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AVPR1A Variation in Chimpanzees (*Pan troglodytes*): Population Differences and Association with Behavioral Style

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Abstract Primates and other mammals show measurable, heritable variation in behavioral traits such as gregariousness, timidity, and aggression. Connections among behavior, environment, neuroanatomy, and genetics are complex, but small genetic differences can have large effects on behavioral phenotypes. One of the best examples of a single gene with large effects on natural variation in social behavior is *AVPR1A*, which codes for a receptor of the peptide hormone arginine vasopressin. Work on rodents shows a likely causal association between *AVPR1A* regulatory polymorphisms and social behavior. Chimpanzees also show variation in the *AVPR1A* regulatory region, with some individuals lacking a *ca.* 350-bp segment corresponding to a putative functional element. Thus, chimpanzees have a "short" allele (segment deletion) and a "long" allele (no deletion) at this locus. Here we compare *AVPR1A* variation in two chimpanzee populations, and we examine behavioral and hormonal data in relation to *AVPR1A* genotypes. We genotyped *AVPR1A* in a captive population of western chimpanzees (*Pan troglodytes verus*, New Iberia Research Center; $N = 64$) for which

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we had quantitative measures of personality (based on 15 behavioral style indices, calculated from 3 yr of observational data), dominance rank, and baseline testosterone levels. We also provide the first assessment of *AVPR1A* genotype frequencies in a wild eastern chimpanzee population (*Pan troglodytes schweinfurthii*, Ngogo community, Kibale National Park, Uganda; $N = 26$). Our results indicated that the *AVPR1A* long allele was associated with a "smart" social personality in captive western chimpanzees, independent of testosterone levels. Although the frequency of the long allele was relatively low in captive western chimpanzees (0.23), it was the major allele in wild eastern chimpanzees (0.62). Our finding that allele and genotype frequencies for the *AVPR1A* polymorphism differ among chimpanzee populations also highlights the need for comparative studies —across subspecies and research sites— in primate behavioral genetics.

Keywords Apes · Behavioral genetics · Gene expression · Hormones · Personality · Vasopressin

Background

Primates and other mammals show measurable variation in behavioral traits such as gregariousness, timidity, and aggression, and much evidence points to genetic influences on this variation (Fairbanks *et al*. 2004; Fitzpatrick *et al*. 2005; Newman *et al*. 2005; Suomi *et al*. 2006). Connections among behavior, environment, neurochemistry, and genetics are complex (Flint *et al*. 2010), but identifying the genes that contribute to variation in behavioral tendencies is not an untenable task. Several studies have shown that small genetic changes can have large effects on behavioral phenotypes. For example, variation at specific candidate genes in key neuromodulatory pathways has been associated with aggression (*MAOA*), social anxiety (*SLC6A4*), and risk taking (*DRD4*) in humans and other mammals (Brent *et al*. 2013; Cases *et al*. 1996; Lesch *et al*. 1996; Newman *et al*. 2005; Rogers *et al*. 2004; Wendland *et al*. 2006).

One of the best examples of a gene with striking effects on natural variation in social behavior is *AVPR1A* (Entrez Gene ID: 552), which codes for a receptor of arginine vasopressin. Arginine vasopressin is a peptide hormone with both peripheral and central effects in vertebrates (Caldwell *et al*. 2008). It has important effects on affiliative, agonistic, and sexual behaviors in animals as diverse as fish (Godwin and Thompson 2012), rodents (Winslow *et al*. 1993), and humans (Ebstein *et al*. 2009; Heinrichs *et al*. 2009; Prichard *et al*. 2007; Walum *et al*. 2008).

Variation in the regulation and distribution of central vasopressin receptors —particularly receptor 1a, the product of *AVPR1A*— may help explain inter- and intraspecific variation in social behavior in mammals (Young and Hammock 2007; *cf*. Fink *et al*. 2006; Rosso *et al*. 2008). In rodents, differences in the pattern of *AVPR1A* expression in the brain are determined in part by length polymorphisms, e.g., microsatellites, in the regulatory region of this gene, and these small sequence length differences can underlie normal variation in gregariousness and affiliative behavior (Donaldson and Young 2008; Insel *et al*. 1994; Young *et al*. 1997, 1999; *cf*. Ophir *et al*. 2008).

This association between *AVPR1A* regulatory polymorphisms and social behavior has been most convincingly demonstrated by a series of studies on voles (Bielsky *et al*.

2005; Hammock and Young 2005, 2006; Lim *et al.* 2004; Young *et al.* 1997, 1999; *cf.* Solomon *et al.* 2009). Closely related montane and prairie voles have promiscuous (nonmonogamous) and monogamous mating systems, respectively, and show corresponding differences in vasopressin receptor expression: prairie voles have higher vasopressin receptor expression in the ventral pallidum and other brain regions than montane voles (Young and Wang 2004). The coding sequence of *AVPR1A* does not differ markedly between these vole species, but sequence length variation in the *AVPR1A* regulatory region is associated with differences in social bonding: prairie voles, the monogamous species, exhibit a *ca.* 400-bp sequence in the 5′ flanking region that is absent in montane and other non-partner-forming voles (Young and Wang 2004; *cf.* Fink *et al.* 2006). Functional assays indicate that this simple length polymorphism in the regulatory region directly alters gene expression (Hammock and Young 2002).

