



Androgen receptor and monoamine oxidase polymorphism in wild bonobos



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ABSTRACT

Androgen receptor gene (*AR*), monoamine oxidase A gene (*MAOA*) and monoamine oxidase B gene (*MAOB*) have been found to have associations with behavioral traits, such as aggressiveness, and disorders in humans. However, the extent to which similar genetic effects might influence the behavior of wild apes is unclear. We examined the loci *AR* glutamine repeat (*ARQ*), *AR* glycine repeat (*ARG*), *MAOA* intron 2 dinucleotide repeat (*MAin2*) and *MAOB* intron 2 dinucleotide repeat (*MBin2*) in 32 wild bonobos, *Pan paniscus*, and compared them with those of chimpanzees, *Pan troglodytes*, and humans. We found that bonobos were polymorphic on the four loci examined. Both loci *MAin2* and *MBin2* in bonobos showed a higher diversity than in chimpanzees. Because monoamine oxidase influences aggressiveness, the differences between the polymorphisms of *MAin2* and *MBin2* in bonobos and chimpanzees may be associated with the differences in aggression between the two species. In order to understand the evolution of these loci and *AR*, *MAOA* and *MAOB* in humans and non-human primates, it would be useful to conduct future studies focusing on the potential association between

Abbreviations: AR, androgen receptor gene; MAOA, monoamine oxidase A gene; MAOB, monoamine oxidase B gene; ARQ, repeat locus coding glutamine in androgen receptor gene; ARG, repeat locus coding glycine in androgen receptor gene; MAin2, repeat locus in intron 2 of monoamine oxidase A gene; MBin2, repeat locus in intron 2 of monoamine oxidase B gene; PCR, polymerase chain reaction.

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aggressiveness, and other personality traits, and polymorphisms documented in bonobos.

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Introduction

Numerous studies have examined the association between neurotransmitter- or hormone-related gene polymorphisms and behavioral traits and disorders in humans (D'Souza and Craig, 2006; Ebstein, 2006). Accordingly, androgen receptor gene (*AR*), monoamine oxidase A gene (*MAOA*) and monoamine oxidase B gene (*MAOB*) are candidate genes.

Androgen receptor is a DNA-binding transcription factor, the main regulator of androgen signaling in the cell, activated mostly by testosterone and 5 α -dihydrotestosterone. Androgen receptor is encoded by the X-chromosome-located *AR*, that has two polymorphic trinucleotide repeat regions – CAG encoding glutamine and GGN encoding glycine (*ARQ* and *ARG*, respectively) – on the N-terminal domain in the first exon in humans (Chang et al., 1988). *ARQ* and *ARG* have been found to be associated with several personality disorders (Aluja et al., 2011; Comings et al., 1999), prostate cancer (Zhai et al., 2014) and certain personality traits like aggression and dominance (Jönsson et al., 2001; Vermeersch et al., 2010), or neuroticism and extraversion (Westberg et al., 2009). In search of the reasons for the numerous phenotypic associations of the trinucleotide repeats (for review, see Rajender et al., 2007), it has been found that both *ARQ* and *ARG* have transcription activation modulating effect (Chamberlain et al., 1994; Lundin et al., 2007).

Monoamine oxidase is an enzyme located throughout the brain in the outer membrane of mitochondria, responsible for monoamine degradation. Monoamine oxidase A degrades mostly serotonin, norepinephrine and dopamine, while monoamine oxidase B has higher affinity toward phenylethylamine, benzylamine, and deprenyl (for review see Shih et al., 1999). The genes encoding these enzymes are located on the X-chromosome (*MAOA* and *MAOB*, respectively). Mutations in *MAOA* have been associated with aggressiveness and borderline mental retardation (Brunner et al., 1993; for review see Fan et al., 2010), while *MAOB* has been implicated in Parkinson's disease (Hotamisligil et al., 1994; Liu et al., 2014). In humans, both genes have a polymorphic dinucleotide repeat motif in intron 2 (*MAin2* and *MBin2*, respectively). *MAin2* has been linked to bipolar disorder (Furlong et al., 1999; Kawada et al., 1995; Lin et al., 2000) and to attention-deficit hyperactivity disorder (Jiang et al., 2001), while *MBin2* has been related to bipolar disorder (Lin et al., 2000) and to Parkinson's disease (Chan et al., 2003; Mellick et al., 2000). Even though the associations can be questioned given the intronic location of these loci (for example see Muramatsu et al., 1997), there is a strong possibility that *MAin2* and *MBin2* have biological functions that remain to be confirmed.

