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Evolutionary divergence of neuroanatomical organization and related genes in chimpanzees and bonobos

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ABSTRACT

Given their close genetic relatedness to humans, bonobos (*Pan paniscus*) and chimpanzees (*Pan troglodytes*) offer an essential comparative framework for studying the evolution of uniquely human traits. These two species differ markedly in their socio-behavioral repertoires, which is reflected in neuroanatomical differences that have been reported in the literature. However, phylogenetic comparative methods have not yet been used to map the evolution of neuroanatomical traits in bonobos and chimpanzees, limiting our ability to understand which neural systems are derived in each species in relation to the last common ancestor of *Pan* (*Pan*-LCA). Here, we examine evolutionary changes in neuroanatomical traits of bonobos and chimpanzees relative to ancestral character reconstructions of the *Pan*-LCA using comparative datasets from hominoids. We found that bonobo brains are derived in showing reduction of whole brain and white matter volumes, with particularly striking reduction of male brain size compared to the inferred *Pan*-LCA value. Brain structures related to social cognition and emotional regulation, like the insular cortex and amygdala, display a mosaic pattern of evolution with certain traits changing to a greater extent in each species. Examination of potential genetic mechanisms underlying divergence of neural and social traits did not reveal clear differences in protein evolution patterns between the two species. These findings suggest that the brain anatomy of extant bonobos and chimpanzees show lineage-specific specializations and neither can be considered to more closely retain the ancestral state of *Pan*. Consequently, this raises questions about the extent that modern chimpanzees or bonobos may serve as referential models for the neuroanatomy of the LCA of humans and apes.

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1. Introduction

Since the split of modern humans, chimpanzees (*Pan troglodytes*), and bonobos (*Pan paniscus*) from their last common ancestor approximately 6–8 million years ago, the human brain has undergone significant changes in size and organization that are related to cognitive and behavioral specializations (Rilling, 2014; Sherwood, Subiaul, & Zawidzki, 2008; Sousa, Meyer, Santpere, Gulden, & Sestan, 2017; Stout & Hecht, 2017). This raises questions regarding the selective pressures that shaped human brain evolution. Due to their close phylogenetic relatedness to humans, chimpanzees and bonobos are key reference species for investigating our evolutionary roots (Hare & Wrangham, 2017; Wrangham & Pilbeam, 2001). By comparing traits in bonobos, chimpanzees, and other great apes, with those in humans, inferences can be made regarding the biology of our common ancestor and of human-specific evolutionary changes.

Bonobos and chimpanzees diverged from each other between 1 and 2 million years ago (Prado-Martinez et al., 2014). Despite their recent split, the two sister-species show substantial differences in behavior and cognition (Boesch, Hohmann, & Marchant, 2002; Hare & Yamamoto, 2017; Stumpf, 2007). Bonobos perform better at solving tasks related to theory of mind, understanding social causality, and receptive joint attention, all indicators of higher social sensitivity (Herrmann, Hare, Call, & Tomasello, 2010; Hopkins, Stimpson, & Sherwood, 2017). Studies in bonobos have also found more flexible vocal and gestural systems (Pika, Liebal, & Tomasello, 2005; Pollick & de Waal, 2007), and lower levels of inter- and intragroup aggression compared to chimpanzees (Furuichi, 2011; Gruber & Clay, 2016). Chimpanzees, on the other hand, perform at a higher level on tasks related to understanding physical causality, like tool-use and spatial memory (Haun, Nawroth, & Call, 2011; Herrmann et al., 2010; Rosati & Hare, 2012). Finally, some reports have concluded that bonobos are more prosocial and tolerant than chimpanzees, but findings are debated as outcomes differ across studies (Cronin, De Groot, & Stevens, 2015; Hare & Kwetuenda, 2010; Hare, Melis, Woods, Hastings, & Wrangham, 2007; Jaeggi, Stevens, & Van Schaik, 2010).

Studies investigating the neuroanatomy that potentially underlies these behavioral specializations have identified differences between bonobos and chimpanzees in brain structure and connectivity (Hopkins et al., 2017; Hopkins, Lyn, & Cantalupo, 2009; Rilling et al., 2012; Stimpson et al., 2016). While bonobos have higher grey matter volumes in regions that are involved in social cognition like the amygdala, insular cortex, and dorsolateral and dorsomedial prefrontal cortex, chimpanzees have greater grey matter volumes in parts of the primary motor cortex, visual cortex, and hippocampus, regions that play a role in motor control, tool use, and spatial memory (Bauernfeind et al., 2013; de Sousa, Sherwood, Mohlberg, et al., 2010; Hopkins et al., 2009; Rilling et al., 2012).