Humans also show sequence length polymorphisms in the upstream regulatory region of this vasopressin receptor gene. Specifically, length variation in the RS3 microsatellite, 3625bp upstream of the transcription start site (Thibonnier *et al.* 1996, 2000), has been associated with differences in human social behavior. Variation at this RS3 microsatellite has been linked to autism (Meyer-Linderber *et al.* 2009; Yirmiya *et al.* 2006), musical aptitude (Ukkola *et al.* 2009), altruistic tendencies, e.g., performance in dictator games (Israel *et al.* 2008), and the propensity of men to form satisfying long-term partnerships (Walum *et al.* 2008).

The *AVPR1A* gene of chimpanzees (*Pan troglodytes*; Ensembl gene ID: ENSPTRG00000005167) exhibits especially interesting variation (Fig. 1; Donaldson *et al.* 2008; Hong *et al.* 2009; Rosso *et al.* 2008). Some individuals completely lack a *ca.* 350-bp segment corresponding to the region containing the RS3 microsatellite. This deletion results in a "short" allele (sometimes referred to as the $DupB^{-}$ allele), whereas the ancestral "long" allele (sometimes referred to as the $DupB^{+}$ allele) retains the segment containing RS3 (Fig. 1). Given that the RS3 microsatellite is thought to influence *AVPR1A* gene expression and aspects of behavioral tendencies in humans (Knafo *et al.* 2008), we can speculate that a complete deletion of this region would affect behavior in chimpanzees.

A previous study (Donaldson *et al.* 2008) genotyped wild-born chimpanzees living at the Yerkes National Primate Center and M. D. Anderson Cancer Center ($N = 47$) and found the frequency of the short allele (the deletion; 0.76) was much higher than the

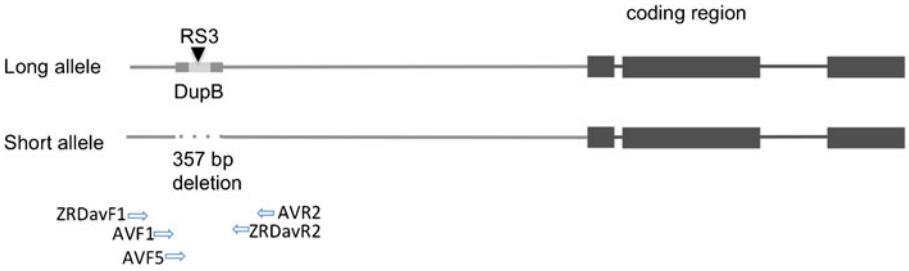

Fig. 1 Length variation in the *AVPR1A* regulatory region of chimpanzees. A 344-bp region, DupB (dark gray box), has been deleted creating a short allele and a long allele. The deleted region of the short allele contains the RS3 microsatellite (light gray), which, like the orthologous human RS3, is *ca.* 3620 bp upstream of the transcription start site. Small arrows correspond to primer annealing sites. Schematic based on Donaldson *et al.* (2012), Thibonniern *et al.* (2000), and Ensembl annotation ENSPTRG00000005167.

frequency of the long allele (no deletion; 0.24) (based on the raw data given in Figure 4 of Donaldson *et al*. 2008). Allele frequencies were in Hardy-Weinberg equilibrium, and only 3 of the 47 individuals were homozygous for the long allele. Most, if not all, of these captive chimpanzees belonged to the West African chimpanzee subspecies (*Pan troglodytes verus*; Donaldson *et al*. 2008), as do 95% of captive chimpanzees in the United States (Ely *et al*. 2005). However, the extent to which this genetic variation underlies variation in behavior is unclear.

Social bonding, especially among males, is a hallmark of chimpanzee social behavior and individuals vary in overall gregariousness and in specific aspects of behavior, such as how much they groom with others and how often they participate in coalitions (Langergraber *et al*. 2009; Mitani 2009; Mitani *et al*. 2000, 2002; Watts 2000a,b). The only study to date to examine potential associations between behavior and *AVPR1A* genotype (Hopkins *et al*. 2012) used personality questionnaires in which human observers rated their impressions of individual chimpanzees, as described in Weiss *et al*. (2007). Even with this subjective assessment of personality, male chimpanzees — but not females— at the Yerkes National Primate Research Center ($N = 83$) showed a positive association between genotype (having the long allele) and "conscientiousness" (Hopkins *et al*. 2012), suggesting a potential role for this *AVPR1A* variant in shaping aspects of personality.

Here we present genotype data for two additional chimpanzee populations, and we examine *AVPR1A* genotypes within the context of quantitative measures of behavioral style (personality; Sapolsky and Ray 1989). We also include data on dominance rank and baseline testosterone levels: addition factors that potentially influence chimpanzee social behavior (Anestis 2006). Because the RS3 microsatellite (present in the chimpanzee long allele but missing in the chimpanzee short allele) purportedly influences *AVPR1A* expression and personality in humans (Ebstein *et al*. 2012), we specifically ask: Does genotype, perhaps in combination with testosterone, show an association with behavioral style in chimpanzees? Our sample includes a captive population (western chimpanzees, *Pan troglodytes verus*, living at the New Iberia Research Center, part of the University of Louisiana system: NIRC) for which we have detailed quantitative data on behavioral style/personality and testosterone levels. We also provide the first assessment of genotype frequencies in a wild chimpanzee population (eastern chimpanzees, *Pan troglodytes schweinfurthii*, living at the Ngogo research site, Kibale National Park, Uganda: Ngogo), providing a comparison of genotype frequencies between subspecies.

