Int J Primatol (2018) 39:269–284
https://doi.org/10.1007/s10764-018-0037-7

# No Evidence of Copy Number Variation in Acidic Mammalian Chitinase Genes (*CHIA*) in New World and Old World Monkeys

**Mareike C. Janiak**[1]

Received: 14 December 2017 / Accepted: 29 March 2018 / Published online: 27 April 2018


**Abstract** Copy number variation may be the most common form of structural genetic variation in the genome. Numerous studies have shown that gene copy number variation can correlate with phenotypic variation, where higher copy numbers correspond to increased expression of the protein and vice versa. Examples include some digestive enzyme genes, where variation in copy numbers and protein expression may be related to dietary differences. Increasing the expression of a digestive enzyme through higher gene copy numbers may thus be a potential mechanism for altering an organism's digestive capabilities. I investigated copy number variation in genes coding for acidic mammalian chitinase, a chitinolytic digestive enzyme that may be used for the digestion of insect exoskeletons, in nonhuman primates with varying levels of insect consumption. I hypothesized that *CHIA* copy number correlates positively with level of insectivory, predicting higher copy numbers in more insectivorous primates. I assessed copy number variation with the QuantStudio 3D digital PCR platform, in a comparative sample of Old World and New World primate species ($N = 10$ species, one or two individuals each). Contrary to my prediction, no evidence of copy number variation was found and all species tested had two gene copies per diploid genome. These findings suggest that if acidic mammalian chitinase expression varies according to insect consumption in primates, it may be up- or downregulated through another mechanism.

**Keywords** AMCase · Digestive enzymes · Insectivory

Handling Editor: Joanna M. Setchell

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10764-018-0037-7) contains supplementary material, which is available to authorized users.

✉ Mareike C. Janiak
mareike.janiak@rutgers.edu

[1] Department of Anthropology, Rutgers University, New Brunswick, NJ 08901, USA

## Introduction

Copy number variation (CNV) is a form of structural variation in the genome, in which a genomic region is present in higher or lower numbers compared to a reference genome or another species' genome (Clop et al. 2012; Stankiewicz and Lupski 2010). Research on CNV has emerged over the past 10 years, as cheaper next-generation sequencing methods and other techniques have allowed for the identification of these structural variants on a broader scale (Clop et al. 2012). Although it is still unclear how common CNV is, some research suggests that it might be the most common form of structural genetic variation (Stankiewicz and Lupski 2010). Some work shows an accelerated rate of CNV in the great apes (Cheng et al. 2005; Marques-Bonet et al. 2009; Sudmant et al. 2013), and species-specific patterns of structural variation across great apes and other primates have been found (Dumas et al. 2007; Gazave et al. 2011; Gokcumen et al. 2013; Gschwind et al. 2017). Although the phenotypic or functional effects of much CNV are not yet understood (Zarrei et al. 2015), comparative studies of human and nonhuman primates have identified potential effects of structural variation on gene expression and transcription (Dumas et al. 2007; Gokcumen et al. 2011, 2013; Gschwind et al. 2017; Iskow et al. 2012; Lee et al. 2008).

Numerous studies have provided evidence that gene CNV can correlate with phenotypic variation (e.g., higher copy numbers can correspond to increased expression of the protein and vice versa) (Hollox et al. 2003; Linzmeier and Ganz 2006; Perry et al. 2006, 2007). CNV has been associated with diseases and developmental disorders in humans (Cantsilieris and White 2013; Jacquemont et al. 2011; Malhotra and Sebat 2012; Pinto et al. 2010), immune function across mammals (Bickhart et al. 2012; Hardwick et al. 2011; Leonard et al. 2012; Wang et al. 2013), and appearance in domestic animals (Elferink et al. 2008; Fontanesi et al. 2009, 2011; Giuffra et al. 1999; Wright et al. 2009).

CNV has also been implicated in digestive enzyme expression. Research indicates that there is variation in the types and amounts of digestive enzymes that are secreted among primates (and other mammals), and that the driving selective pressure on this variation is diet (Axelsson et al. 2013; Behringer et al. 2013; Zhang et al. 2002). Phenotypic variation relating to digestive enzymes can strongly affect an organism's digestive abilities (Ingram et al. 2009; Mandel and Breslin 2012), which in humans is illustrated most famously through the example of lactase persistence (Enattah et al. 2008; Itan et al. 2009, 2010; Ranciaro et al. 2014; Schlebusch et al. 2013; Tishkoff et al. 2007). Pepsin A, a protein-digesting enzyme produced in the stomach, has been shown to be highly polymorphic at both the protein level and the genetic level in various primates (Narita et al. 2000; Taggart et al. 1985). The genetic basis of the observed protein variation has not been completely resolved yet, but one study suggests that in *Pongo pygmaeus* pepsin A is encoded by two different genes, which may have three and five copies, respectively (Narita et al. 2010). Human populations with high-starch diets produce higher levels of salivary amylase, an enzyme that digests starch, than populations that do not commonly include starch in their diets (Perry et al. 2007). This increased secretion correlates with a larger number of copies of the salivary amylase gene, *AMY1* (Mandel et al. 2010; Perry et al. 2007). A relationship between pancreatic amylase secretion and *AMY2* gene copy number has been identified in dogs, but not in wolves, which have only the standard two copies (Axelsson et al. 2013). It is possible that this change allowed dogs to digest potentially starch-rich food scraps provided by humans (Axelsson et al. 2013).

This work suggests that increasing the expression of a digestive enzyme through higher copy numbers may be a potential mechanism for altering an organism's digestive capabilities, and provides an incentive for exploring CNV in other digestive enzyme genes, such as the acidic mammalian chitinase genes (*CHIA*). Acidic mammalian chitinase (AMCase) is a chitinolytic enzyme produced in the stomach (Boot et al. 2001, 2005; Krykbaev et al. 2010; Ohno et al. 2016; Strobel et al. 2013), and work on mice, insectivorous bats, poultry, and pigs has shown that it is used for the digestion of chitinous insect exoskeletons (Ohno et al. 2016; Strobel et al. 2013; Tabata et al. 2017a, b, 2018). In other tissues, such as the lungs, acidic mammalian chitinase degrades environmental chitin, and genetic variation in human *CHIA* genes has been linked to asthma and other lung diseases (Okawa et al. 2016; Reese et al. 2007; Seibold et al. 2009; Zhu et al. 2004). In nonhuman primates, most species retain at least one protein-coding *CHIA* gene, while some of the more insectivorous species have between two and five protein-coding *CHIA* paralogs (*hCHIA*, *mCHIA*, *CHIA3*, *CHIA4*, and *CHIA5*) (Janiak et al. 2018). The only species in which all *CHIA* paralogs have been pseudogenized are some of the folivorous colobine monkeys (*Rhinopithecus bieti* and *Nasalis larvatus*), which are not known to feed on insects (Janiak et al. 2018). However, there is also significant variation in insect consumption across species that share the same *CHIA* genotype, such as the cercopithecines, in which only *mCHIA* remains protein coding. Likewise, platyrrhines, in some of which both *mCHIA* and *hCHIA* remain protein coding, vary in their mean insect intake (Table I). For example, insects reportedly make up only 7.2% of the diet of *Callithrix jacchus*, while it is 53.4% for *Saimiri sciureus* (Table I). Another platyrrhine, *Sapajus apella*, retains only one protein-coding *CHIA* gene, despite a mean insect consumption of >30% (Table I). It is therefore possible that in primate species with higher insect consumption, the production of acidic mammalian chitinase is increased via higher copy numbers of the *mCHIA* or *hCHIA* genes, similar to what has been found for salivary amylase and the *AMY1* gene (Perry et al. 2007).