With these reported differences in behavior and underlying neuroanatomy, there is keen interest in determining whether bonobos or chimpanzees represent a better model for the *Pan-Homo* LCA. Three major hypothesis have been proposed, each with varying degrees of support (Hare & Wrangham, 2017; Pilbeam & Lieberman, 2017, pp. 22–141; Wrangham & Pilbeam, 2001). The mosaic hypothesis proposes that a mixture of traits currently present in bonobos and chimpanzees was likely present in the *Pan*-LCA (Prüfer et al., 2012; Weiss et al., 2015). This hypothesis, for example, finds support in comparative investigation of the genomes of each species, and in the structure of personality dimensions, where neither species is more similar to humans, but rather show a mosaic of traits that are more similar between [bonobo – human], [chimpanzee – human], or [bonobo – chimpanzee] (Prüfer et al., 2012; Weiss et al., 2015). The second hypothesis suggests that bonobos are a better model for the *Pan-Homo* LCA, and is largely based on socio-cognitive similarities between humans and bonobos (Hare & Wrangham, 2017; Parish & De Waal, 2000), although it has also been suggested based on musculoskeletal anatomical similarities between the two species (Diogo, Molnar, & Wood, 2017). The third hypothesis suggests that chimpanzees are the better model for the *Pan-Homo* LCA, assuming they have undergone relatively little evolutionary change since their split from bonobos, while bonobos have undergone higher rates of changes (Wrangham & Pilbeam, 2001). In support of this last hypothesis, a wide variety of studies of skeletal biology have recently concluded that bonobos are the more derived species (Pilbeam & Lieberman, 2017, pp. 22–141). Central to testing these hypotheses is determining whether the socio-cognitive capacities – and corresponding proximate mechanisms – of one *Pan* species are more derived than those of the other.

The first aim of this paper was to investigate the evolutionary change in neuroanatomical traits of bonobos and chimpanzees since their split from the *Pan*-LCA. If changes are minimal in one of the two species, this would be an indicator that it is more evolutionarily conserved and thus potentially a better model for the *Pan-Homo* LCA. To calculate evolutionary change in neuroanatomical traits along each *Pan* lineage, we incorporated all published data from comparative studies that included volumetric or cytoarchitectural brain data for six extant ape genera (humans, chimpanzees, bonobos, gorillas, orang-utans and gibbons). Our second aim was to examine potential genetic mechanisms underlying *Pan-Homo* differences, similarities, and variation in evolutionary rate. Using genomic data for bonobos, chimpanzees, and humans, we asked whether genes involved in brain development, neural function, and social cognition exhibit signals of accelerated protein evolution on one *Pan* lineage but not the other and if differential patterns of allele sharing were present in human-chimpanzee versus human-bonobo due to incomplete lineage sorting (ILS) (Pollard, Iyer, Moses, & Eisen, 2006). The

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latter question is based on the observation that 1.7% of the human genome is more similar to chimpanzees than to bonobos, while 1.6% is more similar to bonobos than to chimpanzees (Prüfer et al., 2012). Since such regions of incomplete lineage sorting can influence phenotypic similarities observed between one dyad but not the other (Joyce et al., 2011), we examined whether "fixed" amino acid substitutions (=i.e., substitutions that show no variation in either species) shared between humans and each *Pan* species are found in genes known to be involved in aspects of neurobiology.

2. Materials and methods

2.1. Neuroanatomical data

Neuroanatomical datasets that were included in the analysis were selected based on pre-determined inclusion criteria. To be part of the analysis, datasets had to include information for representatives of all living great ape genera (*Homo sapiens*, *P. troglodytes*, *P. paniscus*, *Gorilla gorilla*, *Pongo pygmaeus*) and have another primate species as an outgroup (typically *Symphalagus syndactylus* or *Hylobates lar*). Inclusion of outgroups can help detect variable rates of evolution and account for them accordingly when estimating ancestral values. In addition, to ensure that neuroanatomical trait values for bonobos and chimpanzees were reasonably well estimated, we restricted our analysis to datasets that included a minimum of 2 adult or late juvenile individuals (age > 7 years) that were largely sex matched (Table 1). In cases where data were only available for one sex in bonobos or chimpanzees, to avoid confounding estimates of sex- and species-specific traits, we removed the other sex from the comparative dataset in all the other species. This resulted in data for a total of 24 variables coming from 11 published studies. Given that our analyses were narrowly focused on changes occurring on the branches from the ancestral reconstruction of the *Pan*-LCA to either bonobos or chimpanzees, we used measures that were unscaled to total brain or body size.

2.2. Statistical analysis

Changes in lineages leading to chimpanzees and bonobos relative to the *Pan*-LCA can be quantified using methods of ancestral estimation. These methods combine observed trait variation across a set of extant species with the phylogenetic tree that describes the evolutionary relationship among these species to estimate the pattern of diversification of the trait in question. In doing so, these methods employ models of the evolution of trait change over time. Traditionally, a standard Brownian motion (BM) model is employed. This

Table 1 – Published neuroanatomical data included in the analysis with age and sex distribution across bonobos and chimpanzees.