Methods

Samples

In total we genotyped the *AVPR1A* regulatory polymorphism for 90 chimpanzees from two populations: a captive chimpanzee population living at NIRC in New Iberia, Louisiana ($N = 64$) and a wild population, Ngogo, Kibale National Park, Uganda ($N = 26$). The samples mostly represent unrelated or distantly related individuals. Breeding records for the captive NIRC population allowed us to focus on individuals that were unlikely to be related, and aside from one pair of maternal brothers, the study subjects

had different mothers. Based on the males in the enclosures at the times of conception, they should also have different fathers; even allowing for the possibility of cross mating through the enclosure mesh, only a small fraction of the 2016 dyads could be related. Genetic analysis of kinship among the wild chimpanzees at Ngogo (Langergraber *et al.* 2007) similarly indicates that only 7 of the 325 dyads (2%) were relatives (half-sibling, sibling, parent–offspring).

The captive population consisted of young (4–10 yr) male (N = 36) and female (N = 28) western chimpanzees (*Pan troglodytes verus*; Ely *et al.* 2005) residing in mixed-sex social groups of 8–12 individuals. Over the course of the study (2000–2002; 2007), subjects resided in *ca.* 10 social groups, which changed slightly each year as a few individuals switched in and out of particular groups. Veterinarians collected the blood samples during routine examinations and shipped the frozen samples to the Yale University Molecular Anthropology Laboratory for analysis.

The wild population consisted of 26 males from the large, well-studied Ngogo community (Watts 2012). We assessed the genotypes of these chimpanzees using fecal samples collected noninvasively and opportunistically during field observations. Fecal samples consisted of *ca.* 5 g (wet weight) of fresh feces, collected from habituated chimpanzees shortly after defecation. The fecal bolus was initially stored in 35 ml of 90% ethanol then transferred to a 50-ml tube prefilled with silica gel beads for desiccation and storage (following Nsubuga *et al.* 2004; Roeder *et al.* 2004). We sent the sealed samples to the Yale Molecular Anthropology Laboratory, where we stored them at +4º C.

Ethical Note

The Uganda Wildlife Authority and the Ugandan National Council of Science and Technology granted permission to collect fecal samples and conduct research at Ngogo. The veterinarians at the New Iberia Research Center collected the blood samples when chimpanzees were anesthetized for other reasons (standard health checks). All individuals at NIRC were housed and handled in strict accordance with good animal practice as defined by the University of Louisiana at Lafayette Institutional Animal Care and Use Committee, following the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, and all animal work was approved by this committee. Protocol approval numbers for Institutional Animal Care and Use Committees are: University of Louisiana at Lafayette IACUC#2010-8707-053; Yale University IACUC#2010-11378.

DNA Extraction and Quantification

Methods of DNA isolation are described in McIntosh *et al.* (2012). Briefly stated, we extracted DNA from 200-µl blood samples using the QIAampDNA Mini Kit automated on a QiaCube (Qiagen). We extracted DNA from *ca.* 100 g fecal material using the QIAamp DNA Stool Mini Kit (Qiagen) after a 48-h incubation period in ASL buffer. For both types of extraction, we recovered the DNA in 200 µl of elution buffer, and we kept samples frozen at –20ºC. Negative extraction controls showed no evidence of DNA contamination. We quantified chimpanzee fecal DNA using a real-time PCR assay targeting the c-*myc* gene (Morin *et al.* 2001) on a Rotor-Gene platform (Qiagen).

We quantified DNA from blood samples using a Nanodrop 2000 (Thermo-Fisher Scientific) spectrophotometer.

Genotyping

Genotyping protocols differed slightly for the high-quality blood-derived DNA and the lower quality fecal-extracted DNA.

Blood Samples Primers for amplifying DNA extracted from blood samples were: forward ZRDavF1: GCATGGTAGCCTCTCTTTAAT and reverse ZRDavR2: CATA CACATGGAAAGCACCTAA (Donaldson *et al*. 2008). These primers target a product of 573 bp (long or $DupB^+$ allele) or 927 bp (short or $DupB^-$ allele). Note that although the main DupB deletion (Fig. 1) is 344 bp, this amplicon includes an additional smaller deletion (10 bp) upstream of DupB. For the sequences examined here, this resulted in a total length difference of 354 bp for this primer pair. This differs slightly from the length difference (357 bp) reported by Donaldson *et al*. (2008), probably because of slight length variation in the RS3 microsatellite of the long allele.

Our polymerase chain reactions (PCRs), in a total volume of 50 μl, contained the Epicentre Failsafe PCR System with Premix I, 2 μl of template (10–50 μg), and a 200 nM concentration of each primer. We amplified PCR targets on Geostorm thermocyclers under the following conditions: initial activation at 95ºC for 2 min, 35 cycles of 30-s denaturation at 95ºC, 30-s annealing at 57ºC, 3-min extension at 72ºC, and a final extension of 7 min at 72ºC. We independently genotyped samples two ($N = 29$), three ($N = 32$), or four ($N = 3$) times to confirm genotypes. Negative controls were always clean. We visualized alleles on a 1.8% agarose gel with a 50–2000 bp ladder (Fisher Low Range). We also ran a subset of blood-derived DNA samples using the alternate sets of primers (those used for the fecal-derived DNA) to confirm that genotypes were consistent regardless of primer pair used.