I investigated CNV at the *mCHIA* and *hCHIA* loci in nonhuman primates, hypothesizing that *CHIA* copy number correlates positively with level of insectivory. I predicted that primates that are relatively more insectivorous (*Erythrocebus patas*, *Miopithecus talapoin*, *Macaca nigra*, *Saguinus fuscicollis*, *Saimiri sciureus*, and *Sapajus apella*) will have higher copy numbers of the *mCHIA* gene and the *hCHIA* gene (only in species with a protein-coding *hCHIA* sequence) than primates that are relatively less insectivorous (*Callicebus moloch*, *Callithrix jacchus*, *Macaca mulatta*, and *M. nemestrina*).

## Methods

### Samples and Sample Preparation

I obtained high-quality DNA samples from seven nonhuman primate species from Coriell Biorepositories: *Callithrix jacchus*, *Callicebus moloch*, *Saguinus fuscicollis*, *Saimiri sciureus*, *Macaca mulatta*, *M. nemestrina*, and *M. nigra*. Dr. George Perry (Penn State University) provided extracted DNA from *Sapajus apella* and Dr. Todd Disotell (New York University) provided extracted DNA from *Erythrocebus patas* (two individuals) and *Miopithecus talapoin*. I quantified all DNA samples using the Qubit

**Table I** Mean percentage of annual feeding time devoted to insect consumption for species included in this study

| Species | Site | Reference | % insect consumption | |
|---|---|---|---|---|
| | | | Study | Species mean |
| *Callicebus moloch (brunneus)* | Manú National Park, Peru | P. C. Wright (1985) | 17.0 | 12.0 |
| | Tambopata, Madre de Dios, Peru | Crandlemire-Sacco (1988) | 11.0 | |
| | Los Amigos, Madre de Dios, Peru | Lawrence (2007) | 8.0 | |
| *Callithrix jacchus* | Joao Pessoa, Brazil | Alonso and Langguth (1989) | 5.4 | 7.2 |
| | Nisia Floresta, Brazil | Digby et al. (2011) | 9.0 | |
| *Erythrocebus patas* | Segera Ranch, Laikipia, Kenya | Isbell (1998) | 35.0 | 23.5 |
| | Kala Maloue National Park, Cameroon | Nakagawa (1989) | 12.0 | |
| *Macaca mulatta* | Dunga Gali, Murree Hills, Pakistan | Goldstein and Richard (1989) | 0.0 | 0.0 |
| *Macaca nemestrina* | Pasoh Forest Reserve, Malaysia | Caldecott (1986) | 12.2 | 12.2 |
| *Macaca nigra* | Tangkoko-DuaSudara Nature Reserve, Sulawesi, Indonesia | O'Brien and Kinnaird (1997) | 32.1 | 32.1 |
| *Miopithecus ogouensis/talapoin* | Makokou, Gabon | Gautier-Hion (1988) | 35.0 | 35.0 |
| *Saguinus fuscicollis* | Estación Biológica Quebrada Blanco, Peru | Knogge and Heymann (2003) | 5.8 | 28.3 |
| | Rio Blanco Research Station, Peru | Garber (1988) | 53.1 | |
| | San Sebastian, Pando, Bolivia | Porter (2001) | 26.0 | |
| *Saimiri sciureus* | Gunma Ecological Park, Brazil | Lima and Ferrari (2003) | 45.0 | 53.4 |
| | Ananim, Brazil | Stone (2007) | 61.8 | |
| *Sapajus apella* | El Rey National Park, Brazil | Brown and Zunino (1990) | 40.3 | 32.6 |
| | Iguazú National Park, Brazil | Brown and Zunino (1990) | 24.9 | |

Data are for individual studies and the mean for each species

dsDNA BR assay kit (Invitrogen) and a Qubit 2.0 fluorometer (Invitrogen). I diluted samples with water to a final concentration of 10 ng/μl.

### Assay Design

To design primers for use across multiple species, I identified a conserved region around exons 9 and 10 of both the *mCHIA* and *hCHIA* genes (Krykbaev et al. 2010). To design specific primers, I aligned the *mCHIA* exon 9 and 10 sequences, including the intronic region, of 13 catarrhine primates (*Chlorocebus aethiops*, *C. sabaeus*, *Allenopithecus nigroviridis*, *Cercocebus atys*, *Colobus guereza*, *Allochrocebus lhoesti*, *Macaca fascicularis*, *M. nemestrina*, *M. mulatta*, *Miopithecus ogouensis*, *Cercopithecus mitis*, *Papio anubis*, and *Rhinopithecus roxellana*) and, separately, of four platyrrhine primates (*Aotus nancymaae*, *Callithrix jacchus*, *Cebus capucinus*, and *Saimiri boliviensis*). I likewise aligned the corresponding region of the *hCHIA* gene for *Callithrix jacchus*, *Saimiri sciureus*, and *Saguinus fuscicollis*, the only species in my sample in which *hCHIA* is not pseudogenized. All sequences were either available via reference genomes on GenBank or had been generated de novo for another study (Janiak et al. 2018). Accession numbers for the species included in this study are listed in Electronic Supplementary Material (ESM) Table SI and the alignments generated for assay design are available in ESM Table SII. I masked any bases that were not identical across all sequences in the alignment by replacing the corresponding nucleotide with "N" in the consensus sequence. I entered the masked consensus sequences in the Custom TaqMan Assay Design Tool (ThermoFisher Scientific) and submitted them to the TaqMan design pipeline. I conducted primer BLASTs of the assays against primate reference genomes to ensure that the assays were specific to the region of interest and would not amplify other, unrelated, loci or paralogous *CHIA* genes. Table II lists primer sequences and assay IDs and ESM Fig. S1 shows the locations of the primers along the gene.

I used the RNase P TaqMan copy number reference assay (ThermoFisher) as the reference assay in all experiments. This assay detects the ribonuclease P (RNase P) RNA component H1 gene, *RPPH1*, a gene with known copy number of two copies per diploid genome. A reference assay for a gene with known copy number is necessary to

**Table II** Primer and probe sequences used in custom TaqMan assays to measure copy numbers of *mCHIA* and *hCHIA* genes in 10 primate species

| Gene | Taxon | Assay | Sequence |
|---|---|---|---|
| *hCHIA* | Platyrrhines | hCHIA_NW_CD47VWA_F | ATGGTCTGGGCCATTGATCTG |
| | | hCHIA_NW_CD47VWA_R | CCTTCTTCAGGGTGGAGATTAGG |
| | | hCHIA_NW_CD47VWA_M | ATGACTTCACTGGCACTTTCT |
| *mCHIA* | Catarrhines | CHIA_OWM_CDGZE6W_F | GAGTGGCTTGGATATGATAACACCAA |
| | | CHIA_OWM_CDGZE6W_R | GGGAACATGCTCACAGGCA |
| | | CHIA_OWM_CDGZE6W_M | ACACAGTCTACCTTGATTTGGAAACT |
| | Platyrrhines | CHIA_NWM_CDPRJ2G_F | AATTATTACAGGCTGATTGGTTAAAGA |
| | | CHIA_NWM_CDPRJ2G_R | AGGGAATTTTCCTTGGTTGCAGAA |
| | | CHIA_NWM_CDPRJ2G_M | CCATTGACTTGGATGATTTCAC |

determine the number of copies of the gene of interest and other studies of nonhuman primate CNV have also used the RNase P assay (Yoshida et al. 2016).

### 3D Digital PCR

I assessed CNV using the QuantStudio 3D Digital polymerase chain reaction (PCR) system, with QuantStudio 3D Digital PCR 20 K chips and QuantStudio 3D Digital PCR Master Mix. I prepared reactions in a total volume of 34.8 μl, containing 17.4 μl of Master Mix, 1.74 μl of custom assay, 1.74 μl of RNase P assay, 1.92 μl of water, and 12 μl of template (10 ng/μl). I incubated this super mix at room temperature for 15 min before loading it onto the chips. I ran all reactions in duplicate with 14.5 μl of the super mix loaded onto each chip. I loaded and sealed chips according to the manufacturer's protocol and ran them under the following conditions: 96 °C for 10 min, followed by 39 cycles of 60 °C for 2 min, 98 °C for 30 s, and 60 °C for 2 min. Chips remained at 10 °C until reading. The chips were read on the QuantStudio3D Digital PCR Instrument and analyzed with QuantStudio 3D Analysis Suite Cloud. I calculated the number of gene copies per diploid genome (CN) as follows:

$$\mathrm{CN} = 2\left(\frac{\text{Copies per μl } \textit{CHIA}}{\text{Copies per μl RNase P}}\right)$$

I divided the measure of copies/μl for the custom assay, *mCHIA* or *hCHIA* in this case, by the copies/μl measured by the reference assay, RNase P, and multiplied by 2 to arrive at the total number of copies per diploid genome.