Variable	Bonobo		Chimpanzee		Study
Region	Male N (age)	Female N (age)	Male N (age)	Female N (age)	
Whole brain volume	28	30	159	204	(Holloway, 1999)
Neocortical gray matter volume	2 (adult)	2 (adult)	3 (adult)	3 (adult)	(Rilling & Insel, 1999b)
Cerebellar vermis volume	1 (adult)	4 (adult)	6 (adult)	7 (adult)	(MacLeod, Zilles, Schleicher, Rilling, & Gibson, 2003)
Cerebellar hemisphere volume	1 (adult)	4 (adult)	6 (adult)	7 (adult)	"
Cerebral white matter volume	2 (adult)	2 (adult)	3 (adult)	3 (adult)	(Rilling & Insel, 1999b)
Whole brain gyrification index	2 (adult)	2 (adult)	3 (adult)	3 (adult)	"
Corpus callosum surface area	2 (adult)	2 (adult)	3 (adult)	3 (adult)	(Rilling & Insel, 1999a)
Primary visual cortex volume		2 (11–25)		3 (adult)	(de Sousa, Sherwood, Mohlberg, et al., 2010)
Lateral geniculate nucleus volume		2 (11–25)		3 (adult)	"
Dorsal frontal cortex volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	(Schenker, Desgouttes, & Semendeferi, 2005)
Mesial frontal cortex volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	"
Orbital frontal cortex volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	"
Frontal white matter volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	"
Temporal cortex volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	"
Temporal white matter volume	2 (8–25)	1 (11)	3 (20–39)	2 (36–39)	"
Granular insular cortex volume	1 (adult)	2 (11–25)	2 (39–40)	1 (45)	(Bauernfeind et al., 2013)
Dysgranular insular cortex volume	1 (adult)	2 (11–25)	2 (39–40)	1 (45)	"
Agranular and frontoinsular cortex volume	1 (adult)	2 (11–25)	2 (39–40)	1 (45)	"
Neuron count in lateral nucleus of amygdala	1 (adult)	3 (2–25)		5 (adult, 24–42)	(Barger et al., 2012)
Neuron count in basal nucleus of amygdala	1 (adult)	3 (2–25)		5 (adult, 24–42)	"
Neuron count in accessory basal nucleus of amygdala	1 (adult)	3 (2–25)		5 (adult, 24–42)	"
Neuron count in central nucleus of amygdala	1 (adult)	3 (2–25)		5 (adult, 24–42)	"
VEN count in left frontoinsular cortex	2 (25–34)	4 (11–52)	4 (19–39)	3 (35–45)	(Allman et al., 2010; Issa et al., 2018)
VEN count in left anterior cingulate cortex	2 (25–34)	3 (13–52)	4 (17–39)	3 (13–35)	"

" Indicates data come from study mentioned above.

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model assumes that average trait change is proportional to the square root of time and the rate of evolution is stochastically constant across all branches. The advantage of this model is that it is mathematically tractable. The disadvantage is that it does not allow quantifying differential change across individual lineages; something which is commonly agreed to characterize trait evolution (Harvey & Purvis, 1991). To overcome this drawback of the standard BM model, several authors have emphasized the usefulness of more flexible models that account for the possibility of rate heterogeneity (e.g., Chira & Thomas, 2016). To quantify putative differential change in chimpanzees versus bonobos since the *Pan*-LCA, we use an extension of the standard BM model that allows quantifying rate parameters along individual lineages. This approach (multiple variance BM; 'mvBM') employs a heuristic algorithm that introduces a correction factor that estimates the extent to which lineage-specific patterns of trait change deviate from a baseline BM assumption (Smaers & Mongle, 2018; Smaers, Mongle, & Kandler, 2016). mvBM therefore allows quantifying lineage-specific rates of evolution and inferring ancestral values of extinct nodes in the tree accordingly. The mvBM procedure has been demonstrated to show equivalent results to standard BM when data is simulated according to BM and to outperform BM when differential change along different lineages are included in the simulation procedure. Importantly, mvBM reduces the error at locations where differential change occurs, and maintains accuracy in the ancestral estimation at other locations. This advantage in accuracy of mvBM has been shown to occur with sample sizes as low as four (Smaers & Mongle, 2017).

Here we use mvBM to estimate lineage-specific trait changes in the chimpanzee and bonobo lineages relative to the *Pan*-LCA. We include information for seven species in the analysis (*H. lar*, *Symphalangus syndactylus*, *P. pygmaeus*, *Gorilla gorilla*, *P. paniscus*, *P. troglodytes*, and *H. sapiens*). Considering information on multiple individuals per species, we iterated the estimation procedure across all possible combinations of individuals per species. Results comprise a distribution of estimates of trait change for each lineage in the phylogeny. We then calculated the overlap between the empirical distributions of trait change for the chimpanzee lineage and the bonobo lineage. Trait change was calculated as a relative ratio of the tip value to the estimated value of the LCA [(*tip value* − *LCA value*)/*LCA value*]. This procedure does not allow for hypothesis testing, but does accurately represent putative differential trait change in the bonobo versus chimpanzee lineages relative to their LCA. Furthermore, by using the *Pan*-LCA as a baseline comparison, we focus our analysis on differential change in the bonobo and chimpanzee lineages. This overcomes some of the uncertainties inherent to ancestral estimation because it does not focus primarily on the precise value of the LCA, but rather on differential change in descendant lineages relative to the LCA.

2.3. Comparisons of protein evolution

Since we were specifically interested in genes involved in neurobiology and social cognition, we selected a "top 100" list of candidate loci for analysis using two approaches. First, we searched the SFARI (Simons Foundation Autism Research Initiative) Gene Database, which categorizes autism candidate genes using an evidence-based systems biology approach (Abrahams et al., 2013). We compiled a list of 83 candidate genes that included all of the loci scored in the SFARI Gene Database as "high confidence genes" or "strong candidate genes" associated with Autism Spectrum Disorder (Table S1). Second, we supplemented our initial search with an additional 17 genes from the NHGRI-EBI (National Human Genome Research Institute-European Bioinformatics Institute) Catalog of published genome-wide association studies (GWAS) (MacArthur et al., 2017). We searched for GWAS-identified genes found to be associated with autism related traits. From the total list of 904 genes, we selected the top 17 genes that appeared the most times in the database search, indicating that the gene–behavior associations were replicated in multiple studies, and/or the genes contained multiple loci associated with autism related traits. Alternatively, we also ran an analysis including only GWAS genes that showed associations with p values $<5 \times 10^{-8}$, which yielded highly similar results. In GWAS studies this p value is considered the statistical significance threshold at which one can differentiate true positives from false positives (Pe'er et al., 2008).