Fecal Samples Because fecal DNA is more degraded than blood DNA, we designed new primers targeting a smaller product. We had two variants of the forward primer (AVF1: CCACATATAAACGCTGACCCGC; AVF5: TCAGAGGGATCCTGTAGAGA), each with the same reverse primer (AVR2: CAGAAAATGCTTAGTGACTGG). The primer pairs, tested first using blood samples with confirmed genotypes (see earlier), generated consistent results (long allele = *ca.* 460 bp or *ca*. 430 bp; short allele = 116 bp or 90 bp, for AVF1 and AVF5 respectively). PCRs, in a total volume of 20 μl, contained 3–5 μl of template (>100 pg), a 400 nM concentration of each primer, 1.0X SuperTaq® buffer, 0.8 mM $MgCl_2$, 250 μM dNTPs, 16 μg of BSA, and 0.25 Units of SuperTaq DNA Polymerase (Life Technologies). Thermocycler (Geostorm) conditions were: initial activation at 95ºC for 5 min, 40–45 cycles of 30-s denaturation at 95ºC, 30-s annealing at 52ºC, 1-min extension at 72ºC, and a final extension of 7 min at 72ºC. We visualized alleles on a 1.8% agarose gel as described in the preceding text, and genotypes were confirmed with two to nine independent PCRs (following Morin *et al*. 2001).

To confirm that the PCR amplicons represented the region of interest, we Sanger sequenced PCR products for homozygous short and homozygous long individuals from each population (captive and wild) on an Applied Biosystems 3730×l DNA Genetic Analyzer at the Yale DNA Analysis Facility.

Hormone Analysis

We obtained hormone levels for a subset of the captive population as part of a previous study of the relationship between hormones and behavior (Anestis 2005, 2006). Briefly stated, our testosterone (males only; $N = 30$) measurements come from urine analysis (per individual: minimum $N = 5$, mean N = 17) using validated radioimmunoassays and enzyme immunoassays in the Yale Reproductive Ecology Laboratory. Raw data were corrected for urine concentration using creatinine or specific gravity. This study included the 17 males analyzed in Anestis (2006), plus an additional 13 males for which we had corresponding behavioral and genotypic data. Baseline testosterone concentrations (mean = 326 ng/ml) were categorized into three rank classes (low: 37–164 ng/ml; medium: 220–321 ng/ml; high: 37 –1068 ng/ml). Details of the hormone methodology and analyses can be found in Anestis (2005, 2006).

Behavioral Data

Our measures of personality, or behavioral style, are based on data collected at NIRC by S. F. Anestis in 2000–2002 and by M. B. Fontenot in 2007. S. F. Anestis used all-occurrences sampling to collect data on social interactions for 41 individuals as part of a study in which she systematically quantified behavioral style (personality) in chimpanzees and examined the relationship between hormone levels, rank, and personality (Anestis 2005, 2006; Table I). M. B. Fontenot used focal sampling to collect data on rank, grooming rate, and affiliation (degree to which individuals spent time in close proximity to others) for another 23 individuals in 2007 as part of a different study. These data were not directly comparable to those collected for the personality study and thus were not our primary focus, but they allowed us to test for associations between genotype and some basic measures of rank and affiliation with a larger sample. These methods contrast sharply with those of Hopkins *et al.* (2012), who asked human observers to describe chimpanzee personalities via questionnaires consisting of 43 adjectives, e.g., dominant, active, lazy, gentle, etc.

Dominance Rank Chimpanzees show clear signals of dominance and subordinance in social groups, and hierarchies tend to be linear (Nishida 1979; Watts 2002; Wittig and Boesch 2003). We assessed dominance relationships for the NIRC groups by constructing matrices of pant grunts (formal, unidirectional vocal signals of subordinate status; Nishida 1979) and supplants. We used the results to divide individuals into three rank classes (low, medium, and high). Focal subjects were all immature, and though males were dominant to females in some mixed-sex dyads, females were dominant to males in others (Anestis 2005).

Behavioral Style We (S. F. Anestis) collected behavioral data with the specific intention of objectively measuring behavioral style (described in detail in Anestis 2005, 2006). Using all-occurrences data, we calculated 15 behavioral style indices that capture the broad spectrum of chimpanzee behavior (Table I). These measures are intended to describe aspects of individual behavioral style, especially those that presumably do not vary with age or group size. To avoid any biases that might have resulted from effects of group size on behavioral frequencies, we standardized the data by dividing the total

Table I Fifteen behavioral style measures based on observational data from the New Iberia Research Center chimpanzees

Is affiliative	% of initiated social interactions that were affiliative
Spreads affiliation	% of affiliation with primary partner
Has friends	No. of different friends divided by the total number of individuals in the group
Participates in play	% of total no. of group play sessions in which individual was a participant
Play partners	No. of different play partners over the study period, corrected for total number of individuals in group
Play offers accepted	% of play offers that are accepted
Gets groomed	% of individual's total grooming sessions as groomee
Participates in grooming	% of total no. of group grooming sessions in which individual was a participant
Does not react to aggression	% of "no reaction" responses to aggression against self
Does not react to approaches	% of "no reaction" responses to approaches
Initiates aggression	% of initiated social interactions that were aggressive
Coalition partners	No. of different coalition partners used in the study period, corrected for number of individuals in the group
Uses coalitions	% of aggressive interactions in which individual formed a coalition either as initiator or by joining a third part
Initiates winnable agonistic interactions	% of agonistic interactions initiated and won (opponent pant grunts, moves, screams, cries, gives fear face)
Chooses safe interactions	Approaches lower-ranking (LR) individuals more often than expected based on the no. of available LR individual

Based on Anestis (2005).