**Data Availability** Gene sequences were generated for a previous study (Janiak et al. 2018) and are deposited in GenBank. The accession numbers for species included in this study are provided in ESM Table SI. The sequence alignments used to design primers are provided in ESM Table SII.

## Ethical Note

This research was approved by the Rutgers University Institutional Animal Care and Use Committee(Protocol #13-043). I have no conflict of interest to declare with respect to the content in this article.

## Results

The *mCHIA* and *hCHIA* TaqMan assays successfully amplified the loci of interest in all species I studied. Absolute numbers amplified by the custom and reference assays ranged from 224.2 to 1961.6 copies/μl (Table III, Table SIII). Measurements for the numbers of copies per microliter and per reaction were very variable between species, but very similar within species across the different assays (Figs. 1 and 2, Table SIII). There was no difference in the copies per microliter detected by the custom assay and the copies/μl detected by the

**Table III** QuantStudio 3D copy number results (copies/μl of DNA, mean and 95% confidence interval) using custom assays for *mCHIA* and *hCHIA* genes and an RNase P reference assay for 10 primate species

| Gene | Species | Copies/μl | | | |
|---|---|---|---|---|---|
| | | Custom assay | | Reference assay | |
| | | Mean | 95% CI | Mean | 95% CI |
| *mCHIA* | *Saimiri sciureus* | 1042.00 | 1027.2–1057.0 | 1061.80 | 1046.8–1077.0 |
| | *Sapajus apella* | 1580.40 | 1559.7–1601.4 | 1603.50 | 1582.5–1624.8 |
| | *Saguinus fuscicollis* | 294.63 | 287.82–301.61 | 285.11 | 278.42–291.96 |
| | *Callicebus moloch* | 567.50 | 557.47–577.71 | 579.61 | 569.45–589.95 |
| | *Callithrix jacchus* | 1637.90 | 1616.5–1659.5 | 1606.60 | 1585.7–1627.9 |
| | *Erythrocebus patas* | 1961.60 | 1940.5–1982.9 | 1864.50 | 1844.0–1885.3 |
| | *Macaca nigra* | 580.39 | 570.18–590.77 | 591.48 | 581.16–601.99 |
| | *Macaca mulatta* | 231.92 | 225.95–238.05 | 224.21 | 218.35–230.23 |
| | *Macaca nemestrina* | 1376.60 | 1358.1–1395.4 | 1402.30 | 1383.5–1421.3 |
| | *Miopithecus talapoin* | 1547.50 | 1527.4–1567.9 | 1567.00 | 1546.6–1587.6 |
| *hCHIA* | *Saimiri sciureus* | 1116.40 | 1100.8–1132.2 | 1119.00 | 1103.3–1134.8 |
| | *Saguinus fuscicollis* | 313.86 | 306.88–321.01 | 308.76 | 301.84–315.84 |
| | *Callithrix jacchus* | 1345.00 | 1326.8–1363.5 | 1348.20 | 1329.9–1366.7 |

RNase P reference assay for any of the samples (Figs. 1 and 2, Table SIII). For all species in the study, the total number of copies calculated for *mCHIA* and *hCHIA* was closest to two copies per diploid genome (Fig. 3).

## Discussion

I found no evidence of CNV in the primate *CHIA* genes, *mCHIA* and *hCHIA*, in any of the 10 primate species I studied, suggesting that they all have only two gene copies per diploid genome. This does not support my prediction that more insectivorous primates would have higher copy numbers of *mCHIA* and *hCHIA* than less insectivorous primates.

Although previous work on other enzymes suggests that higher copy numbers may be a useful way of increasing the expression of a digestive enzyme (Axelsson et al. 2013; Mandel et al. 2010; Mandel and Breslin 2012; Perry et al. 2007), my results suggest this may not be the case for acidic mammalian chitinase. However, an important caveat to this conclusion is that I tested only one individual for most species (I included two *Erythrocebus patas* individuals), so additional research is needed to test whether the result applies more generally.

That said, a possible explanation for this negative result is that an increase in acidic mammalian chitinase expression may not be necessary for some of the species in my sample. According to the Jarman–Bell principle, an animal's nutritional requirements negatively correlate with body mass (Bell 1971; Jarman 1974). Larger-bodied animals, although needing an absolutely larger amount of energy, have a relatively lower nutrient and energy requirement per unit body mass compared to smaller-bodied

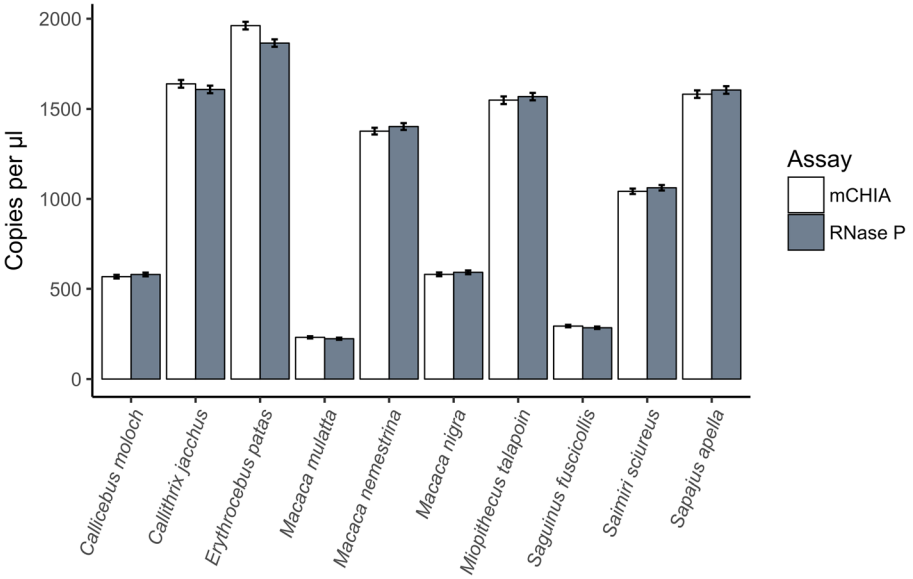


**Fig. 1** Mean number of copies of *mCHIA* and an RNase P reference assay per microliter of DNA in 10 primate species. Whiskers indicate 95% confidence intervals.

animals, meaning that they can afford to subsist on poorer quality, but abundant, foods. Smaller-bodied animals require less energy in absolute terms, but need to focus on high-quality foods to meet their relatively higher nutrient demands (Bell 1971; Jarman 1974). Hence, the ability to collect and consume a large amount of food is the main

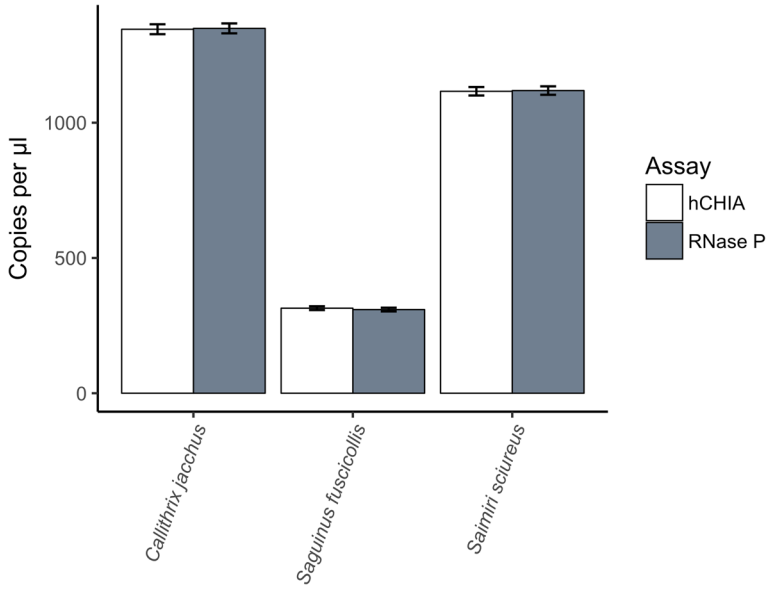


**Fig. 2** Mean number of copies of *hCHIA* and an RNase P reference assay per microliter of DNA in three primate species. Whiskers indicate 95% confidence intervals.