Behavioral trait- and disorder-related candidate genes have also been examined in animals, notably in the phylogenetically closest animals to humans, the non-human primates (Anestis et al., 2014; Hopkins et al., 2012; Inoue-Murayama, 2009). The abovementioned four loci (*ARQ*, *ARG*, *MAin2* and *MBin2*) have also been studied in non-human primates, including chimpanzees (Hong et al., 2006, 2008). However, as to the best of our knowledge, bonobos have never been studied for these four loci with the exception of 2 individuals for loci *ARQ* and *ARG* (Hong et al., 2006).

The divergence between bonobos and chimpanzees, the closest living relatives of humans, has been estimated to be approximately 1 million years ago (Hey, 2010). Bonobos and chimpanzees share several traits, such as living in fission–fusion social system and residing in a male philopatric society (Goodall, 1983; Kano, 1982), but the two species also differ in important ways that might be related to their aggressive tendencies. In contrast to chimpanzees, bonobo communities have lower levels of both intra- and inter-group aggression, while female bonobos have high social status, prolonged estrus periods and maintain strong social bonds with each other, all characteristics lacking in chimpanzees (Furuichi, 1997, 2011; Kano, 1992). Recently Hare et al. (2012) have proposed the self-domestication hypothesis, which speculates that the psychological, behavioral and morphological differences between bonobos and chimpanzees can be the consequences of selection against aggression, which is a typical characteristic of domestication.

Because *AR*, *MAOA* and *MAOB* influence aggressiveness along with other traits, they are among the ideal candidate genes to study the reasons for behavioral differences in chimpanzees and bonobos, which in turn can help

us to understand the functions and evolution of these genes. As a first step toward this approach, we analyzed the loci *ARQ*, *ARG*, *MAIn2* and *MBin2* in bonobos for comparison with those of chimpanzees and humans.

Materials and methods

Sample collection

Fecal sample collection took place in Wamba, Luo Scientific Reserve, Democratic Republic of the Congo between November 2012 and November 2013. The research camp is located at 0°11'08" N, 22°37'58" E. Details of the field site have been described (Hashimoto et al., 2008; Kano, 1992). Fecal samples were collected from 22 females and 10 males from 2 communities of wild bonobos (*Pan paniscus*), 13 females and 7 males from P group, and 9 females and 3 males from E1 group (for further information see Tokuyama et al., 2012 for P group, and Furuichi et al., 2012; Ryu et al., in press for E1 group). All the individuals in the analyzed communities were individually recognized. In P group, 4 females and 2 males were infants of 5 females, while in E1 group, a male was an infant of a female among the samples. The rest of the individuals were believed to be unrelated. The sampling procedure has been described by Kawamoto et al. (2013).