For all 100 selected candidate genes implicated in cognitive or social development, we compiled protein similarity measurements and dN/dS ratios (calculated within Ensembl via PAML, Yang, 1997) for both bonobo and chimpanzee genomic data using humans, gorillas, or rhesus macaques (*Macaca mulatta*) as the reference species. We used the Genomicus portal (Muffato, Louis, Poisnel, & Crollius, 2010) to query these data for each gene using the ancestral primate node as the root. We tested for statistical differences in mean dN/dS scores between the two species using one-way analysis of variance (ANOVA) in SPSS. Since the bonobo reference genome is less complete than the chimpanzee and human reference genomes, for genes showing differences of interest, we also assessed the completeness of protein data via visual alignments using Wasabi (Veidenberg, Medlar, & Löytynoja, 2015) in Ensembl.

2.4. Shared differences via incomplete lineage sorting

Previous comparisons of the human, chimpanzee, and bonobo reference genomes identified 18 fixed amino acid differences shared between human - chimpanzee and 18 fixed amino acid differences shared between human - bonobo (Prüfer et al., 2012; Table S2). These genes and amino acid substitutions were highlighted by Prüfer et al. (2012) as strong candidates for understanding the genetic bases of phenotypic traits humans share with one *Pan* species but not the other. Thus, we compiled data on the likely function (biological process, molecular function, cellular component) of each of these 36 ILS loci using the Gene Ontology database (i.e., GO terms; Gene Ontology Consortium et al., 2004) and the GeneCards portal (Safran et al., 2010). We were specifically interested in knowing whether the GO terms for the 36 ILS loci were related to neurobiology, social behavior, cognition, or brain development.

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3. Results

3.1. Evolutionary changes in neuroanatomy

We calculated the percent difference between the estimated value of the *Pan*-LCA to extant bonobo and chimpanzee values for neuroanatomical traits and iterated the estimation procedure across all possible combinations of individuals per species to generate a distribution. Changes among descendent lineages are expected, and our focus was identifying the direction, strength, and significance of those changes. For a total of seven out of 24 neuroanatomical traits, a distribution overlap of <.05 was found indicating a clearly distinct evolutionary pattern between the two species for these traits (Fig. 1, Table 2). Compared to the estimated *Pan*-LCA for total brain volume, a decrease was found in bonobos and an increase in chimpanzees for both sexes. The largest difference in brain volume change was found for decreased brain size in bonobo males. A similar pattern of increase in chimpanzees and decrease in bonobos was found for total cerebral white matter volume (sexes combined), but not gray matter volume. Among insular cortex subregions, granular insular cortex volume displayed an increase in chimpanzees and a decrease in bonobos, whereas dysgranular and agranular + frontoinsular cortex volumes were increased in bonobos and decreased in chimpanzees. The number of neurons in the central nucleus of the amygdala increased in chimpanzees and showed a decrease in bonobos. The evolutionary changes in the remaining traits were more modest and showed greater overlap in distributions between species (Table 2, Fig. 2). Trait evolution trees that include all great ape species are shown in Supplementary figure S1.

3.2. Comparison of protein evolution and incomplete lineage sorting differences

Bonobos and chimpanzees did not differ significantly in mean dN/dS ratios for our list of 100 candidate genes involved in neurobiology and social cognition, whether using human [$F(1,138) = .10$, $p = .76$], gorilla [$F(1,140) = .01$, $p = .93$] or macaque [$F(1,168) = .04$, $p = .85$] sequences as a reference (Table 3). Detailed inspection of protein alignments for those individual genes that showed especially large differences in protein similarity levels between the two species revealed in many cases the presence of truncated ends or proteins that were not yet annotated in the bonobo genome. Therefore these differences (due to reference genome coverage rather than biological differences) were not assessed via statistical analysis. Results for protein evolution and similarities for all

