Supplementary Information

Genetic origins of social networks in rhesus macaques

Authors: Lauren J.N. Brent, Sarah R. Heilbronner, Julie E. Horvath, Janis Gonzalez-Martinez, Angelina Ruiz-Lambides, Athy G. Robinson, J.H. Pate Skene, Michael L. Platt

DESCRIPTION OF SUPPLEMENTARY FILES

Supplementary Table S1: Phenotypic correlations between social network metrics

		Grooming				Aggression				Proximity		
		IS	OS	B	E	IS	OS	B	E	S	B	E
Grooming	IS	X	0.26 (< 0.001)	0.52 (< 0.001)	0.63 (< 0.001)	-0.17 (0.02)	0.22 (0.03)	-0.03 (0.70)	0.14 (0.06)	0.47 (< 0.001)	0.13 (0.081)	0.46 (< 0.001)
	OS		X	0.39 (< 0.001)	0.55 (< 0.001)	-0.03 (0.70)	0. 00 (0.97)	-0.02 (0.79)	0.05 (0.54)	0.47 (< 0.001)	0.12 (0.09)	0.46 (< 0.001)
	B			X	0.22 (0.002)	-0.03 (0.67)	-0.03 (0.68)	0.03 (0.68)	-0.07 (0.35)	0.24 (< 0.001)	0.25 (< 0.001)	0.18 (0.01)
	E				X	-0.1 (0.18)	0.24 (0.001)	-0.04 (0.59)	0.28 (0.001)	0.50 (< 0.001)	0.00 (0.96)	0.60 (< 0.001)
Aggression	IS					X	-0.35 (< 0.001)	-0.02 (0.81)	0.17 (0.02)	0.08 (0.29)	-0.01 (0.89)	0.00 (0.98)
	OS						X	0.33 (< 0.001)	0.74 (< 0.001)	0.16 (0.03)	-0.12 (0.10)	0.13 (0.07)
	B							X	0.32 (< 0.001)	-0.22 (0.002)	-0.12 (0.12)	-0.18 (0.01)
	E								X	0.28 (< 0.001)	-0.06 (0.42)	0.31 (< 0.001)
Proximity	S									X	0.31 (< 0.001)	0.91 (< 0.001)
	B										X	0.24 (0.001)
	E											X

Value in cell is correlation coefficient (p value). Phenotypic correlations calculated using Pearson's correlation coefficient in R software. IS = instrength, OS = outstrength, B = betweenness, E = eigenvector, S = strength.

Supplementary Table S2: Genetic correlation matrix for network metrics with heritability greater than zero

	Grooming Betweenness	Grooming Eigenvector	Aggression Outstrength	Proximity Eigenvector
Grooming Betweenness	-----	-0.332 (0.366, 106, 0.314)	-0.228 (0.405, 107, 0.664)	-0.426 (0.639, 107, 0.472)
Grooming Eigenvector		----	0.029 (0.465, 107, 0.952)	1.00 (0.898, 106, 0.122)†
Aggression Outstrength			-----	-0.886 (0.878, 107, 0.104)
Proximity Eigenvector				----

Values are additive genetic correlations (standard error, N, p). * $P \leq 0.05$. No bivariate correlations were statistically significant († *despite a correlation of 1.00, a large error term resulted in a non-significant relationship*). Genotypic correlations calculated using quantitative genetic analysis in SOLAR (Almasy & Blangero 1998). We estimated genetic correlation (rA) between traits x and y as rA(xy) = COVA(xy) / sqrt(VA(x)*VA(y)), where COVA gives the genetic covariance (Lynch & Walsh 1998). Only traits with significant additive genetic variance were examined as these are the only traits, by definition, which may co-vary genotypically (Lynch & Walsh 1998). Bivariate models included network metrics as dependent variables. Coefficients of relatedness (h^2) were included in all models, along with age, sex and dominance rank as fixed effects.

References:

Almasy, L. and Blangero, J. 1998. Multipoint quantitative-trait linkage analysis. Am J Hum Genet. 62:1198-1211.