Genotyping

DNA was extracted by using a QIAamp DNA Stool Mini Kit (Qiagen, California, USA) according to the manufacturer's instructions. *ARQ* and *ARG* were amplified by applying the following primer sets: ARhf (5'-TCCAGAATCTGTTCAGAGCGTGC-3') and ARhr (5'-GCTGTGAGGGTTGCTGTTCTCAT-3') for *ARQ*, and ARGf (5'-CAGTCCGCTATGGGACCTGGCGA-3') and ARGr (5'-GGACTGGGATAGGGCACTCTGCTCACC-3') for *ARG* (Hong et al., 2006). The primer sets for monoamine oxidase were MAIn2F (5'-TAGCTCCAGGAAGGAAATGTT-3') and MAIn2R (5'-CACCCAGGGAAAAGTAGGTTA-3') for *MAIn2*, and MBin2F (5'-GATTGAAAGATTGTGTGGTG-3') and MBin2R (5'-CCAGTTTGCTTCCTTCAA-3') for *MBin2* (Hong et al., 2008). A 10 µl PCR reaction contained 2 µl DNA with a concentration of > 150 pg/µl (Step 3 in Table S1 in Kawamoto et al., 2013), 0.5 µM forward primer, 0.5 µM reverse primer, 0.5 U *LA* Taq polymerase, 400 µM of each of the dNTP and GC buffer I (TaKaRa, Shiga, Japan). Following the initial incubation at 95 °C for 2 min, PCR amplification was performed for 35 cycles of 95 °C for 30 s, 60 °C (*ARQ*) or 65 °C (*ARG*) for 1 min and 75 °C for 2 min for *AR* loci, while 35 cycles (*MAIn2*) or 40 cycles (*MBin2*) of 95 °C for 30 s, 50 °C for 1 min and 75 °C for 2 min for *MAOA* and *MAOB* loci. For all loci PCR amplification was followed by a final extension at 74 °C for 10 min.

For genotyping we applied the ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions by using labeled primers. We sequenced the major alleles in the ABI 3130xl Genetic Analyzer. Sequencing was repeated at least once in the case of each sequenced allele. We named the alleles by the number of repeats counted from nucleotide sequences or estimated from allele lengths. For androgen receptor, a registered human sequence (access no. NM_000044) and a registered chimpanzee sequence (access no. NM_001009012), for *MAOA*, a registered human sequence (access no. AB302097) and two registered chimpanzee sequences (access nos. AB302099 and AB302100), and for *MAOB*, two registered human sequences (access nos. X63276 and AB302115), and two registered chimpanzee sequences (access nos. AB302116 and AB2117) were obtained from the GenBank database. Repeats were counted by the longest uninterrupted trinucleotide (androgen receptor) or dinucleotide repeats (monoamine oxidase). We used CLUSTAL W for sequence alignment (Thompson et al., 1994).

In order to compare the bonobo allele frequencies with those of chimpanzees and humans, we used the published allele frequency; in the case of androgen receptor 33 females and 19 males of humans (Comings et al., 1999), while 32 females and 25 males of chimpanzees (Hong et al., 2006), and in the case of *MAOA* and *MAOB*, 25 females and 30 males of humans, while 24 females and 18 males of chimpanzees (Hong et al., 2008).

To the best of our knowledge, none of the genes analyzed in this study have been analyzed in bonobos before. Hence we registered the nucleotide sequences in DDBJ/EMBL/GenBank nucleotide sequence database with the accession numbers AB970511–AB970525.

Statistical analysis

The statistical analysis was conducted using R software (version 2.15.2). The allele frequency was calculated as allele numbers/total number of chromosomes, that is 1 for males, 2 for females. To compare allele frequencies of the P and E1 communities, we used the chi-square test. Since we did not find any significant differences between the communities for any of the regions ($p > 0.24$), for the further analysis we handled the samples as belonging to one single population. We used two indices, Shannon's equitability (EH) and expected heterozygosity (H_e , Nei and Roychoudhury, 1974), to examine the diversity among humans, chimpanzees and bonobos on the loci *ARQ*, *ARG*, *MAin2* and *MBin2*.

Results

The frequencies, Shannon's equitability and expected heterozygosity of the *ARQ*, *ARG*, *MAin2* and *MBin2* alleles in bonobos, along with those of humans and chimpanzees obtained from published data (Comings et al., 1999; Hong et al., 2006, 2008) are presented in Tables 1, 2, 3 and 4, respectively.

ARQ

We found 6 lengths in *ARQ* in bonobos, containing 11, 12, 15, 16, 17 and 18 CAG repeats (Table 1, typical peak patterns of PCR products in Supplementary Fig. S1). Bonobos had the fewest number of alleles per locus among the three species, in spite of the high values of expected heterozygosity and the Shannon's equitability ($H_e = 0.762$, $EH = 0.634$) compared with the other examined loci regarding all the three species.

Table 1
Allele frequencies of androgen receptor CAG repeats in human, chimpanzee and bonobo.