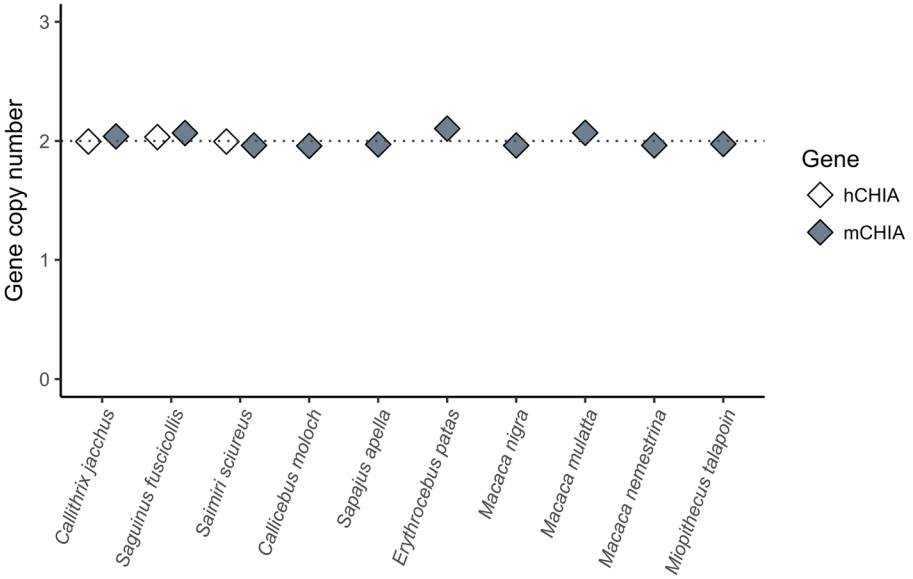


**Fig. 3** Relative copy number of *mCHIA* and *hCHIA* per diploid genome in 10 primate species. The relative copy number is estimated based on comparison with an RNase P reference assay, which detects *RPPH1*, a gene that is known to have only two copies per diploid genome. The dotted line indicates a gene copy number of two per diploid genome.

challenge for large-bodied animals, whereas for smaller-bodied animals it is the efficient and quick extraction of nutrients from their foods (Gaulin 1979).

Insects are a high-quality food (Raubenheimer et al. 2014; Rothman et al. 2014), but with the exception of social insects, they are scattered across the environment, and their chitinous exoskeletons may pose a digestive challenge (Rothman et al. 2014; Strait and Vincent 1998). Hence, smaller primates are expected to rely heavily on insects, as long as they can effectively cope with their exoskeletons, while insectivory in larger primates should focus on social insects, such as ants and termites (Fleagle 2013; Gaulin 1979; Isbell 1998; Kay 1984). The finding that all cercopithecines, regardless of insect consumption, have only one functional *CHIA* paralog, *mCHIA*, and possibly no CNV may therefore be consistent with primatological theory (Gaulin 1979). *Erythrocebus patas* and *Macaca nigra* are among the Old World monkeys with the highest mean insect consumption, but a mean adult female body mass of 6500 g and 5470 g, respectively (Smith and Jungers 1997). This is well above the 500 g threshold associated with insectivorous primates (Fleagle 2013; Gingerich 1980; Kay 1984), and maximally efficient digestion of their insect prey is likely to be less important for these cercopithecines than for a smaller primate (Gaulin 1979). Even *Miopithecus talapoin*, which is the smallest Old World monkey (Fleagle 2013) at 1120 g (Smith and Jungers 1997), is above this threshold and larger than many New World monkeys. The amount of acidic mammalian chitinase expressed from a single *CHIA* paralog may thus be sufficient to digest insect prey adequately, even for more insectivorous Old World monkeys.

New World monkeys tend to be both more insectivorous and smaller-bodied than Old World monkeys (Fleagle 2013; Gaulin 1979). In line with predictions of the

Jarman–Bell principle and Kay's threshold (Bell 1971; Gingerich 1980; Jarman 1974; Kay 1984), previous work showed that some species retain two functional *CHIA* paralogs, theoretically doubling their ability to digest insect exoskeletons with acidic mammalian chitinase (Janiak et al. 2018). Platyrrhine species that do not retain the second *CHIA* paralog also tend to be less insectivorous (Janiak et al. 2018). One exception to this is the *Sapajus apella*, which retains only one functional *CHIA* gene, *mCHIA*, despite a fairly insectivorous diet (Table I). Results of the CNV analyses show that this is not made up for by additional copies of the *mCHIA* gene (Figs. 1 and 3). Possibly, this is due to the comparatively large body size of *Sapajus apella*. Adult females have a mean body mass of 2520 g, much more than similarly insectivorous platyrrhine species, such as the *Saimiri sciureus* (662 g) or the *Saguinus fuscicollis* (358 g) (Smith and Jungers 1997), which retain two functional *CHIA* paralogs (Janiak et al. 2018). However, the closely related *Cebus capucinus* retains two functional *CHIA* paralogs (Janiak et al. 2018), despite having similar levels of insect consumption (Chapman and Fedigan 1990; Mallott 2016; McCabe and Fedigan 2007; Rose 1994) and being almost identical in size to *Sapajus apella* (Smith and Jungers 1997). What causes this difference in genotype is currently unclear.

Although the results of this study suggest that the *CHIA* genes in primates are not subject to CNV, it is plausible that acidic mammalian chitinase enzyme expression is up- or downregulated through another mechanism, such as variable mRNA expression in response to feeding behaviors (Tabata et al. 2018). A controlled feeding experiment in birds show that expression of intestinal enzymes is somewhat plastic in response to diet changes, while expression of pancreatic enzymes is not, suggesting that pancreatic enzyme expression is under stricter genetic control (Brzęk et al. 2013). Therefore, CNV as a mechanism for upregulation may be more likely in pancreatic enzymes, such as amylase, as opposed to intestinal enzymes such as acidic mammalian chitinase. In addition, increased copy number does not always lead to increased protein expression, and the effects of gene duplications are often more complicated (Dennis et al. 2012), and my results cannot exclude the possibility of partial CNV in another region of the *CHIA* genes. Future studies should investigate differences in *CHIA* promoter regions, mRNA expression in primate stomach tissues, and functional effects of polymorphisms in *CHIA* paralogs. Previous work showed that there are small interspecies differences in primate *mCHIA* and *hCHIA* sequences, from single nucleotide polymorphisms to multi-basepair insertions and deletions (Janiak et al. 2018). The effects, if any, these changes have on the resulting protein and its functionality are unclear and an avenue for future research.

## Conclusion

I investigated CNV in the *CHIA* paralogs, *mCHIA* and *hCHIA*, in Old World and New World primate species with varying levels of insect consumption. I found no evidence that the *CHIA* genes had higher copy numbers in more insectivorous primate species. All primate species I studied had two gene copies per diploid genome, suggesting that CNV is not commonly found in primate *CHIA* genes, but additional research with a larger sample size per species is needed to confirm this.

