepsin compares to chymosin in milk clotting activity suggests that chymosin is not essential for the digestion of milk in neonate
mammals and begs the question 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. Experiments with porcine pepsin have shown that this protease cleaves immunoglobulins into smaller fragments and it is likely that pepsin from other species has the same effect. 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 of critical importance to many mammalian species, in which immunoglobulins are not transferred through the placenta prior to birth. Placental transfer of immunity occurs in primates, rabbits, and possibly other mammals, but species in which neonatal chymosin activity has been demonstrated tend to rely on postnatal transfer through the colostrum. The presence of trypsin inhibitors in the colostrum provides further evidence that proteases are disadvantageous to the transfer of immunoglobulins, and that chymosin may have evolved as a neonate-specific digestive enzyme that effectively clots milk, while having a general proteolytic activity that is low enough to prevent damaging the immunoglobulins.

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

Both humans and rabbits transfer (some) immunoglobulins through the placenta prior to birth, which may explain why the neonates of both taxa lack chymosin. Rabbits, however, do
express a different neonate-specific protease, pepsinogen F. Kageyama and colleagues suggest that the presence of a neonate-specific pepsin might be due to the high protein content of rabbit milk. Primate milk is comparatively low in protein and high in lactose, therefore specific proteases may not be necessary to efficiently digest it. While this is certainly a reasonable explanation for the lack of chymosin found 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.

**Ribonuclease and Lysozyme**

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

Lysozyme is usually expressed in macrophages, where it digests the peptidoglycan cell walls of bacteria as part of the immune defense system of the host animal. 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, as they have
undergone extremely similar changes without sharing a common origin.\textsuperscript{107} As opposed to ruminant artiodactyls, which have up to ten different genes for lysozyme, colobines have retained the single gene that is found in most mammals.\textsuperscript{106,107} This gene has accumulated nine amino acid substitutions,\textsuperscript{107} that appear to create functional differences improving 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 non-digestive lysozymes, which function best at a neutral pH.\textsuperscript{108} Additionally, digestive lysozyme is more resistant to digestion by pepsin. Stomach lysozymes of the cow (\textit{Bos taurus}) and the langur monkey (\textit{Presbytis entellus}) retained around 75\% of their activity after an hour of exposure to pepsin, as opposed to the lysozymes of rats and humans which retained less than 7.5\% activity.\textsuperscript{108}

Similar to lysozyme, ribonuclease has been exapted for a digestive function in ruminants and colobines. However, the digestive function of ribonuclease was not only acquired in parallel by ruminants and leaf-monkeys, but actually evolved independently at least twice within the colobine lineage, once in the Asian and once in the African colobines (Fig. 4).\textsuperscript{105,109,110} Gene duplications were followed by parallel functional changes in the daughter genes, but unlike in digestive lysozyme, these functional changes are caused by different amino acid substitutions in the ruminants versus the colobines.

All primates share one gene for pancreatic ribonuclease called \textit{RNASE1}. In numerous Asian colobines a second pancreatic ribonuclease gene, \textit{RNASE1B}, has been found.\textsuperscript{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.\textsuperscript{111} In the African colobines \textit{RNASE1} was duplicated twice, resulting in two additional pancreatic ribonuclease genes, \textit{RNASE1β} and
RNASE1γ. \textsuperscript{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 at a pH of 7.4 in all primates, while the Asian colobine enzyme, RNASE1B, and the African colobine enzymes, RNASE1β and RNASE1γ, have an optimal pH of 6.3 and 6.7, respectively (Fig. 4). \textsuperscript{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.\textsuperscript{109}

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

Interestingly, RNASE1 duplications have also been found in carnivores and bats, animals that are not foregut fermenters.\textsuperscript{113,114} In the superfamily Musteloidea (order Carnivora), RNASE1 was duplicated independently in four families: the Procyonidae (raccoons), Ailuridae (red panda), Mephitidae (skunks) and Mustelidae (weasels).\textsuperscript{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).\textsuperscript{114} All of these bat species are insectivorous, suggesting the possibility of a dietary adaptation, however, at this point there is no conclusive evidence to support this hypothesis.\textsuperscript{114} Likewise, more research is needed to understand the functional significance of the RNASE1 duplications in carnivores.\textsuperscript{115} Nevertheless, the parallel evolution of a digestive system
and the convergent adaptation of digestive enzymes in ruminants and colobines discussed above illustrate the power common selective pressures can have, especially when related to diet.