"Affiliative" interactions involve nonaggressive touching, hugging and/or grooming. "Aggressive" interactions are those involving hitting, threatening, attacking and/or chasing. Dyads whose combined affiliative (touch, groom, hug) frequencies were greater than the mean for all dyads in their group were categorized as "friends."

number of acts of a given type of behavior by the number of individuals in the group or by using percentages calculated on an individual or group-wide basis (Table I). For example, the measurement "is affiliative" represents the number of affiliative social interactions that a given individual initiated divided by the total number of social interactions it initiated.

Some behavioral style indices are interdependent, though covariance may not be immediately apparent. To investigate the relationships between variables in the sample, we used principal components analysis (Varimax rotation with Kaiser normalization, factors were only included if eigenvalues >1) to reduce the data —including all index scores for all individuals in the sample— to a set of principal factors. We log-transformed the data to reduce skew and kurtosis (Anestis 2006). We defined the dominant indices —those driving variation in components— as those with values >0.6 or <−0.6.

Based on this method, six behavioral style components (Table II) emerged from the 15 indices, which were labeled according to the indices driving each: "smart" (uses coalitions, gets groomed often, and has play offers frequently accepted); "affiliative" (grooms often relative to total grooming in group, and a high percentage of his/her initiated interactions are affiliative); "playful" (has many play partners, and

Table II Behavioral style components, and the behavioral indices driving each, for the New Iberia Research Center chimpanzees

Affiliative	□ Grooms and gets groomed frequently □ Initiates affiliation more than aggression
Aggressive	□ Has many coalition partners □ Initiates aggression more than affiliation
Friendly	□ Directs affiliative behaviors to all group members
Mellow	□ Exhibits low reactivity to both neutral approaches and aggression
Playful	□ Has many play partners □ Spends a lot of time playing
Smart	□ Uses coalitions □ Gets groomed frequently □ Has play offers accepted

Modified from Anestis (2005).

plays often relative to total play in group); "aggressive" (has many coalition partners and a high percentage of his/her initiated interactions are aggressive); "friendly" (spreads affiliative interactions among all group members); and "mellow" (tends to not react when being threatened).

Behavioral styles can be conceptualized as spectra along which individuals fall between the two possible extremes, similar to human personality classifications along scales such as introvert/extrovert and neuroticism/emotional stability (Eysenck 1947). In the analysis of behavioral style, individuals' scores served as independent variables in tests of genotype–behavior associations. Additional details about the observational methodology and the behavioral styles can be found in Anestis (2005, 2006).

Statistical Analyses

We tested for population adherence to Hardy–Weinberg equilibrium and consistency with genotype frequencies previously reported for a captive population of western chimpanzees (Yerkes National Primate Research Center and M.D Anderson Cancer Center; Donaldson *et al*. 2008). We assessed differences in allele frequencies between populations/subspecies using chi-squared contingency tables and the fixation index (Weir and Cockerham 1984). Population genetics calculations were run on GenoDive (Meirmans and VanTienderen 2004).

We used general linear models to examine the importance of genotype on behavioral style in the captive population. Because only four of the NIRC individuals had the LL genotype, we did not have a sufficient sample to use three categories of genotype (SS, SL, LL). We followed Hopkins *et al*. (2012) in classifying genotypes as having (LL and SL) or lacking (SS) the long allele. We first conducted analyses using the presence or absence of the long allele, i.e., genotype, as a sole binary independent variable predicting each of the six behavioral style indices. We examined additional models using dominance rank, sex, and testosterone as covariates. Ideally, all of the covariates would be included in the same model but this was not possible because the number of individuals with testosterone data was far fewer than the numbers for which

we had data on the other variables. Therefore, we used two models, one that contained genotype and testosterone as predictors plus one interaction term and a second that used genotype, dominance rank, and sex as predictors and included four interaction terms. If the interaction terms were not significant, we re-ran the analysis without them and interpreted the results of the main effects only. If an interaction term was significant, we interpreted the importance of the interaction effect, but did not do so for the main effects (Quinn and Keough 2002).

We used Akaike's Information Criterion (AIC) to judge the best models explaining each behavioral style when more than one model exhibited a $P < 0.05$. The model with the lowest AIC score is considered the best and any model within two AIC units is considered equally good (Burnham and Anderson 2002). We did not calculate AIC values for the models with testosterone as a predictor because these models contained a smaller sample size and AIC values can be compared only when using the same dependent variable and sample size. Primatological research is increasingly using AIC for model selection (Kamilar *et al.* 2013; Tecot *et al.* 2012; Wheeler *et al.* 2011) as it has several benefits when compared to stepwise procedures (Garamszegi 2011).

We examined diagnostic plots (residuals vs. leverage, QQ plots, etc.) for each model to confirm that the data conformed to the assumptions of linear models. We treated individuals as outliers if they exhibited a standardized residual greater than an absolute value of three. We removed any outliers and re-ran the respective models. We performed all linear models using the lm function in the "stats" package of R (R Development Core Team 2011).

Results

Genotype frequencies for the captive western chimpanzee population were in Hardy–Weinberg equilibrium ($\chi^2 = 0.260$, df = 1, $P = 0.61$), and the short allele was more common (frequency: 0.77) than the long allele (0.23). This remained true when we excluded the one related dyad ($\chi^2 = 0.222$, df = 1, $P = 0.64$) and when we calculated Hardy–Weinberg equilibrium for only males ($\chi^2 = 0.323$, df = 1, $P = 0.57$) or only females ($\chi^2 = 0.912$, df = 1, $P = 0.34$). Moreover, genotype and allele frequencies did not differ significantly between males and females (males: 0.79 S, 0.21 L; females: 0.73 S, 0.27 L; $\chi^2 = 0.17$, df = 1, $P = 0.92$). The NIRC allele and genotype frequencies were almost identical to those of a previous study (Donaldson *et al.* 2008) of captive western chimpanzees at Yerkes and M.D. Anderson Cancer Center ($\chi^2 = 0.116$, df = 2, $P = 0.94$; Fig. 2).