<i>ARQ</i>	Human <i>N</i> = 85	Chimpanzee <i>N</i> = 89	Bonobo <i>N</i> = 54
CAG11			0.037
CAG12			0.019
CAG14	0.023	0.045	
CAG15	0.012	0.011	0.278
CAG16			0.296
CAG17		0.056	0.130
CAG18		0.326	0.241
CAG19	0.059	0.079	
CAG20	0.109	0.011	
CAG21	0.137 ^a	0.146 ^a	
CAG22	0.117	0.112	
CAG23	0.125	0.124	
CAG24	0.098	0.045	
CAG25	0.070	0.034	
CAG26	0.102	0.011	
CAG27	0.059		
CAG28	0.066		
CAG29	0.023		
H_e	0.903	0.830	0.762
EH	1	0.852	0.634

Sequences of alleles with underlined frequencies were determined.

Human and chimpanzee allele frequencies were obtained from published data (human from Comings et al., 1999, chimpanzee from Hong et al., 2006).

H_e : the expected heterozygosity.

EH : the Shannon's equitability.

N: the number of X chromosomes.

^a GenBank accession numbers: human CAG21 NM_000044, chimpanzee CAG21 NM_001009012, bonobo CAG15–CAG18 AB970511–AB970514.

Table 2

Allele frequencies of androgen receptor GGN repeats in human, chimpanzee and bonobo.

ARG	Human	Chimpanzee	Bonobo
	N = 85	N = 89	N = 54
GGN14		0.011	
GGN16	0.013		
GGN17		0.551 ^a	
GGN18		0.022	0.870
GGN19	0.089	0.393	<u>0.130</u>
GGN20		0.011	
GGN22	0.032	0.011	
GGN23	0.446 ^a		
GGN24	0.382		
GGN25	0.013		
GGN26	0.013		
GGN27	0.013		
H_e	0.646	0.541	0.234
EH	1	0.726	0.309

Sequences of alleles with underlined frequencies were determined.

Human and chimpanzee allele frequencies were obtained from published data (human from Comings et al., 1999, chimpanzee from Hong et al., 2006).

Refer to Table 1 for abbreviations H_e , EH and N.^a GenBank accession numbers: human GGN23 NM_000044, chimpanzee GGN17 NM_001009012, bonobo GGN18 AB970515, GGN19 AB970516.

The nucleotide sequence alignments are shown in Fig. 1. A preceding sequence of 63 bp was uniform in bonobos, chimpanzees and humans, followed by a CTG repeat region that was (CTG)₂ in bonobos, CTG or (CTG)₂ in chimpanzees, while (CTG)₃ in humans. The polymorphic CAG repeat region was followed by a monomorphic CAG repeat region: (CAG)₅ in the *Pan* genus and (CAG)₆ in humans. The sequence of 18 bp between the two CAG repeat regions was uniform in the three species. The following sequence of 27 bp was common in the three species.

Table 3

Allele frequencies of monoamine oxidase A intron 2 dinucleotide repeats in human, chimpanzee and bonobo.

MAOA	Human		Chimpanzee		Bonobo	
	N = 80		N = 66		N = 54	
	Sequence	fr	Sequence	fr	Sequence	fr
CN14			(CA)10CG(CA)3	0.020		
CN15			(CA)11CG(CA)3	0.740 ^a		
CN16			(CA)12CG(CA)3	0.230 ^a	(CN)16	0.037
CN17					(CA)13CG(CA)3	0.241
CN18			(CA)14CG(CA)3	0.020	(CA)14CG(CA)3	<u>0.148</u>
CN19	(CA)13(CGCA)2(CA)2	0.010			(CA)15CG(CA)3	<u>0.370</u>
CN20	(CA)14(CGCA)2(CA)2	0.280 ^a				
CN21	(CA)15(CGCA)2(CA)2	0.160				
CN22	(CA)16(CGCA)2(CA)2	0.080			(CA)22	<u>0.111</u>
CN23	(CA)17(CGCA)2(CA)2	0.060			(CN)23	0.019
CN24	(CA)18(CGCA)2(CA)2	0.310 ^a			(CN)24	0.019
CN25	(CA)19(CGCA)2(CA)2	0.090			(CN)25	0.056
CN26	(CA)20(CGCA)2(CA)2	0.010				
H_e		0.782		0.399		0.766
EH		1		0.424		0.985

Sequences of alleles with underlined frequencies were determined.