**Acknowledgements** I thank the editor and two anonymous reviewers for their thoughtful and constructive comments. This work was supported by funding from the Center for Human Evolutionary Studies at Rutgers University, the International Primatological Society, and a National Science Foundation DDRIG (Award #1650864). I thank Dr. P. J. Perry, Dr. Todd Disotell, and Coriell Institute (Repository numbers: NG05311, NG05313, NG06115, NG07101, NG07107, NG07921) for providing samples. Drs. Rob Scott, Erin Vogel, and Ryne Palombit provided helpful feedback on earlier versions of this article.

## References

Alonso, C., & Langguth, A. (1989). Ecologia e comportamento de *Callithrix jacchus* (Primates: Callitrichidae) numa ilha de floresta Atlântica. *Revista Nordestina de Biologia, 6*(2), 105–137.

Axelsson, E., Ratnakumar, A., Arendt, M.-L., Maqbool, K., Webster, M. T., Perloski, M., Liberg, O., Arnemo, J. M., Hedhammar, Å., & Lindblad-Toh, K. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature, 495*(7441), 360–364.

Behringer, V., Borchers, C., Deschner, T., Möstl, E., Selzer, D., & Hohmann, G. (2013). Measurements of salivary alpha amylase and salivary cortisol in hominoid primates reveal within-species consistency and between-species differences. *PLoS One, 8*(4), e60773.

Bell, R. H. V. (1971). A grazing ecosystem in the Serengeti. *Scientific American, 225*(1), 86–93.

Bickhart, D. M., Hou, Y., Schroeder, S. G., Alkan, C., Cardone, M. F., Matukumalli, L. K., Song, J., Schnabel, R. D., Ventura, M., Taylor, J. F., Garcia, J. F., van Tassell, C. P., Sonstegard, T. S., Eichler, E. E., & Liu, G. E. (2012). Copy number variation of individual cattle genomes using next-generation sequencing. *Genome Research, 22*(4), 778–790.

Boot, R. G., Blommaart, E. F., Swart, E., Ghauharali-van der Vlugt, K., Bijl, N., et al (2001). Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *Journal of Biological Chemistry, 276*(9), 6770–6778.

Boot, R. G., Bussink, A. P., Verhoek, M., de Boer, P. A. J., Moorman, A. F. M., & Aerts, J. M. F. G. (2005). Marked differences in tissue-specific expression of chitinases in mouse and man. *Journal of Histochemistry and Cytochemistry, 53*(10), 1283–1292.

Brown, A. D., & Zunino, G. E. (1990). Dietary variability in *Cebus apella* in extreme habitats: Evidence for adaptability. *Folia Primatologica, 54*(3–4), 187–195.

Brzęk, P., Ciminari, M. E., Kohl, K. D., Lessner, K., Karasov, W. H., & Caviedes-Vidal, E. (2013). Effect of age and diet composition on activity of pancreatic enzymes in birds. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 183*(5), 685–697.

Caldecott, J. O. (1986). An ecological and behavioural study of the pig-tailed macaque. *Contributions to Primatology, 21*, 1–259.

Cantsilieris, S., & White, S. J. (2013). Correlating multiallelic copy number polymorphisms with disease susceptibility. *Human Mutation, 34*(1), 1–13.

Chapman, C. A., & Fedigan, L. M. (1990). Dietary differences between neighboring *Cebus capucinus* groups: Local traditions, food availability or responses to food profitability. *Folia Primatologica, 54*(3–4), 177–186.

Cheng, Z., Ventura, M., She, X., Khaitovich, P., Graves, T., Osoegawa, K., Church, D., DeJong, P., Wilson, R. K., Pääbo, S., Rocchi, M., & Eichler, E. E. (2005). A genome-wide comparison of recent chimpanzee and human segmental duplications. *Nature, 437*(7055), 88–93.

Clop, A., Vidal, O., & Amills, M. (2012). Copy number variation in the genomes of domestic animals. *Animal Genetics, 43*(5), 503–517.

Crandlemire-Sacco, J. (1988). An ecological comparison of two sympatric primates: *Saguinus fuscicollis* and *Callicebus moloch* of Amazonian Peru. *Primates, 29*(4), 465–475.

Dennis, M. Y., Nuttle, X., Sudmant, P. H., Antonacci, F., Graves, T. A., Nefedov, M., Rosenfeld, J. A., Sajjadian, S., Malig, M., Kotkiewicz, H., Curry, C. J., Shafer, S., Shaffer, L. G., de Jong, P. J., Wilson, R. K., & Eichler, E. E. (2012). Evolution of human-specific neural *SRGAP2* genes by incomplete segmental duplication. *Cell, 149*(4), 912–922.

Digby, L. J., Ferrari, S. F., & Saltzman, W. (2011). Callitrichines. In C. J. Campbell, A. Fuentes, K. C. MacKinnon, S. K. Bearder, & R. M. Stumpf (Eds.), *Primates in perspective* (pp. 91–107). Oxford: Oxford University Press.

Dumas, L., Kim, Y. H., Karimpour-Fard, A., Cox, M., Hopkins, J., Pollack, J. R., & Sikela, J. M. (2007). Gene copy number variation spanning 60 million years of human and primate evolution. *Genome Research, 17*(9), 1266–1277.

Elferink, M. G., Vallée, A. A. A., Jungerius, A. P., Crooijmans, R. P. M. A., & Groenen, M. A. M. (2008). Partial duplication of the *PRLR* and *SPEF2* genes at the late feathering locus in chicken. *BMC Genomics, 9*, 391.

Enattah, N. S., Jensen, T. G. K., Nielsen, M., Lewinski, R., Kuokkanen, M., Rasinpera, H., el-Shanti, H., Seo, J. K., Alifrangis, M., Khalil, I. F., Natah, A., Ali, A., Natah, S., Comas, D., Mehdi, S. Q., Groop, L., Vestergaard, E. M., Imtiaz, F., Rashed, M. S., Meyer, B., Troelsen, J., & Peltonen, L. (2008). Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *American Journal of Human Genetics, 82*(1), 57–72.

Fleagle, J. G. (2013). *Primate adaptation and evolution*, 3rd ed. San Diego: Academic Press.

Fontanesi, L., Beretti, F., Riggio, V., González, E. G., Dall'Olio, S., et al (2009). Copy number variation and missense mutations of the agouti signaling protein (*ASIP*) gene in goat breeds with different coat colors. *Cytogenetic and Genome Research, 126*(4), 333–347.

Fontanesi, L., Dall'Olio, S., Beretti, F., Portolano, B., & Russo, V. (2011). Coat colours in the Massese sheep breed are associated with mutations in the agouti signalling protein (*ASIP*) and melanocortin 1 receptor (*MC1R*) genes. *Animal, 5*(1), 8–17.

Garber, P. A. (1988). Diet, foraging patterns, and resource defense in a mixed species troop of *Saguinus mystax* and *Saguinus fuscicollis* in Amazonian Peru. *Behaviour, 105*(1), 18–34.

Gaulin, S. J. (1979). A Jarman/bell model of primate feeding niches. *Human Ecology, 7*(1), 1–20.

Gautier-Hion, A. (1988). Diet and dietary habits of forest guenons. In *A primate radiation: Evolutionary biology of the African guenons* (pp. 257–283). Cambridge: Cambridge University Press.

Gazave, E., Darre, F., Morcillo-Suarez, C., Petit-Marty, N., Carreno, A., Marigorta, U. M., Ryder, O. A., Blancher, A., Rocchi, M., Bosch, E., Baker, C., Marques-Bonet, T., Eichler, E. E., & Navarro, A. (2011). Copy number variation analysis in the great apes reveals species-specific patterns of structural variation. *Genome Research, 21*(10), 1626–1639.

Gingerich, P. D. (1980). Dental and cranial adaptations in Eocene Adapidae. *Zeitschrift für Morphologie und Anthropologie, 71*(2), 135–142.

Giuffra, E., Evans, G., Törnsten, A., Wales, R., Day, A., Looft, H., Plastow, G., & Andersson, L. (1999). The belt mutation in pigs is an allele at the dominant white (I/KIT) locus. *Mammalian Genome, 10*(12), 1132–1136.