Genotype frequencies for the wild eastern chimpanzee population were also in Hardy–Weinberg equilibrium ($\chi^2 = 0.491$, df = 1, $P = 0.48$; excluding related dyads: $\chi^2 = 0.159$, df = 1, $P = 0.69$), but were strikingly different from the two captive populations ($\chi^2 = 0.22.1$, df = 2, $P < 0.001$; Fig. 2). The long allele was more common (frequency: 0.62) and a greater portion of the population was heterozygous (Ngogo: 0.54; NIRC: 0.33; Yerkes/MDACC: 0.30). However, F_{st} at this locus was still relatively low (0.10).

Significant behavioral differences emerged in the NIRC sample between individuals with and without a copy of the long *AVPR1A* allele (Tables III and IV; Electronic Supplementary Material Tables SI–SIV). In particular, two models (from which we

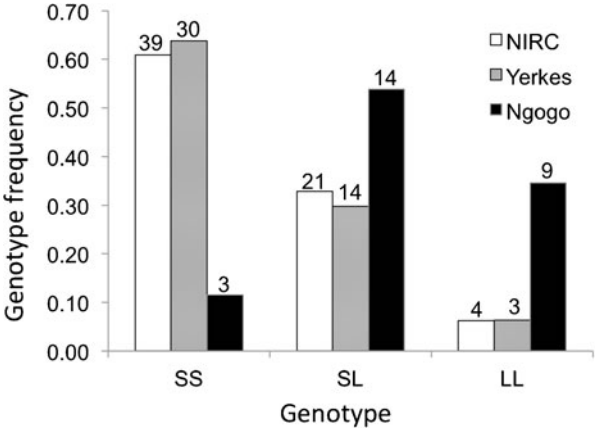

Fig. 2 Comparison of *AVPR1A* genotype frequencies and sample sizes across populations. White bars: captive western chimpanzees at New Iberia Research Center; light gray bars: captive western chimpanzees at Yerkes National Primate Center and M. D. Anderson Cancer Center (from Donaldson *et al.* 2008); black bars: wild eastern chimpanzees at Ngogo. The number of individuals with a given genotype is above each bar. S = short allele, or $DupB^-$; L = long allele or $DupB^+$. Although genotype frequencies differ between the captive and wild populations, all three populations are in Hardy–Weinberg equilibrium.

excluded one outlier) significantly predicted the "smart" index. Model 1 contained only genotype (model $P = 0.034$) and Model 2 contained genotype, sex, and dominance rank (model $P = 0.019$). Model 2 had the lowest AIC values (69.82), with Model 1 being within 2.01 AIC units of the best model, suggesting that genotype alone is nearly as good as the more complex model for predicting the "smart" component (Table III).

Only one behavioral style component ("friendly") was best predicted by a model containing interaction terms. A model containing genotype, dominance rank, and sex, plus all possible interactions of these variables, predicted the "friendly" component and exhibited the lowest AIC value, although it was not strictly significant ($P = 0.053$), suggesting that the explanatory value of this model is limited (Table IV). Importantly,

Table III Predicting the "smart" component of chimpanzee (New Iberia Research Center; 2000–2002) behavioral style using general linear models

Model	Predictors	Slope	SE	*t* value	*P* value	Model r^2	Model *F*	Model *P* value	df	AIC
1	Long allele	0.420	0.191	2.202	0.034	0.113	4.849	0.034	1, 38	71.83
2	Long allele	0.344	0.186	1.852	0.072	0.237	3.728	0.019	3, 36	69.82
	Sex	–0.404	0.187	–2.167	0.037					
	Dominance rank	–0.228	0.129	–1.763	0.086					
3	Long allele	0.477	0.254	1.878	0.076	0.156	1.766	0.198	2, 19	—
	Testosterone	0.059	0.166	0.356	0.726					

Model results without outlier.

Interaction terms were not statistically significant in any model.

The model with the lowest AIC value is considered the best and additional models within two AIC units are considered equally good. Note that model 1 is 2.01 AIC units from the best model.

Table IV Predicting the "friendly" component of chimpanzee (New Iberia Research Center; 2000–2002) behavioral style using general linear models

Model	Predictors	Slope	SE	*t* value	*P* value	Model r^2	Model *F*	Model *P* value	df	AIC
1	Long allele	0.188	0.224	0.838	0.407	0.018	0.702	0.407	1, 39	87.25
2	Long allele	–3.582	1.274	–2.811	0.008	0.325	2.269	0.053	7, 33	83.87
	Sex	–1.016	0.761	–1.335	0.191					
	Dominance rank	–0.191	0.276	–0.696	0.491					
	Long allele × sex	3.101	1.505	2.061	0.047					
	Long allele × DR	1.620	0.556	2.914	0.006					
	DR × sex	0.403	0.342	1.180	0.246					
	Long allele × sex × DR	–1.131	0.694	–1.631	0.112					
3	Long allele	–0.055	0.285	–0.192	0.850	0.063	0.674	0.521	2, 20	—
	Testosterone	0.203	0.189	1.074	0.295					

For model 2, there are statistically significant interaction terms demonstrating that individuals with the long allele have a positive relationship between dominance rank and friend index, and individuals with the long allele have higher friendly scores for males but not females. In addition, the main effects should not be interpreted because of the presence of significant interaction terms.