**Conclusion and Directions for Future Research**

Digestive enzymes play an important role 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 been evolved independently in response to convergent digestive 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 in our knowledge of primate, and more broadly, mammalian digestive enzymes remain. 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 found 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 for understanding what traits are ancestral or derived and our ability to evaluate evolutionary hypotheses.

For example, additional research on strepsirrhines and platyrrhines may explain why the
neonate-specific enzyme chymosin persists throughout adulthood in New World monkeys. The hypotheses put forward thus far are limited and without a more complete picture of which primate taxa express chymosin in adulthood, it is impossible to propose and test any alternatives. For instance, because no digestive enzyme research has included the strepsirrhines, it is unknown whether they express chymosin at all, and, if yes, for how long. Do they share the primitive mammalian condition or either of the derived states found in platyrrhines and catarrhines? Uncovering a differential pattern of chymosin expression across primates would help to devise a more directional hypothesis of the adaptive benefits of this digestive enzyme.

The presence or absence of salivary amylase has yet to be confirmed in both colobines and strepsirrhines. Again, knowing if 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 levels of salivary amylase that are higher than those of humans, even though their gene lacks the retroviral insertion that is presumably 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 the lactase persistence trait in humans, 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; however, none of the known LP-alleles appear to be found here. This implies that an alternative haplotype is present that allows adults to digest milk in this population.

More research is also needed to elucidate the evolution of chitinases. As most previous
research on chitinase has been done on bats, I think that 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 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 found in the digestive enzymes of primates and other mammals, and how this variation is distributed phylogenetically, the next step is 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 be a useful alternative initially.

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. Additionally, knowledge of inter- and intraspecific variation in primate digestive enzymes can provide insight into the evolution of dietary ecologies and dietary adaptations of primates past and present, as well as give us a better grasp of the true digestive capabilities of different species.

Acknowledgements
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104 Beintema JJ. 1990. The primary structure of langur (Presbytis entellus) pancreatic


119 Lambert JE. 2005. Competition, predation, and the evolutionary significance of the

Table 1. Endogenously produced digestive enzymes of primates.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene symbol</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-Amylase</td>
<td>AMY1</td>
<td>Starch</td>
</tr>
<tr>
<td>Maltase</td>
<td>MGAM</td>
<td>Disaccharide maltose (product of starch digestion by α-amylase)</td>
</tr>
<tr>
<td>Chitinase</td>
<td>CHIA</td>
<td>Chitin (present in cell walls of fungi and exoskeletons)</td>
</tr>
<tr>
<td>Pepsin A</td>
<td>PGA</td>
<td>Protein</td>
</tr>
<tr>
<td>Chymosin</td>
<td>CYM</td>
<td>Protein</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>RNASE1</td>
<td>In ruminants and colobines: foregut bacteria</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>LYZ, LZM</td>
<td>In ruminants and colobines: foregut bacteria</td>
</tr>
<tr>
<td>Trypsin</td>
<td>PRSS1</td>
<td>Protein</td>
</tr>
<tr>
<td>Gastricsin</td>
<td>PGC</td>
<td>Protein</td>
</tr>
<tr>
<td>Lipases</td>
<td>PNLIP, CEL</td>
<td>Lipids</td>
</tr>
<tr>
<td>Lactase</td>
<td>LCT</td>
<td>Lactose (main carbohydrate in milk)</td>
</tr>
<tr>
<td>Trehalase</td>
<td>TREH</td>
<td>Trehalose (disaccharide found in insects, fungi, and plants)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>SI</td>
<td>Sucrose, maltose</td>
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</tbody>
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Table 2. Ages and origins of LP alleles and archeological evidence for advent of dairying.