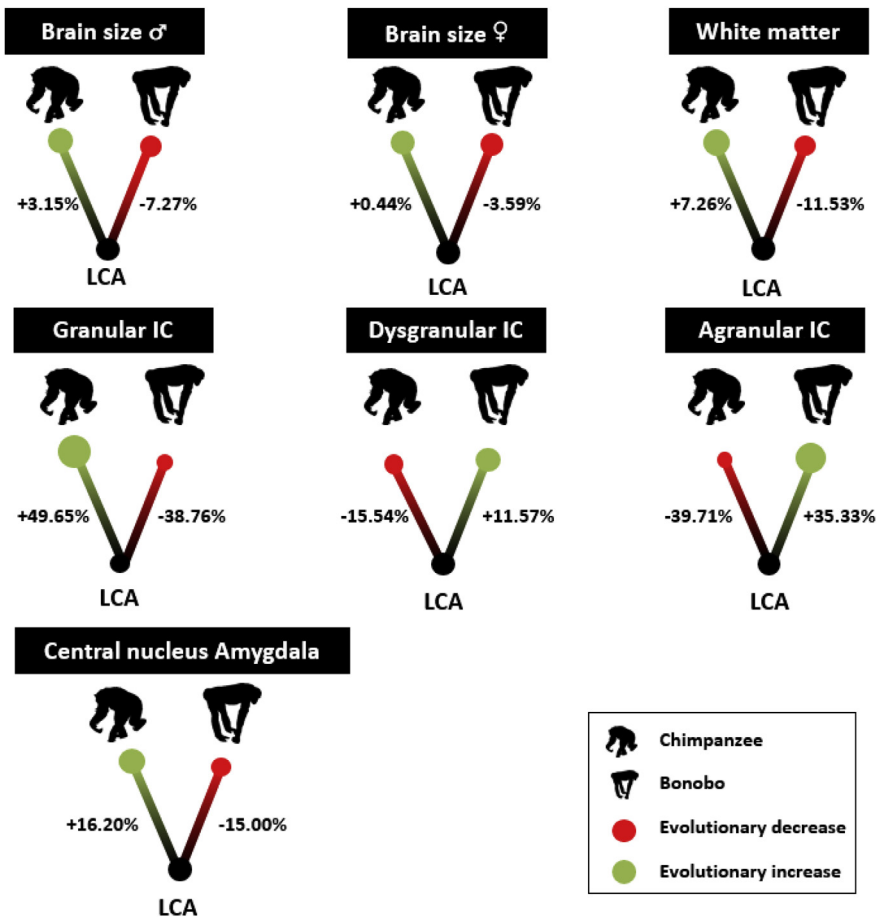

Fig. 1 – Evolutionary patterns of neuroanatomical change in bonobos and chimpanzees since their split from the *Pan*-LCA for traits that show a distribution overlap <.05. Dots are scaled to match the percentage change difference from the ancestral state (the *Pan*-LCA). IC = Insular cortex. White matter refers to whole brain white matter, ♂ = male, ♀ = female.

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Table 2 – Estimated mean evolutionary change in bonobo and chimpanzee neuroanatomical traits since the split from the *Pan* last common ancestor.

Region	Mean change chimpanzee	Mean change bonobo	Distribution overlap
Whole brain volume, male	**.031**	**−.073**	**.000**
Whole brain volume, female	**.004**	**−.036**	**.000**
Neocortical gray matter volume	−.008	−.035	.690
Whole brain gyrification index	.001	−.007	.309
Cerebral white matter volume	**.073**	**−.115**	**.008**
Corpus callosum surface area	−.015	−.017	.743
Cerebellar vermis volume	−.126	.094	.309
Cerebellar hemisphere volume	.017	−.061	.454
Dorsal frontal cortex volume	−.030	−.030	.612
Mesial frontal cortex volume	−.079	.020	.338
Orbital frontal cortex volume	−.070	.025	.546
Frontal white matter volume	−.035	−.017	.713
Temporal cortex volume	−.037	−.007	.683
Temporal white matter volume	.076	−.116	.306
Granular insular cortex volume	**.496**	**−.388**	**.006**
Dysgranular insular cortex volume	**−.155**	**.116**	**.014**
Agranular and frontoinsular cortex volume	**−.397**	**.353**	**.000**
VEN count in left frontoinsular cortex	.212	−.096	.268
VEN count in left anterior cingulate cortex	−.071	−.009	.845
Primary visual cortex volume	−.121	.099	.136
Lateral geniculate nucleus volume	.164	−.172	.084
Neuron count in lateral nucleus of amygdala	−.046	.031	.314
Neuron count in basal nucleus of amygdala	.069	−.010	.592
Neuron count in accessory basal nucleus of amygdala	.047	−.076	.473
Neuron count in central nucleus of amygdala	**.162**	**−.150**	**.000**

Bold face indicates distribution overlap <.05, italic indicates $p < .1$.

genes included in the study can be found in Table S1. Assessment of the 36 ILS genes (Prüfer et al., 2012) showing fixed amino acid differences shared either by human-chimpanzee (18 genes, Table S2) or human-bonobo (18 genes, Table S3) revealed no functional relationship to neural processes or development that could be directly associated with the neuroanatomical differences between the two species.

4. Discussion

The aim of this paper was to investigate evolutionary patterns associated with differences in brain structure of bonobos and chimpanzees since their split from the *Pan*-LCA. To estimate these evolutionary patterns, we analyzed comparative neuroanatomical data from hominoids. The results of this study suggest that the bonobo lineage is derived in being characterized by marked reduction of whole brain and white matter volumes, whereas the chimpanzee lineage shows modest brain size and white matter increase. Additionally, both lineages show changes compared to the estimated ancestral state for regions related to social cognition and emotional regulation, i.e., the insular cortex and amygdala. The remaining neuroanatomical traits did not show evidence of strong differences in evolutionary change between bonobos and chimpanzees.