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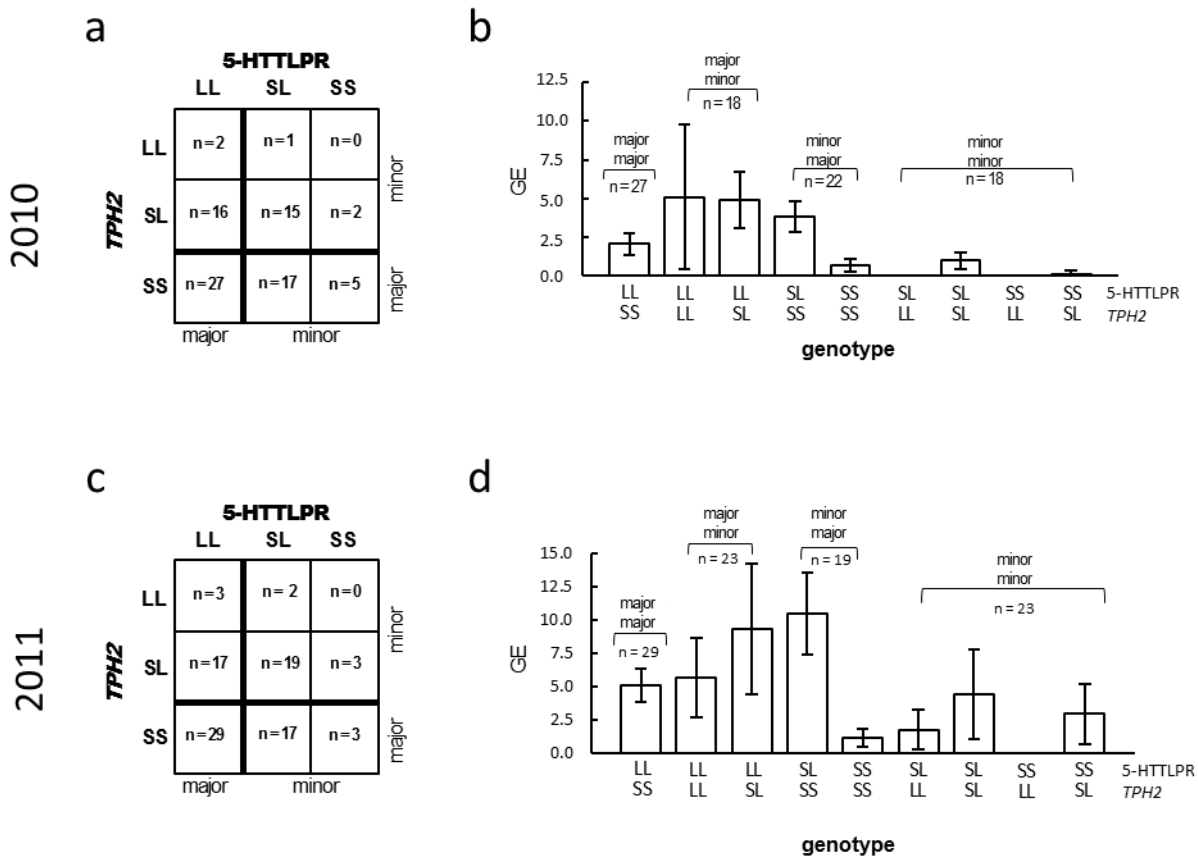
Supplementary Table S3: Additive genetic variance and selection differentials of social network measures (full results)