<table>
<thead>
<tr>
<th></th>
<th>Estimates of Allele Origin (years BP)</th>
<th>Likely Location/Population of Origin</th>
<th>Archeological Evidence for Advent of Dairying in Region of Origin</th>
</tr>
</thead>
</table>
| T-13910        | ~8000-9000⁵⁸                         | Central Balkans¹²                   | Slaughtering age profiles of sheep, goats, and cattle suggest milk use ~11,000 years BP¹¹⁶
|                | ~5000-12000⁶⁵                        |                                     | Organic residues on pottery provide evidence for milk use by 9000 years BP¹¹⁷ |
|                | ~6256-8683¹²                         |                                     |                                                                     |
| G-13915        | ~4000²,⁶⁶                            | Arabian Peninsula²,⁶⁶               | Domestication of the camel occurred ~6000 years BP⁶⁶                |
| G-13907        | ~5000³                              | Cushitic Speakers/Eastern Ethiopia⁵ | Stable isotope analyses of potsherds indicate dairying in Saharan Africa began by 7000 years BP¹¹⁸ |
| G-14009        |                                     | Ethiopia/East Africa (?)⁶²         |                                                                     |
| C-14010        | ~2700-6800⁵⁸                        | Cushitic Speakers/East Africa⁵     |                                                                     |
Fig. 1. Salivary amylase expression in primates (relative to humans) and the proposed timing of the two insertions thought to cause amylase expression in saliva.\textsuperscript{11} Cercopithecines express salivary amylase despite lacking the retroviral insertion, suggesting the possibility of an alternate mechanism.
Fig. 2. Frequency of the lactase persistence phenotype and frequencies of known associated alleles of the *LCT* gene. Phenotype frequencies predicted by surface interpolation based on measurements at 235 locations. Allele frequencies based on data from the Global Lactase Persistence Association Database and Jones et al.
Fig. 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 answered. Photo by Mickey Samuni-Blank via Wikimedia Commons.
Fig. 4. The independently duplicated ribonucleases in Asian and African colobines and ruminants exhibit parallel functional changes, including similar optimal pH.\textsuperscript{105,109}
Box 1. Cheek pouches

The cercopithecines are a sub-group of the Old World monkeys that are characterized by the evolution of cheek pouches, which they often utilize to store food.\textsuperscript{3,119} Lambert\textsuperscript{119} has suggested that cheek pouches may facilitate the digestion of unripe fruit, seeds, or underground storage organs, foods that contain high levels of starch.\textsuperscript{31,119} The high levels of salivary amylase expressed in these species\textsuperscript{31} provide evidence to support this hypothesis. \textit{Papio hamadryas} (hamadryas baboons) and \textit{Theropithecus gelada} (gelada baboons) exhibit salivary amylase levels twice as high as humans and about eight times higher than in \textit{Pan troglodytes} (common chimpanzees), consistent with the diets of these species, which in addition to being high in starch, are also likely to be high in tannins.\textsuperscript{31} Evidence that cheek pouches are an important site for the initial digestion of polysaccharides in cercopithecine primates has been provided by one study.\textsuperscript{120} A potato inserted into the cheek pouch of a restrained bonnet macaque (\textit{Macaca radiata}) has over 50\% of its starch converted into simpler sugars within five minutes, indicating that the high amylase levels found in the primate cheek pouch are extremely effective at starch digestion.\textsuperscript{120}
Image for Box 1. Long-tailed macaques (*Macaca fascicularis*) on Sumatra with filled cheek pouches. Photo by Joram Berlowitz.