Gokcumen, O., Babb, P. L., Iskow, R. C., Zhu, Q., Shi, X., Mills, R. E., Ionita-Laza, I., Vallender, E. J., Clark, A. G., Johnson, W. E., & Lee, C. (2011). Refinement of primate copy number variation hotspots identifies candidate genomic regions evolving under positive selection. *Genome Biology, 12*(5), R52.

Gokcumen, O., Tischler, V., Tica, J., Zhu, Q., Iskow, R. C., Lee, E., Fritz, M. H. Y., Langdon, A., Stutz, A. M., Pavlidis, P., Benes, V., Mills, R. E., Park, P. J., Lee, C., & Korbel, J. O. (2013). Primate genome architecture influences structural variation mechanisms and functional consequences. *Proceedings of the National Academy of Sciences of the USA, 110*(39), 15764–15769.

Goldstein, S. J., & Richard, A. F. (1989). Ecology of rhesus macaques (*Macaca mulatta*) in Northwest Pakistan. *International Journal of Primatology, 10*(6), 531–567.

Gschwind, A. R., Singh, A., Certa, U., Reymond, A., & Heckel, T. (2017). Diversity and regulatory impact of copy number variation in the primate *Macaca fascicularis*. *BMC Genomics, 18*, 144.

Hardwick, R. J., Machado, L. R., Zuccherato, L. W., Antolinos, S., Xue, Y., Shawa, N., Gilman, R. H., Cabrera, L., Berg, D. E., Tyler-Smith, C., Kelly, P., Tarazona-Santos, E., & Hollox, E. J. (2011). A worldwide analysis of beta-defensin copy number variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. *Human Mutation, 32*(7), 743–750.

Hollox, E. J., Armour, J. A. L., & Barber, J. C. K. (2003). Extensive normal copy number variation of a beta-defensin antimicrobial-gene cluster. *American Journal of Human Genetics, 73*(3), 591–600.

Ingram, C. J. E., Mulcare, C. A., Itan, Y., Thomas, M. G., & Swallow, D. M. (2009). Lactose digestion and the evolutionary genetics of lactase persistence. *Human Genetics, 124*(6), 579–591.

Isbell, L. A. (1998). Diet for a small primate: Insectivory and gummivory in the (large) patas monkey (*Erythrocebus patas pyrrhonotus*). *American Journal of Primatology, 45*(4), 381–398.

Iskow, R. C., Gokcumen, O., Abyzov, A., Malukiewicz, J., Zhu, Q., Sukumar, A. T., Pai, A. A., Mills, R. E., Habegger, L., Cusanovich, D. A., Rubel, M. A., Perry, G. H., Gerstein, M., Stone, A. C., Gilad, Y., & Lee, C. (2012). Regulatory element copy number differences shape primate expression profiles. *Proceedings of the National Academy of Sciences of the USA, 109*(31), 12656–12661.

Itan, Y., Powell, A., Beaumont, M. A., Burger, J., & Thomas, M. G. (2009). The origins of lactase persistence in Europe. *PLoS Computational Biology, 5*(8), e1000491.

Itan, Y., Jones, B. L., Ingram, C. J., Swallow, D. M., & Thomas, M. G. (2010). A worldwide correlation of lactase persistence phenotype and genotypes. *BMC Evolutionary Biology, 10*(1), 36.

Jacquemont, S., Reymond, A., Zufferey, F., Harewood, L., Walters, R. G., Kutalik, Z., Martinet, D., Shen, Y., Valsesia, A., Beckmann, N. D., Thorleifsson, G., Belfiore, M., Bouquillon, S., Campion, D., de Leeuw, N., de Vries, B. B. A., Esko, T., Fernandez, B. A., Fernández-Aranda, F., Fernández-Real, J. M., Gratacòs, M., Guilmatre, A., Hoyer, J., Jarvelin, M. R., Frank Kooy, R., Kurg, A., le Caignec, C., Männik, K., Platt, O. S., Sanlaville, D., van Haelst, M. M., Villatoro Gomez, S., Walha, F., Wu, B. L., Yu, Y., Aboura, A., Addor, M. C., Alembik, Y., Antonarakis, S. E., Arveiler, B., Barth, M., Bednarek, N., Béna, F., Bergmann, S., Beri, M., Bernardini, L., Blaumeiser, B., Bonneau, D., Bottani, A., Boute, O., Brunner, H. G., Cailley, D., Callier, P., Chiesa, J., Chrast, J., Coin, L., Coutton, C., Cuisset, J. M., Cuvellier, J. C., David, A., de Freminville, B., Delobel, B., Delrue, M. A., Demeer, B., Descamps, D., Didelot, G., Dieterich, K., Disciglio, V., Doco-Fenzy, M., Drunat, S., Duban-Bedu, B., Dubourg, C., el-Sayed Moustafa, J. S., Elliott, P., Faas, B. H. W., Faivre, L., Faudet, A., Fellmann, F., Ferrarini, A., Fisher, R., Flori, E., Forer, L., Gaillard, D., Gerard, M., Gieger, C., Gimelli, S., Gimelli, G., Grabe, H. J., Guichet, A., Guillin, O., Hartikainen, A. L., Heron, D., Hippolyte, L., Holder, M., Homuth, G., Isidor, B., Jaillard, S., Jaros, Z., Jiménez-Murcia, S., Joly Helas, G., Jonveaux, P., Kaksonen, S., Keren, B., Kloss-Brandstätter, A., Knoers, N. V. A. M., Koolen, D. A., Kroisel, P. M., Kronenberg, F., Labalme, A., Landais, E., Lapi, E., Layet, V., Legallic, S., Leheup, B., Leube, B., Lewis, S., Lucas, J., MacDermot, K. D., Magnusson, P., Marshall, C., Mathieu-Dramard, M., McCarthy, M. I., Meitinger, T., Antonietta Mencarelli, M., Merla, G., Moerman, A., Mooser, V., Morice-Picard, F., Mucciolo, M., Nauck, M., Coumba Ndiaye, N., Nordgren, A., Pasquier, L., Petit, F., Pfundt, R., Plessis, G., Rajcan-Separovic, E., Paolo Ramelli, G., Rauch, A., Ravazzolo, R., Reis, A., Renieri, A., Richart, C., Ried, J. S., Rieubland, C., Roberts, W., Roetzer, K. M., Rooryck, C., Rossi, M., Saemundsen, E., Satre, V., Schurmann, C., Sigurdsson, E., Stavropoulos, D. J., Stefansson, H., Tengström, C., Thorsteinsdóttir, U., Tinahones, F. J., Touraine, R., Vallée, L., van Binsbergen, E., van der Aa, N., Vincent-Delorme, C., Visvikis-Siest, S., Vollenweider, P., Völzke, H., Vulto-van Silfhout, A. T., Waeber, G., Wallgren-Pettersson, C., Witwicki, R. M., Zwolinksi, S., Andrieux, J., Estivill, X., Gusella, J. F., Gustafsson, O., Metspalu, A., Scherer, S. W., Stefansson, K., Blakemore, A. I. F., Beckmann, J. S., & Froguel, P. (2011). Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature, 478*(7367), 97–102.

Janiak, M. C., Chaney, M. E., & Tosi, A. J. (2018). Evolution of acidic mammalian chitinase genes (*CHIA*) is related to body mass and insectivory in primates. *Molecular Biology and Evolution, 35*(3), 607–622.

Jarman, P. J. (1974). The social organisation of antelope in relation to their ecology. *Behaviour, 48*(1), 215–267.

Kay, R. F. (1984). On the use of anatomical features to infer foraging behavior in extinct primates. In J. Cant & P. S. Rodman (Eds.), *Adaptations for foraging in nonhuman primates* (pp. 21–53). New York: Columbia University Press.

Knogge, C., & Heymann, E. W. (2003). Seed dispersal by sympatric tamarins, *Saguinus mystax* and *Saguinus fuscicollis*: Diversity and characteristics of plant species. *Folia Primatologica, 74*(1), 33–47.