The model with the lowest AIC value is considered the best and additional models within two AIC units are considered equally good.

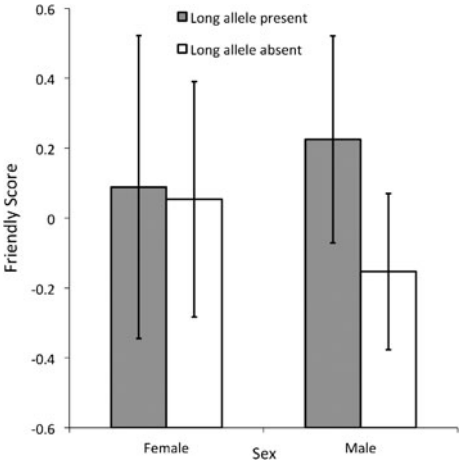

Fig. 3 *AVPR1A* genotype and scores on the "friendly" component of behavioral style. Chimpanzee males with the long allele (gray bars) display a positive relationship between genotype and "friendly" score, but females do not. Data come from observations of chimpanzees at the New Iberia Research Center (2000–2002).

two interaction terms were significant, genotype × sex ($P = 0.047$) and genotype × dominance rank ($P = 0.006$). The genotype × sex interaction showed that males with a long allele had higher "friendly" scores than males without the long allele, though this effect was not present in females (Fig. 3). The genotype × rank effect showed that individuals with the long allele displayed a positive relationship between dominance rank and "friendly" index value. Individuals without the long allele did not exhibit this relationship (Fig. 4).

Significant models also existed for two other behavioral style components, "aggressive" and "mellow," though in both cases dominance rank was the most important predictor and genotype had little effect (Electronic Supplementary Material Tables SI–SIV). The "playful" and "affiliative" components were not significantly predicted by any model. Similarly, no significant relationship was found for genotype and the general measures of "affiliation"

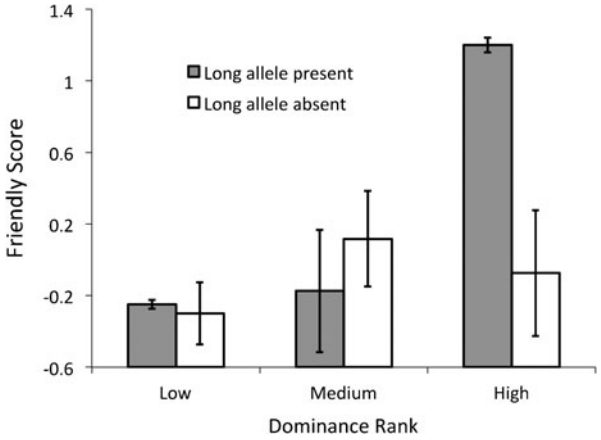

Fig. 4 Relationship between dominance rank and scores on the "friendly" component of behavioral style. Chimpanzees with the long allele (gray bars) display a positive relationship between dominance rank and "friendly" score. Genotypes without the long allele (SS; white bars) do not show this pattern. Data come from observations of chimpanzees at the New Iberia Research Center (2000–2002). See also Table IV.

based on the larger data set. We also found no relationship between testosterone and behavioral style (testosterone was not a significant predictor; Tables III and IV; Electronic Supplementary Material Tables S1–SIV).

Discussion

Our results indicate striking differences in genotype frequencies across populations and potential associations between *AVPR1A* genotype and aspects of behavioral style in chimpanzees.

Behavioral Associations with *AVPR1A* Genotype

Our previous studies documented variation in chimpanzee behavioral style, i.e., personality, and suggested that this was associated with variation in hormone levels (Anestis 2005, 2006). This study builds upon those results, showing an association between genotype and aspects of behavioral style. The presence of the long allele was associated with a higher "smart" score. Individuals scoring high in the "smart" component are those that use coalitions in their aggressive encounters, receive more grooming than they return, and are likely to initiate play successfully with peers. In our previous study (Anestis 2005, 2006), we found that individuals with high scores for this component invested relatively heavily in social relationships and social monitoring. The association of the *AVPR1A* long allele with this component is intriguing, as this genomic region is associated with social behavior in other species (Young and Wang 2004) and with "conscientiousness" scores in a previous study of captive western chimpanzees (Hopkins *et al.* 2012).

High-ranking chimpanzees with the long allele also showed higher scores in the "friendly" behavioral style component, a measure defined by high levels of positive interaction with all group mates. Captive chimpanzees show great variation in how equitably they groom, touch, and sit near the members of their social group (Anestis 2005). High-ranking individuals might have more choice in whom they interact with and thus genotype-based differences in ability to negotiate the social group would be more apparent in high-ranking than low-ranking individuals, though this hypothesis requires further testing.

Moreover, the fact that males show a relationship between genotype and "friendly" score, but females do not, is interesting given that Hopkins *et al.* (2012) also found a sex difference in personality traits associated with genotype. In that study, males with the long allele had higher "dominance" and showed "less conscientiousness." Because our study and that of Hopkins *et al.* (2012) took very different approaches to measuring behavioral study (observational data vs. handler questionnaires, respectively), the results are difficult to compare. Yet both highlight the need to consider sex-based differences in analyses of behavioral genetics.

Earlier analyses (Anestis 2006) of this population indicated a positive trend between testosterone levels and scores for the "mellow" index ($r = 0.33$, $P = 0.06$). We found no such association in this analysis, perhaps because 13 additional individuals were included in the present study, and/or we employed a different statistical approach (general linear models).