Whole brain volume shows a significant decrease in bonobos and an increase in chimpanzees relative to their LCA, with sex-specific differences in the degree of these evolutionary changes. While bonobo females also showed a reduction, the reduction in brain volume was more pronounced in male bonobos. Comparison of data for cerebral grey and white matter volumes suggest that the brain size changes are largely attributable to changes in white matter volume, while cortical grey matter volume remains relatively stable. This is in line with previous findings that among great apes, variation in white matter volume is typically greater than variation in neocortical grey matter volume (Rilling & Insel, 1999b). As grey matter is largely composed of cell bodies, and white matter of axons, growing brains require disproportionately more white matter to allow for similar levels of connectivity among cortical neurons (Frahm, Stephan, & Stephan, 1982; Rilling & Insel, 1999b). The significant decrease in brain volume in bonobos is in line with a previous hypothesis that suggests that bonobos may have undergone evolutionary pressures similar to the domestication process (Hare, Wobber, & Wrangham, 2012). Domesticated species typically show a reduction in absolute brain size when compared to their wild ancestors, and contemporary close relatives (Kruska, 2005). Compared to chimpanzees, bonobos also show no sexual dimorphism in cranial capacity (Zihlman, Stahl, & Boesch, 2008) and according to the results in this study, this may be attributable mostly to a decrease in male brain volume.

In chimpanzees, the increase in brain size may partly be linked to selective pressures supporting larger body size. However, body size differences between bonobos and chimpanzees have been shown to vary depending on the subspecies of chimpanzee used in the comparison (Behringer et al., 2016; Zihlman et al., 2008). While bonobos are, for example, taller than eastern chimpanzees (*P. troglodytes schweinfurthii*), they are smaller than western chimpanzees (*P. troglodytes verus*) (Zihlman et al., 2008). In this study, brain size measures for three

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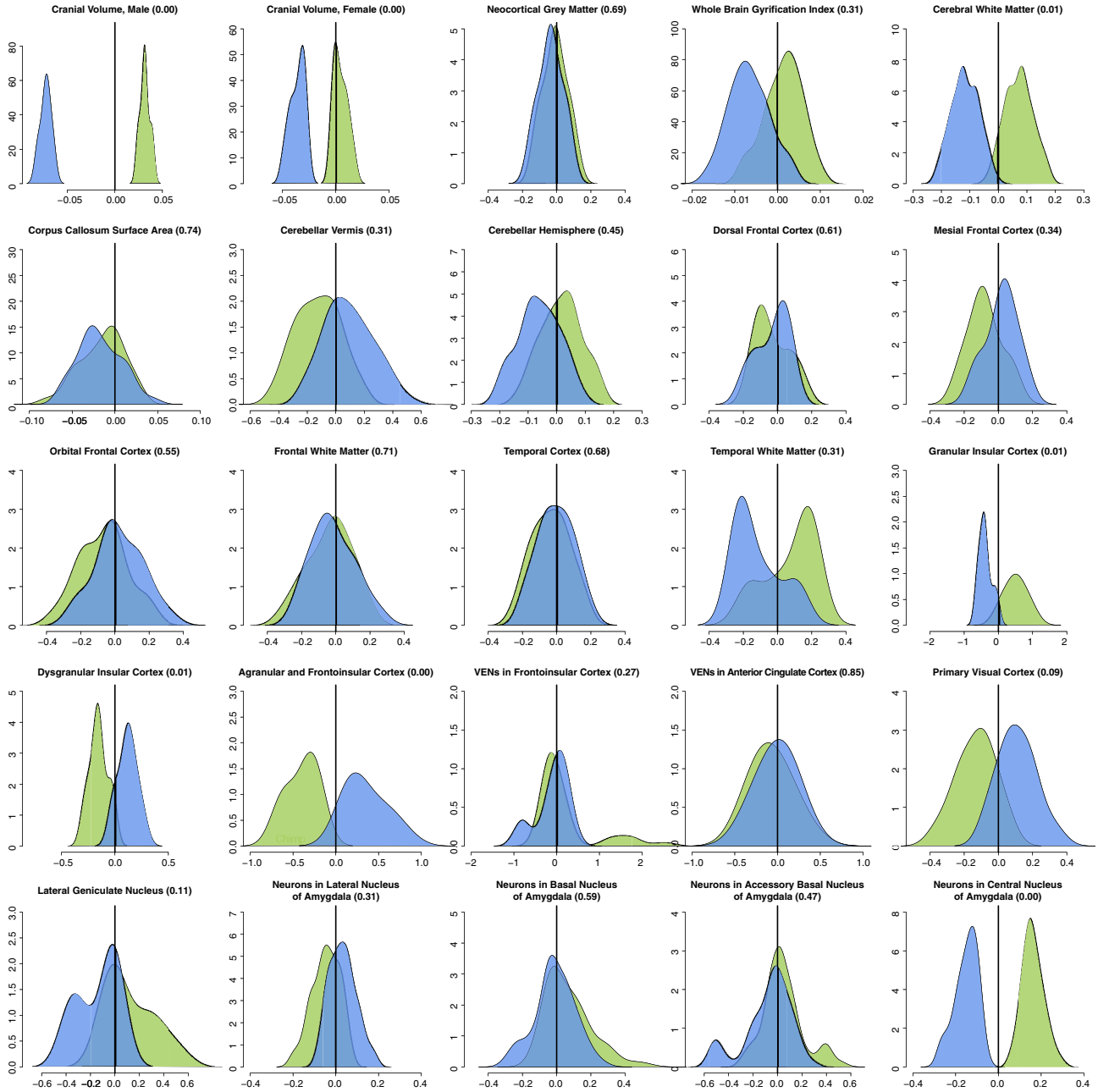

Fig. 2 – Evolutionary distribution patterns of neuroanatomical change in bonobos (blue) and chimpanzees (green) since their split from the *Pan*-LCA.

different subspecies of chimpanzees were combined (*P.t. verus*, *P.t. schweinfurthii* and *P.t. troglodytes*) and the results thus represent a combination of these subspecies, compared to bonobos.