Krykbaev, R., Fitz, L. J., Reddy, P. S., Winkler, A., Xuan, D., Yang, X., Fleming, M., & Wolf, S. F. (2010). Evolutionary and biochemical differences between human and monkey acidic mammalian chitinases. *Gene, 452*(2), 63–71.

Lawrence, J. M. (2007). *Understanding the pair bond in brown titi monkeys* (Callicebus brunneus): *Male and female reproductive interests*. Ph.D. thesis, Columbia University, New York.

Lee, A. S., Gutiérrez-Arcelus, M., Perry, G. H., Vallender, E. J., Johnson, W. E., Miller, G. M., Korbel, J. O., & Lee, C. (2008). Analysis of copy number variation in the rhesus macaque genome identifies candidate loci for evolutionary and human disease studies. *Human Molecular Genetics, 17*(8), 1127–1136.

Leonard, B. C., Marks, S. L., Outerbridge, C. A., Affolter, V. K., Kananurak, A., Young, A., Moore, P. F., Bannasch, D. L., & Bevins, C. L. (2012). Activity, expression and genetic variation of canine ß-defensin 103: A multifunctional antimicrobial peptide in the skin of domestic dogs. *Journal of Innate Immunity, 4*(3), 248–259.

Lima, E. M., & Ferrari, S. F. (2003). Diet of a free-ranging group of squirrel monkeys (*Saimiri sciureus*) in eastern Brazilian Amazonia. *Folia Primatologica, 74*(3), 150–158.

Linzmeier, R. M., & Ganz, T. (2006). Copy number polymorphisms are not a common feature of innate immune genes. *Genomics, 88*(1), 122–126.

Malhotra, D., & Sebat, J. (2012). CNVs: Harbingers of a rare variant revolution in psychiatric genetics. *Cell, 148*(6), 1223–1241.

Mallott, E. K. (2016). *Social, ecological, and developmental influences on fruit and invertebrate foraging strategies and gut microbial communities in white-faced capuchins* (*Cebus capucinus*). Ph.D. thesis, University of Illinois at Urbana-Champaign.

Mandel, A. L., & Breslin, P. A. S. (2012). High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. *Journal of Nutrition, 142*(5), 853–858.

Mandel, A. L., Peyrot des Gachons, C., Plank, K. L., Alarcon, S., & Breslin, P. A. S. (2010). Individual differences in *AMY1* gene copy number, salivary α-amylase levels, and the perception of oral starch. *PLoS One, 5*(10), e13352.

Marques-Bonet, T., Kidd, J. M., Ventura, M., Graves, T. A., Cheng, Z., Hillier, L. D. W., Jiang, Z., Baker, C., Malfavon-Borja, R., Fulton, L. A., Alkan, C., Aksay, G., Girirajan, S., Siswara, P., Chen, L., Cardone, M. F., Navarro, A., Mardis, E. R., Wilson, R. K., & Eichler, E. E. (2009). A burst of segmental duplications in the genome of the African great ape ancestor. *Nature, 457*(7231), 877–881.

McCabe, G. M., & Fedigan, L. M. (2007). Effects of reproductive status on energy intake, ingestion rates, and dietary composition of female *Cebus capucinus* at Santa Rosa, Costa Rica. *International Journal of Primatology, 28*(4), 837–851.

Nakagawa, N. (1989). Activity budget and diet of patas monkeys in Kala Maloue National Park, Cameroon: A preliminary report. *Primates, 30*(1), 27–34.

Narita, Y., Oda, S.-I., Takenaka, O., & Kageyama, T. (2000). Gastric digestive proteinases of the greater horseshoe bat, *Rhinolophus ferrumequinum*. *Mammal Study, 25*(1), 17–26.

Narita, Y., Oda, S.-I., Takenaka, O., & Kageyama, T. (2010). Lineage-specific duplication and loss of pepsinogen genes in hominoid evolution. *Journal of Molecular Evolution, 70*(4), 313–324.

O'Brien, T. G., & Kinnaird, M. F. (1997). Behavior, diet, and movements of the Sulawesi crested black macaque (*Macaca nigra*). *International Journal of Primatology, 18*(3), 321–351.

Ohno, M., Kimura, M., Miyazaki, H., Okawa, K., Onuki, R., Nemoto, C., Tabata, E., Wakita, S., Kashimura, A., Sakaguchi, M., Sugahara, Y., Nukina, N., Bauer, P. O., & Oyama, F. (2016). Acidic mammalian chitinase is a proteases-resistant glycosidase in mouse digestive system. *Scientific Reports, 6*, 37756.

Okawa, K., Ohno, M., Kashimura, A., Kimura, M., Kobayashi, Y., Sakaguchi, M., Sugahara, Y., Kamaya, M., Kino, Y., Bauer, P. O., & Oyama, F. (2016). Loss and gain of human acidic mammalian chitinase activity by nonsynonymous SNPs. *Molecular Biology and Evolution, 33*(12), 3183–3193.

Perry, G. H., Tchinda, J., McGrath, S. D., Zhang, J., Picker, S. R., Caceres, A. M., Iafrate, A. J., Tyler-Smith, C., Scherer, S. W., Eichler, E. E., Stone, A. C., & Lee, C. (2006). Hotspots for copy number variation in chimpanzees and humans. *Proceedings of the National Academy of Sciences of the USA, 103*(21), 8006–8011.

Perry, G. H., Dominy, N. J., Claw, K. G., Lee, A. S., Fiegler, H., Redon, R., Werner, J., Villanea, F. A., Mountain, J. L., Misra, R., Carter, N. P., Lee, C., & Stone, A. C. (2007). Diet and the evolution of human amylase gene copy number variation. *Nature Genetics, 39*(10), 1256–1260.

Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., Regan, R., Conroy, J., Magalhaes, T. R., Correia, C., Abrahams, B. S., Almeida, J., Bacchelli, E., Bader, G. D., Bailey, A. J., Baird, G., Battaglia, A., Berney, T., Bolshakova, N., Bölte, S., Bolton, P. F., Bourgeron, T., Brennan, S., Brian, J., Bryson, S. E., Carson, A. R., Casallo, G., Casey, J., Chung, B. H. Y., Cochrane, L., Corsello, C., Crawford, E. L., Crossett, A., Cytrynbaum, C., Dawson, G., de Jonge, M., Delorme, R., Drmic, I., Duketis, E., Duque, F., Estes, A., Farrar, P., Fernandez, B. A., Folstein, S. E., Fombonne, E., Freitag, C. M., Gilbert, J., Gillberg, C., Glessner, J. T., Goldberg, J., Green, A., Green, J., Guter, S. J., Hakonarson, H., Heron, E. A., Hill, M., Holt, R., Howe, J. L., Hughes, G., Hus, V., Igliozzi, R., Kim, C., Klauck, S. M., Kolevzon, A., Korvatska, O., Kustanovich, V., Lajonchere, C. M., Lamb, J. A., Laskawiec, M., Leboyer, M., le Couteur, A., Leventhal, B. L., Lionel, A. C., Liu, X. Q., Lord, C., Lotspeich, L., Lund, S. C., Maestrini, E., Mahoney, W., Mantoulan, C., Marshall, C. R., McConachie, H., McDougle, C. J., McGrath, J., McMahon, W. M., Merikangas, A., Migita, O., Minshew, N. J., Mirza, G. K., Munson, J., Nelson, S. F., Noakes, C., Noor, A., Nygren, G., Oliveira, G., Papanikolaou, K., Parr, J. R., Parrini, B., Paton, T., Pickles, A., Pilorge, M., Piven, J., Ponting, C. P., Posey, D. J., Poustka, A., Poustka, F., Prasad, A., Ragoussis, J., Renshaw, K., Rickaby, J., Roberts, W., Roeder, K., Roge, B., Rutter, M. L., Bierut, L. J., Rice, J. P., Salt, J., Sansom, K., Sato, D., Segurado, R., Sequeira, A. F., Senman, L., Shah, N., Sheffield, V. C., Soorya, L., Sousa, I., Stein, O., Sykes, N., Stoppioni, V., Strawbridge, C., Tancredi, R., Tansey, K., Thiruvahindrapduram, B., Thompson, A. P., Thomson, S., Tryfon, A., Tsiantis, J., van Engeland, H., Vincent, J. B., Volkmar, F., Wallace, S., Wang, K., Wang, Z., Wassink, T. H., Webber, C., Weksberg, R., Wing, K., Wittemeyer, K., Wood, S., Wu, J., Yaspan, B. L., Zurawiecki, D., Zwaigenbaum, L., Buxbaum, J. D., Cantor, R. M., Cook, E. H., Coon, H., Cuccaro, M. L., Devlin, B., Ennis, S., Gallagher, L., Geschwind, D. H., Gill, M., Haines, J. L., Hallmayer, J., Miller, J., Monaco, A. P., Nurnberger Jr, J. I., Paterson, A. D., Pericak-Vance, M. A., Schellenberg, G. D., Szatmari, P., Vicente, A. M., Vieland, V. J., Wijsman, E. M., Scherer, S. W., Sutcliffe, J. S., & Betancur, C. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. *Nature, 466*(7304), 368–372.