Human and chimpanzee allele frequencies were obtained from published data (Hong et al., 2008).

Refer to Table 1 for abbreviations H_e , EH and N.

fr: frequency.

^a GenBank accession numbers: human CN20 AB302097, CN24 AB302098, chimpanzee CN15 AB302099, CN16 AB302100, bonobo CN17–CN19, CN22 AB970517–AB970520.

Table 4

Allele frequencies of monoamine oxidase B intron 2 dinucleotide repeats in human, chimpanzee and bonobo.

MAOB	Human N = 80		Chimpanzee N = 66		Bonobo N = 54	
	Sequence	fr	Sequence	fr	Sequence	fr
GN19	(GT)17(GA)2	0.010				
GN20	(GT)18(GA)2	0.140			(GT)13(GA)7	<u>0.370</u>
GN21	(GT)19(GA)2	0.040			(GT)15(GA)6	<u>0.093</u>
GN22	(GT)20(GA)2	0.040	(GT)13(GA)9	0.020	(GT)11(GA)11	<u>0.074</u>
GN23	(GT)21(GA)2	0.030			(GT)12(GA)11	<u>0.241</u>
GN24	(GT)22(GA)2	0.080 ^a	(GT)15(GA)9	0.740 ^a	(GT)13(GA)11	<u>0.222</u>
GN25	(GT)23(GA)2	0.080	(GT)16(GA)9	0.230 ^a		
GN26	(GT)24(GA)2	0.150	(GT)17(GA)9	0.020		
GN27	(GT)25(GA)2	0.400 ^a				
GN28	(GT)26(GA)2	0.040				
GN29	(GT)27(GA)2	0.010				
H_e		0.779		0.399		0.743
EH		1		0.375		0.762

Sequences of alleles with underlined frequencies were determined.

Human and chimpanzee allele frequencies were obtained from published data (Hong et al., 2008).

Refer to Table 1 for abbreviations H_e , EH and N .

fr: frequency.

^a GenBank accession numbers: human GN24 X63276, GN27 AB302115, chimpanzee GN24 AB302116, GN25 AB2117, bonobo GN20–GN24 AB970521–AB970525.

ARG

We detected 2 alleles in ARG in bonobos, containing 18 and 19 GGN repeats (Table 2, typical peak patterns in Supplementary Fig. S2). The (GGN)₁₈ allele was common and the expected heterozygosity and the Shannon's equitability were small ($H_e = 0.234$, $EH = 0.309$) compared to those values of humans and chimpanzees.

The nucleotide sequence alignments are shown in Fig. 2. A preceding sequence of 42 bp was identical in the three species. ARG had 4 motifs in bonobos (b–d in Fig. 3): (GGT)₂, (GGG)₂, (GGT)₂ and (GGC)_n from 5' to 3'. This structure was identical to that of the chimpanzee, while in humans the second component of the motif was different: GGTGGG instead of (GGG)₂. Repeat sequence was followed by a sequence of 63 bp that was uniform in the three species.

MAin2

We uncovered 8 alleles in MAin2 in bonobos, containing 16, 17, 18, 19, 22, 23, 24 and 25 CN repeats (Table 3, typical peak patterns in Supplementary Fig. S3). The expected heterozygosity and the Shannon's equitability had high values ($H_e = 0.766$, $EH = 0.985$), exceeding the chimpanzee diversity values and almost equivalent to those of the human population.