In the insular cortex, chimpanzees showed an evolutionary increase in volume of the granular subdivision, whereas bonobos showed increases in volume of the dysgranular and agranular subdivisions. Given the distinct functional differences that have been described for these anatomical subdivisions, these results may be associated with the evolution of different social behavioral strategies between species. While the insular cortex as a whole has been linked to self-recognition, emotional awareness, empathy, and cooperation (Critchley, 2005), fMRI studies in humans reveal that the more posterior granular insula plays a role in somatosensory visceral processing, and the anterior agranular insula cortex (AIC) is critical for processing of emotional and social awareness (Flynn, Benson, & Ardila, 1999; Gu, Hof, Friston, & Fan, 2013). The AIC is among the most differentially expanded neocortical regions in humans (Bauernfeind et al., 2013), and the increase in bonobos follows a similar pattern which might support their greater sensitivity to social cues (Herrmann et al., 2010; Hopkins et al., 2017; Rilling et al., 2012). Despite the increase in size of the AIC, no support was found for differential evolution in the number of von Economo

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Table 3 – Comparison of differences in mean protein evolution and similarity in bonobos and chimpanzees for 83 candidate genes involved in neurobiology and social cognition.

	Reference	*Pan* species	Mean	SE
dN/dS	Human	Chimpanzee	.29	.03
		Bonobo	.30	.03
	Gorilla	Chimpanzee	.31	.05
		Bonobo	.29	.03
	Macaque	Chimpanzee	.19	.02
		Bonobo	.19	.02
Protein similarity	Human	Chimpanzee	99%	.18
		Bonobo	99%	.25
	Gorilla	Chimpanzee	99%	.21
		Bonobo	98%	.26
	Macaque	Chimpanzee	98%	.37
		Bonobo	97%	.41

neurons (VENs), a unique subset of neurons found in the ventral-most subdivision of the AIC known as the frontoinsular cortex (FI) (Allman et al., 2010), and the anterior cingulate cortex (ACC) (Nimchinsky, Vogt, Morrison, & Hof, 1995). These neurons are hypothesized to be involved in empathy, social awareness, and self-control (Allman et al., 2010, 2011), but show no apparent increase or decrease along either *Pan* lineage. This is in line with previous studies showing a relatively large overlap in VEN numbers between bonobos and chimpanzees (Allman et al., 2010; Issa et al., 2018).

Species differences were found for anatomically and functionally distinct nuclei of the amygdala. Chimpanzees showed a substantial increase in neuron numbers in the central nucleus of the amygdala, while bonobos showed a comparable degree of decrease of neurons in this region since their split from the *Pan*-LCA. Previous studies have shown that compared to chimpanzees, bonobos are more similar to humans in amygdala organization, with humans and bonobos both showing significantly lower numbers of neurons in the central nucleus than other apes, including chimpanzees (Barger et al., 2012). The neurons of the central nucleus furnish major projections to autonomic regions in the hypothalamus and brainstem, and therefore this nucleus is considered important for generating visceral and internal-state stress responses (LeDoux, 2007; Rizvi, Ennis, Behbehani, & Shipley, 1991). The divergence of neuron numbers in this region between the two *Pan* species may be linked to differential levels of reactivity and arousal in response to fear-evoking stimuli. Compared to bonobos, chimpanzees are known to take more risks and show higher levels of exploratory behavior when encountered with novel objects and situations (Haun et al., 2011; Herrmann, Hare, Cissewski, & Tomasello, 2011; Rosati & Hare, 2012). In addition, chimpanzees also have a higher propensity for reactive aggression compared to bonobos and humans, as shown in routine occurrences of dyadic fights over resources and dominance status (Goodall, 1986; Wrangham, 2018). Reactive aggression refers to the emotional response to a threat or frustrating event, with the goal to remove the provoking stimulus, and is typically associated with a sudden increase in sympathetic activation (Lickley & Sebastian, 2018; Raine et al., 1998). Proactive aggression, its counterpart, involves purposeful planning of the attack and usually lacks the emotional arousal linked to reactive aggression. The latter aggression type is said to be more prominent in humans compared to chimpanzees, and absent in wild bonobos (Wrangham, 2018).

Comparison of the evolutionary patterns for the remaining neuroanatomical data revealed relatively smaller increases/decreases with a considerable overlap found in the distributions for both species. The pattern difference for the lateral geniculate nucleus (LGN), for example, approached our 5% overlap threshold (8.4%), but was in the opposite direction of the pattern found for the primary visual cortex (V1). Given the strong interconnections of these regions in the visual pathway (de Sousa, Sherwood, Hof, & Zilles, 2013; de Sousa, Sherwood, Schleicher, et al., 2010), we expected both to follow a similar direction of evolutionary change within species. It is notable that these additional neural regions of interest related to sensorimotor systems and executive cognitive function did not display strong evidence of evolutionary divergence between *Pan* species, whereas certain brain structures involved in socio-emotional functions did. This suggests that socio-emotional systems are an especially important target of reorganization in bonobo and chimpanzee brain evolution.