Porter, L. M. (2001). Dietary differences among sympatric Callitrichinae in northern Bolivia: *Callimico goeldii*, *Saguinus fuscicollis* and *S. labiatus*. *International Journal of Primatology, 22*(6), 961–992.

Ranciaro, A., Campbell, M. C., Hirbo, J. B., Ko, W.-Y., Froment, A., Anagnostou, P., Kotze, M. J., Ibrahim, M., Nyambo, T., Omar, S. A., & Tishkoff, S. A. (2014). Genetic origins of lactase persistence and the spread of pastoralism in Africa. *American Journal of Human Genetics, 94*(4), 496–510.

Raubenheimer, D., Rothman, J. M., Pontzer, H., & Simpson, S. J. (2014). Macronutrient contributions of insects to the diets of hunter-gatherers: A geometric analysis. *Journal of Human Evolution, 71*(C), 70–76.

Reese, T. A., Liang, H.-E., Tager, A. M., Luster, A. D., Van Rooijen, N., et al (2007). Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature, 447*(7140), 92–96.

Rose, L. M. (1994). Sex differences in diet and foraging behavior in white-faced capuchins (*Cebus capucinus*). *International Journal of Primatology, 15*(1), 95–114.

Rothman, J. M., Raubenheimer, D., Bryer, M. A. H., Takahashi, M., & Gilbert, C. C. (2014). Nutritional contributions of insects to primate diets: Implications for primate evolution. *Journal of Human Evolution, 71*(C), 59–69.

Schlebusch, C. M., Sjödin, P., Skoglund, P., & Jakobsson, M. (2013). Stronger signal of recent selection for lactase persistence in Maasai than in Europeans. *European Journal of Human Genetics, 21*(5), 550–553.

Seibold, M. A., Reese, T. A., Choudhry, S., Salam, M. T., Beckman, K., Eng, C., Atakilit, A., Meade, K., Lenoir, M., Watson, H. G., Thyne, S., Kumar, R., Weiss, K. B., Grammer, L. C., Avila, P., Schleimer, R. P., Fahy, J. V., Rodriguez-Santana, J., Rodriguez-Cintron, W., Boot, R. G., Sheppard, D., Gilliland, F. D., Locksley, R. M., & Burchard, E. G. (2009). Differential enzymatic activity of common haplotypic versions of the human acidic mammalian chitinase protein. *Journal of Biological Chemistry, 284*(29), 19650–19658.

Smith, R. J., & Jungers, W. L. (1997). Body mass in comparative primatology. *Journal of Human Evolution, 32*(6), 523–559.

Stankiewicz, P., & Lupski, J. R. (2010). Structural variation in the human genome and its role in disease. *Annual Review of Medicine, 61*, 437–455.

Stone, A. I. (2007). Responses of squirrel monkeys to seasonal changes in food availability in an eastern Amazonian forest. *American Journal of Primatology, 69*(2), 142–157.

Strait, S. G., & Vincent, J. (1998). Primate faunivores: Physical properties of prey items. *International Journal of Primatology, 19*(5), 867–878.

Strobel, S., Roswag, A., Becker, N. I., Trenczek, T. E., & Encarnação, J. A. (2013). Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. *PLoS One, 8*(9), e72770.

Sudmant, P. H., Huddleston, J., Catacchio, C. R., Malig, M., Hillier, L. W., Baker, C., Mohajeri, K., Kondova, I., Bontrop, R. E., Persengiev, S., Antonacci, F., Ventura, M., Prado-Martinez, J., Great Ape Genome Project, Marques-Bonet, T., & Eichler, E. E. (2013). Evolution and diversity of copy number variation in the great ape lineage. *Genome Research, 23*(9), 1373–1382.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Imamura, Y., Seki, S., Ueda, H., Matoska, V., Bauer, P. O., & Oyama, F. (2017a). Protease resistance of porcine acidic mammalian chitinase under gastrointestinal conditions implies that chitin-containing organisms can be sustainable dietary resources. *Scientific Reports, 7*(1), 12963.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Kino, Y., Matoska, V., Bauer, P. O., & Oyama, F. (2017b). Gastric and intestinal proteases resistance of chicken acidic chitinase nominates chitin-containing organisms for alternative whole edible diets for poultry. *Scientific Reports, 7*(1), 6662.

Tabata, E., Kashimura, A., Kikuchi, A., Masuda, H., Miyahara, R., Hiruma, Y., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Matoska, V., Bauer, P. O., & Oyama, F. (2018). Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs. *Scientific Reports, 8*(1), 1461.

Taggart, R. T., Mohandas, T. K., Shows, T. B., & Bell, G. I. (1985). Variable numbers of pepsinogen genes are located in the centromeric region of human chromosome 11 and determine the high-frequency electrophoretic polymorphism. *Proceedings of the National Academy of Sciences of the USA, 82*(18), 6240–6244.

Tishkoff, S. A., Reed, F. A., Ranciaro, A., Voight, B. F., Babbitt, C. C., Silverman, J. S., Powell, K., Mortensen, H. M., Hirbo, J. B., Osman, M., Ibrahim, M., Omar, S. A., Lema, G., Nyambo, T. B., Ghori, J., Bumpstead, S., Pritchard, J. K., Wray, G. A., & Deloukas, P. (2007). Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics, 39*(1), 31–40.

Wang, J., Wang, H., Jiang, J., Kang, H., Feng, X., Zhang, Q., & Liu, J. F. (2013). Identification of genome-wide copy number variations among diverse pig breeds using SNP genotyping arrays. *PLoS One, 8*(7), e68683.

Wright, P. C. (1985). *The cost and benefits of nocturnality for* Aotus trivirgatus *(the night monkey)*. Ph.D. thesis, City University of New York.

Wright, D., Boije, H., Meadows, J. R. S., Bed'hom, B., Gourichon, D., et al (2009). Copy number variation in intron 1 of *SOX5* causes the pea-comb phenotype in chickens. *PLoS Genetics, 5*(6), e1000512.

Yoshida, K., Go, Y., Kushima, I., Toyoda, A., Fujiyama, A., et al (2016). Single-neuron and genetic correlates of autistic behavior in macaque. *Science Advances, 2*(9), e1600558–e1600558.

Zarrei, M., MacDonald, J. R., Merico, D., & Scherer, S. W. (2015). A copy number variation map of the human genome. *Nature Reviews Genetics, 16*(3), 172–183.

Zhang, J., Zhang, Y.-P., & Rosenberg, H. F. (2002). Adaptive evolution of a duplicated pancreatic ribonuclease gene in a leaf-eating monkey. *Nature Genetics, 30*(4), 411–415.

Zhu, Z., Zheng, T., Homer, R. J., Kim, Y.-K., Chen, N. Y., Cohn, L., Hamid, Q., & Elias, J. A. (2004). Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science, 304*(5677), 1678–1682.