The nucleotide sequence alignments are shown in Fig. 3. A preceding sequence of 78 bp was identical in the three species with the exception of a C/T SNP in bonobos and a monomorphic G → T single nucleotide change in humans ('a' in Fig. 3). MAin2 contained a polymorphic dinucleotide repeat (CA)_n, followed by a single copy of (CGCA) motif in the Pan genus with a G/A SNP in bonobos (c in Fig. 3), while (CGCA)₂ in humans, followed by a monomorphic (CA)₂ motif in the three species. Repeat sequence was followed by a sequence of 38 bp that was equal in the three species.

MBin2

We discovered 5 alleles in MBin2 in bonobos, containing 20–24 GN repeats (Table 4, typical peak patterns in Supplementary Fig. S4). The expected heterozygosity and the Shannon's equitability values

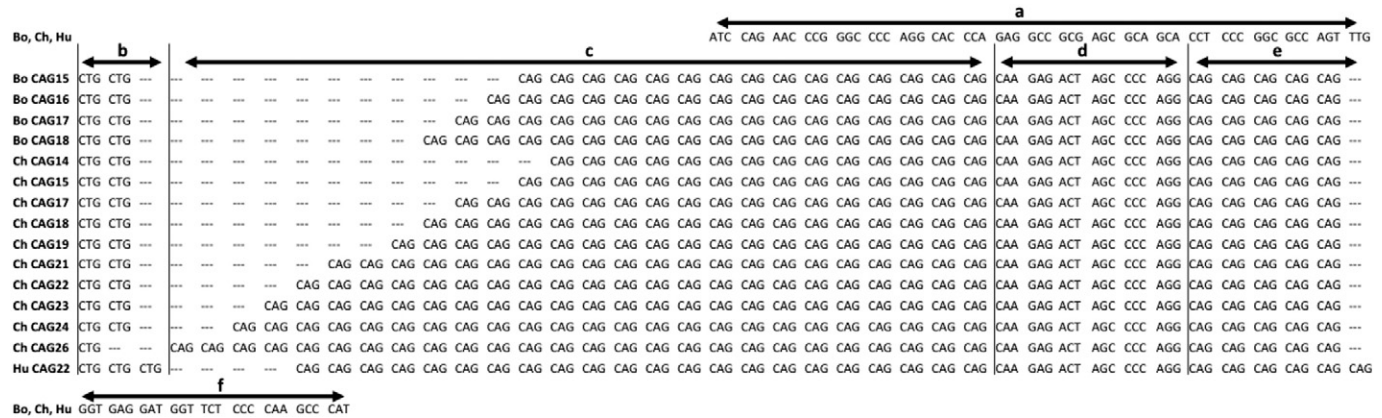


Fig. 1. Comparison of AR CAG repeats among bonobo, chimpanzee and human. *Bo* bonobo, *Ch* chimpanzee, *Hu* human. The number following CAG represents the repeat number of CAG trinucleotide. In the nucleotide sequence a: the preceding sequence that is common in bonobos, chimpanzees and humans, b: the preceding CTG repeats, c: the CAG repeats, d: a following sequence that is common in the three species, e indicates a second, non-polymorphic CAG repeat region, f: the following sequence that is common in the three species. GenBank accession numbers: human CAG21 NM_000044, chimpanzee CAG21 NM_001009012, bonobo CAG15–CAG18 AB970511–AB970514.