A methodological constraint of the current study was that we could only incorporate data from comparative neuroanatomical studies that included measurements from all ape genera. These studies typically have a relatively small sample size per species, which limits the power of the statistical models used in our analyses. Consequently, the results should be interpreted as preliminary, as replication in larger samples is warranted to confirm our findings. Furthermore, due to this limitation, we could not investigate the evolutionary patterns behind additional neuroanatomical differences of interest that have been reported in studies comparing only the two species. For example, it has been shown that bonobos have a smaller putamen and hippocampal volume than chimpanzees (Hopkins et al., 2009). Bonobo brains have also been shown to have a larger pathway linking the amygdala with the ventral anterior cingulate cortex (Rilling et al., 2012), and increased levels of serotonergic innervation of the amygdala (Stimpson et al., 2016). At present, the direction of evolutionary change for these traits in the *Pan* lineages cannot be resolved. Further studies including similar brain phenotypic measures for all ape species are required to investigate the evolutionary divergence pattern underlying these neuroanatomical differences. Finally, with the exception of the whole brain measures used in this study, our comparison of neuroanatomical data was limited to chimpanzees belonging to the western subspecies (*P. troglodytes verus*). As previous studies have suggested that anatomical comparisons between the two *Pan* species are dependent on large within-species variation in chimpanzees (Behringer et al., 2016; Zihlman et al., 2008), we cannot draw conclusions about the brain evolutionary change that occurred in the other chimpanzee subspecies. Similarly, most neuroanatomical measures included in this study come from captive individuals. While our results warrant replication in wild populations, the benefit of a captive environment is that it provides stability, and eliminates potential environmental influences that

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could shape between-species differences in wild bonobos and chimpanzees. Therefore, we would expect the neuroanatomical differences to be even larger in wild populations.

There were also no observable differences between the patterns of protein evolution on the bonobo lineage compared to the chimpanzee lineage using our comparison of protein evolution and similarities via dN/dS ratios based on the reference genomes of each species. Three (*TBL1XR1*, *WAC*, *DEAF1*) of the 100 neurobiology and social cognition-related candidate genes identified in the SFARI Gene Database showed a signal of neutral evolution (dN/dS ≅ 1 in at least one comparison) for one or both of the *Pan* lineages (Table S1). The vast majority of the candidate genes had a typical dN/dS ratio between .2 and .4 as would be expected under stabilizing (or purifying) selection. Given the role of these genes in neurobiological function, it is typically not surprising to find a majority showing a signal of strong purifying selection (Dorus et al., 2004). However, these analyses average selection pressures across the whole sequence, which could mask specific sites under positive selection, and thus further analyses using site-based models, which compare rate heterogeneity across different amino acid positions (e.g., CodeML, Yang, Nielsen, Goldman, & Pedersen, 2000), are warranted. Protein similarity values, when compared to the human proteins, were often lower for bonobos than chimpanzees, but this difference is likely attributable to the bonobo reference genome being less complete than the chimpanzee reference genome. That is, missing data or truncated ends were more common for protein sequences obtained via the bonobo reference genome than via the chimpanzee reference genome.

The 36 genes that showed amino acid substitution in line with incomplete lineage sorting (ILS) between human, bonobos, and chimpanzees, are involved in a wide array of functions and cellular activities, including immune response, circadian cycles, and olfaction (Table S2 and S3). However, none of these 36 genes are implicated in aspects of neurobiology, social behavior, cognition, or brain development. Notably, many of these ILS loci are regulatory proteins (e.g., *ZIM3*, *ANKRD2*, *ZNF345*, *ZNF227*, *ZNF419*) that impact the expression of other genes, and thus are consistent with the idea that gene regulation, as opposed gene coding, can be a primary driver of phenotypic differences between humans and other apes (King & Wilson, 1975). Future studies investigating differences in bonobo and chimpanzee brain gene expression patterns would be informative.

To conclude, the results of this study support a model of mosaic brain evolution in bonobos and chimpanzees, with different patterns of evolutionary changes along each lineage. Neither species displayed the majority of directional changes in neuroanatomy, nor retention of ancestral character states since their split from the *Pan*-LCA. Among the various neuroanatomical traits in our analysis, brain size and neural regions associated with socio-emotional function appear to be the main targets of evolutionary change in brain organization between bonobos and chimpanzees. These findings are congruent with the observed differences in the behavior of these species in the wild and in captive settings (Gruber & Clay, 2016; Hare & Yamamoto, 2017; Herrmann et al., 2010), and potentially demonstrate the effects of differential selection in each lineage for alternative strategies to manage social conflict (Furuichi, 2011; Wrangham, 2018). Our findings emphasize that the brain biology of neither extant bonobos nor chimpanzees necessarily reflects the ancestral state of *Pan*, and urges caution in considering either modern species as an accurate model to represent the LCA with humans (Sayers & Lovejoy, 2014).

Conflict of interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cortex.2018.09.016.

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