The associations we find between genotype and "smart" and "friendly" behavioral styles are intriguing and highlight the need for more detailed analysis of *AVPR1A* variation in chimpanzees and other primates. However, our results must be viewed as only a first step toward understanding potential links between *AVPR1A* and chimpanzee social behavior. The oxytocin and arginine vasopressin pathways are complex and genes involved in this system play varying roles across tissues and cell-type (Ebstein *et al*. 2012). Our demonstration of a statistical association between genotype and aspects of personality in chimpanzees is not direct evidence of a genotype–phenotype link. Given our limited sample size, our lack of gene expression data connecting the polymorphism to neurophysiology, and the mixed-history of replicating results in human behavioral genetics (Flint *et al*. 2010), any interpretation of our data is tentative. Nonetheless, our results add to growing evidence (Hopkins *et al*. 2012) that the regulatory polymorphism of *AVPR1A* could play a functional role in behavioral differences among chimpanzees.

Notably, the long allele, which shows an association with "smart" and "friendly" social skills, is the ancestral allele and is thus most similar to the variants found in other primates, including humans (Babb *et al*. 2010; Donaldson *et al*. 2008; Rosso *et al*. 2008). To date, only the long allele has been found in bonobos (*Pan paniscus*), but only a handful of individuals have been genotyped (Donaldson *et al*. 2008; Rosso *et al*. 2008). This prompts the question: If *AVPR1A* genotype influences social behavior, what drives the maintenance of the short (derived, and seemingly "less social") allele in chimpanzee populations? This is especially interesting given that the short allele is more common in captive western chimpanzees (77%) and the long allele more common in the wild eastern population (62%). Because our analyses compare having (21 SL and 4 LL combined) and lacking (39 SS) the long allele, observed differences may reflect the heterozygous vs. homozygous condition, not simply the presence of the long allele. However, the frequency of the SS genotype (61%) is much greater than the SL genotype (33%) in the NIRC population, which makes it unlikely that the S allele is maintained via heterozygous advantage. A larger sample of homozygous long individuals would be needed to compare among all three genotypes.

Population Differences in Genotype and Allele Frequencies

This study provides the first assessment of variation at this locus in a wild chimpanzee population and the first comparison of *AVPR1A* genotype frequencies across chimpanzee subspecies. Our finding that genotype and allele frequencies differ markedly between the captive western chimpanzees (*Pan troglodytes verus*) and the wild eastern chimpanzees (*Pan troglodytes schweinfurthii*) is noteworthy, although why the differences exist and what effect they might have on behavior is unclear.

Chimpanzee behavior varies considerably in the wild (Hohmann and Boesch 2002; Muller and Mitani 2005; Watts 2012). Some of this variation concerns consistent subspecific differences in aspects of social bonding, which would raise the possibility that differences in selection regimes have favored the long allele in eastern chimpanzees, but the short allele in western chimpanzees. However, subspecific differences could simply reflect flexible behaviors dependent on local ecology and environment. For example, western chimpanzee females at Taï National Park (Côte d'Ivoire) are more gregarious than eastern chimpanzee females at Gombe National Park (Tanzania),

Mahale Mountains National Park (Tanzania), or Kanyawara Research Center (Uganda) (Lehman and Boesch 2008). However, eastern chimpanzee females at Ngogo are as gregarious as those at Taï and many female dyads at Ngogo have strong social bonds (Langergraber *et al.* 2009; Wakefield 2008, 2013). The Ngogo community belongs to the same population, i.e., same genetic background (Langergraber 2011) as that at Kanyawara, and the striking differences in female social behavior between these two sites have a plausible ecological explanation (greater overall food abundance and less variation in fruit availability at Ngogo; Potts *et al.* 2011; Watts *et al.* 2012; Wakefield 2008, 2013). As this indicates, we should be cautious about invoking genetics to explain social variation unless we can rule out the possibility that behavioral variation is shaped by differences in local ecology.

Moreover, genetic differentiation at *AVPR1A* ($F_{st} = 0.10$) is not outside the range of population differences among chimpanzee subspecies at neutral nuclear loci (Bowden *et al.* 2012), and the dissimilar *AVPR1A* genotype frequencies could reflect differences due simply to drift. As a point of comparison, we also sequenced the gene *APOE* for these same samples, and found no population differences in the coding region, but a single intronic nucleotide showed fixed differences between subspecies/populations (McIntosh *et al.* 2012).

Finally, the difference in allele frequencies might have resulted from biased sampling with respect to behavioral genotypes and phenotypes. That is, this might be a difference between captive vs. wild chimpanzees, rather than a difference between subspecies. Perhaps timid individuals were less likely to end up in captivity (an effect documented in other species; Carter *et al.* 2012). Genotype frequencies from a wild western population are needed to clarify this issue.

Conclusions

This is the first study to 1) test for associations between *AVPR1A* genotype and behavior using systematic, quantitative measures of behavioral style; 2) include testosterone as a factor in testing for such associations; 3) assess *AVPR1A* genotype and allele frequencies in a wild chimpanzee population; and 4) compare *AVPR1A* genotype frequencies among chimpanzee subspecies. Understanding the links between genotype, environment, and behavioral variation is challenging (Flint *et al.* 2010), especially for primates, where behaviors are complex, sample sizes are generally small, and environments are difficult to control (Bradley and Lawler 2011). In demonstrating a potential association between *AVPR1A* genotype and personality in chimpanzees, our study provides an early step toward understanding the genetic basis of behavioral variation in primates.

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