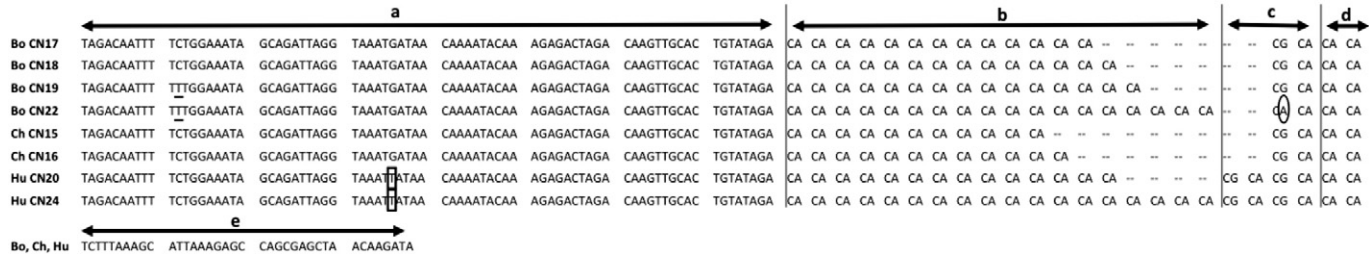


Fig. 3. Comparison of MAOA CN repeats among bonobo, chimpanzee and human. *Bo* bonobo, *Ch* chimpanzee, *Hu* human. The number following CN represents the repeat number of CN dinucleotide. In the nucleotide sequence a: the preceding sequence that is identical in the three species with the exception of a C/T SNP in bonobos (underlined) and a monomorphic G → T single nucleotide change in humans (boxed), b: the CA repeat region, c: CGCA in the *Pan* genus with a G/A SNP in bonobo (circled), (CGCA)₂ in human, d: (CA)₂ in the three species, e: the following sequence that is common in the three species. GenBank accession numbers: human CN20 AB302097, CN24 AB302098, chimpanzee CN15 AB302099, CN16 AB302100, bonobo CN17–CN19, CN22 AB970517–AB970520.

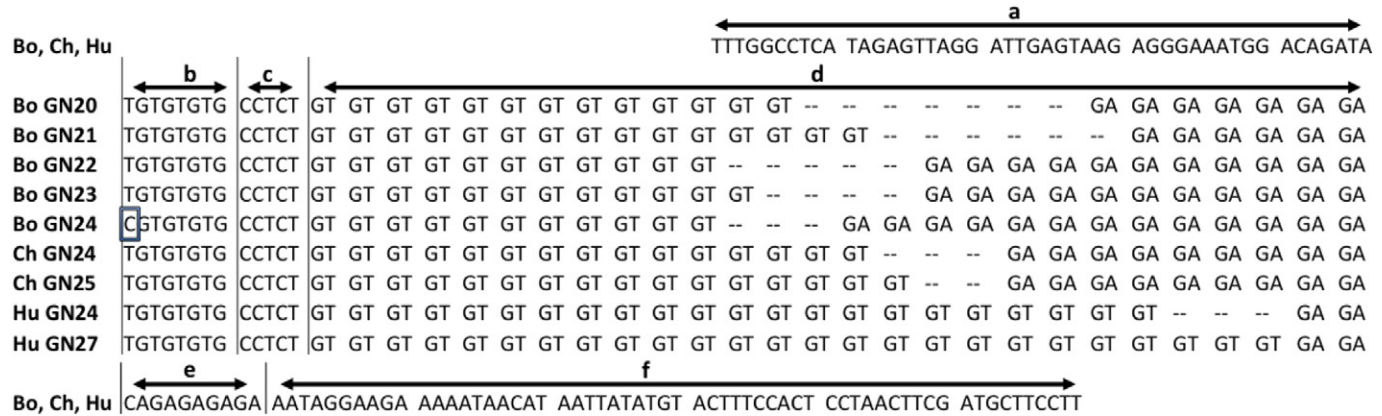


Fig. 4. Comparison of *MAOB* GN repeats among bonobo, chimpanzee and human. *Bo* bonobo, *Ch* chimpanzee, *Hu* human. The number following GN represents the repeat number of GN dinucleotide. In the nucleotide sequence a: the preceding sequence that is common in bonobos, chimpanzees and humans, b: (TG)₄ with a T/C SNP in bonobos (boxed), c: common in the three species, d: the GN repeat region, e: CA(GA)₄, f: the following sequence that is common in the three species. GenBank accession numbers: human GN24 X63276, GN27 AB302115, chimpanzee GN24 AB302116, GN25 AB2117, bonobo GN20–GN24 AB970521–AB970525.

($H_e = 0.743$, $EH = 0.762$) were greater than those of the chimpanzee, but remained below the human diversity values.

The nucleotide sequence alignments are shown in Fig. 4. A preceding sequence of 47 bp was common in the three species. The following (TG)₄ motif with a C/T SNP in bonobos (b in Fig. 4) was followed by a motif of CCTCT in the three species. *MBin2* had 2 motifs that showed different tendencies in the three species: (GT)_n and (GA)_n in the bonobo, (GT)_n and (GA)₉ in the chimpanzee, while (GT)_n and (GA)₂ in the human. The polymorphic GN repeat was followed by a (GA)₄ motif separated by a CA dinucleotide. The following sequence of 60 bp was uniform in the three species.

Discussion

In wild bonobos, the four loci, *ARQ*, *ARG*, *MAin2* and *MBin2*, were all polymorphic. Apart from *ARG*, where the study population had only 2 alleles, the loci were very diverse. We found the flanking regions of *ARG* and *ARQ* highly conservative as in chimpanzees and humans. The flanking regions of *MAin2* and *MBin2* were more diverse in bonobos than in chimpanzees or humans. Bonobos had 2 SNPs in the *MAin2* locus and 1 SNP in the *MBin2* locus, while no SNP was present in either humans or chimpanzees in these flanking regions. In the case of *MBin2* the GN repeat region itself was more diverse than that of humans or chimpanzees. In the latter species, only the GT repeat region has been found to be polymorphic (Grimsby et al., 1992; Hong et al., 2008), while in the bonobos we found that the GA repeat region was also polymorphic. Loci *MAin2* and *MBin2* showed more allelic diversity in bonobos than in chimpanzees. Given that our bonobo population came from only two neighboring communities, while the chimpanzee population samples originated presumably from several communities (Hong et al., 2008), the result was surprising. Based on multiple intergenic autosomal regions, nucleotide diversity of bonobos has been found to be closest to western chimpanzees, who are characterized by the lowest nucleotide diversity among chimpanzee subspecies (Fischer et al., 2006, 2011), which underlines our finding that bonobos showed higher diversity in the case of *MAin2* and *MBin2*. Bonobos have lower level of aggression than chimpanzees (Furuichi, 2009; Hare et al., 2012). Because monoamine oxidase influences aggressiveness (Shih and Thompson, 1999), the differences between the polymorphisms of *MAin2* and *MBin2* in bonobos and chimpanzees may be associated with the differences in aggression between the two species.

ARQ and *ARG* have also been found to be associated with aggressiveness in humans (Vermeersch et al., 2010). We found that locus *ARQ* in bonobos had generally shorter alleles than in humans or chimpanzees. Such a finding could contradict other reports that more aggressive humans tended to have shorter CAG repeats (Jönsson et al., 2001). This unexpected result remains to be explained. Studies are needed focusing on the functions of the alleles in chimpanzees, bonobos and humans in order to understand the evolution of *AR*. Bonobos and chimpanzees are particularly adequate species to compare regarding *AR* polymorphisms, since it has been found that these two species were different in testosterone level change throughout ontogeny (Wobber et al., 2013).

The weak points of this study are that our bonobo samples originated from two neighboring communities in the wild, and 13 individuals were mother–offspring pairs among the 32, while the chimpanzee and human samples that we compared our results with originated from several populations. Even though this raises the significance of the higher diversity that we found in two of the four analyzed loci in bonobos, we have to consider our comparison with great care. The genetic diversity of bonobos shows extensive geographic variation (Kawamoto et al., 2013). Future studies investigating these loci in different bonobo populations are therefore recommended. Individual-based comparison is also necessary to elucidate the function of the candidate loci. For example Hong et al. (2011) have examined a polymorphism of tryptophan hydroxylase 2 gene in chimpanzees, and they reported that neuroticism was associated with it, as in humans.

In this study we found that bonobos were polymorphic on loci *ARQ*, *ARG*, *MAin2* and *MBin2*, and that the diversity of the latter two loci was higher than in chimpanzees. Bonobos and chimpanzees differ in their level of aggressiveness, and aggressiveness could be associated with the genes that we analyzed, as in humans. Therefore, to understand the evolution of these loci and eventually *AR*, *MAOA* and *MAOB* in humans and non-human primates, it would be useful to conduct future studies on the association of aggressiveness

and other personality traits with the polymorphisms found in this study in bonobos and to compare the results with those of chimpanzees and humans.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.mgene.2014.10.005>.

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