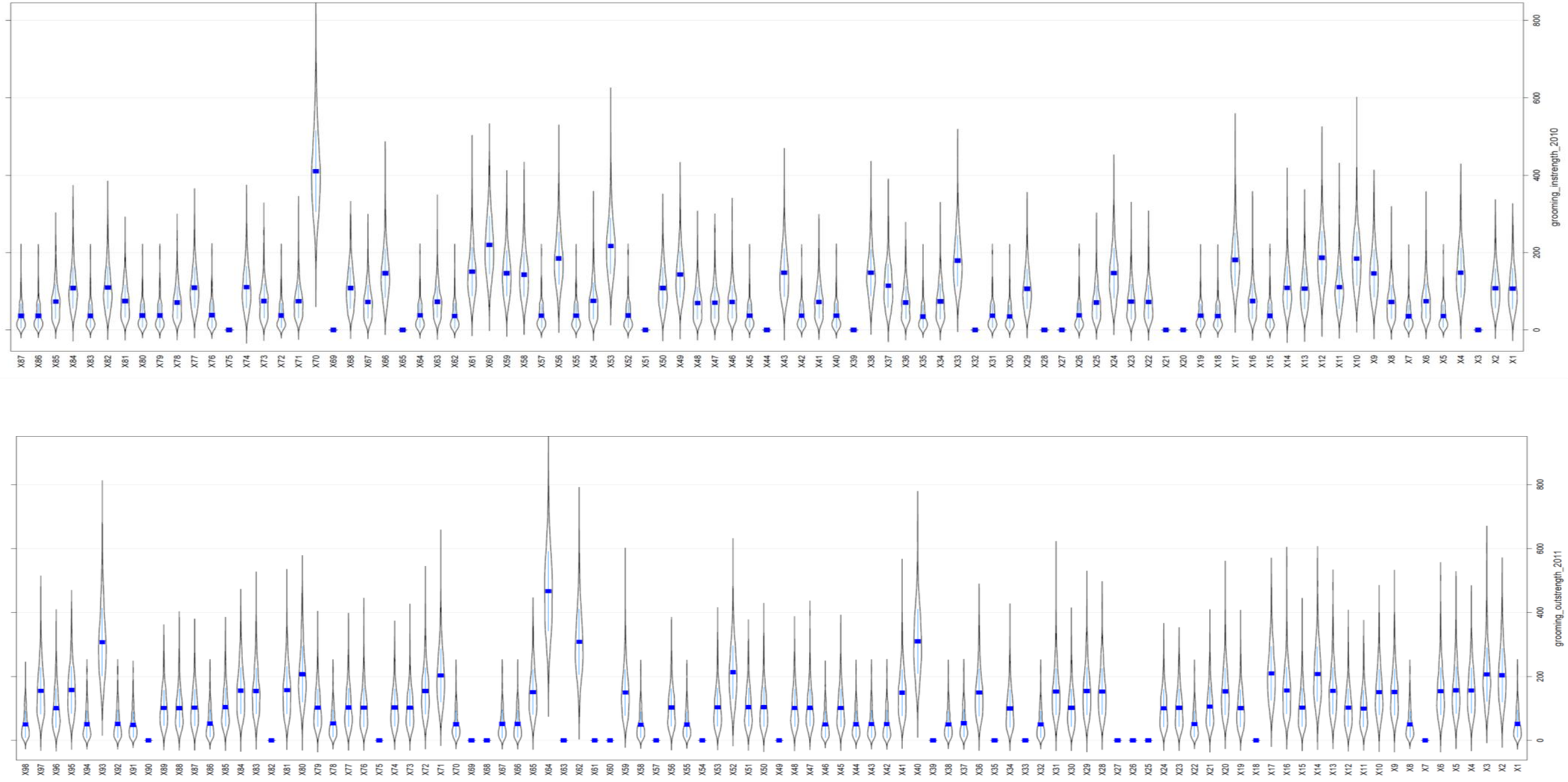
		Proportion of variance											*Selection Differentials*				
	D,I	h^2 (error)	h^2 *p*	c^2 (error)	c^2 *p*	age (error)	Age *p*	sex (error)	Sex *p*	rank (error)	Rank *p*	N1	*S* (error)	S *p*	*C* (error)	C *p*	N2
Grooming																	
Instrength	D	0.00	0.500	0.00	0.500	-0.03 (0.02)	0.251	**0.34 (0.17)***	0.025	-0.005 (0.01)	0.195	183 (106)	**0.002 (0.001)***	**0.036***	-0.23xe^{-5} (0.18xe^{-5})	0.199	79
Outstrength	D	0.31 (0.30)	0.144	0.00	0.500	-0.01 (0.02)	0.135	**1.10 (0.23)****	**0.0009**	**-0.02 (0.001)***	**0.011**	182 (104)	0.001 (0.001)	0.413	-0.53xe^{-5} (0.73xe^{-5})	0.467	79
Betweenness	I	**0.84 (0.31)***	**0.025**	0.00	0.500	-0.04 (0.06)	0.571	0.66 (0.57)	0.375	0.007 (0.02)	0.730	179 (101)	-0.001 (0.001)	0.427	2.90xe^{-5} (2.80xe^{-5})	0.303	79
Eigenvector	I	**0.36 (0.24)**†**	**0.073**	0.00	0.500	0.32 (0.18)	0.103	**12.57 (1.04)****	**<0.0001**	**-0.27 (0.05)****	**<0.0001**	181 (104)	0.16 (0.17)	0.887	3.30 (4.42)	0.456	79
Aggression																	
Instrength	D	0.00	0.500	0.00	0.500	**-0.07 (0.02)****	**0.0004**	**1.00 (0.22)****	**0.0002**	**0.02 (0.01)****	**0.0002**	183 (105)	-0.05 (0.06)	0.483	0.02 (0.05)	0.635	79
Outstrength	D	**0.66 (0.28)***	**0.020**	0.07 (0.14)	0.394	-0.01 (0.03)	0.091	-0.45 (0.32)	0.050	**-0.07 (0.01)****	**<0.0001**	182 (105)	0.05 (0.05)	0.260	**0.02 (0.01)****	**0.004**	79
Betweenness	I	0.00	0.500	0.00	0.500	-0.05 (0.03)	0.111	-0.61 (0.33)	0.073	-0.01 (0.33)	0.138	181 (103)	-0.1xe^{-4}(0.002)	0.977	**0.1xe^{-4} (0.2xe^{-4})****	**0.0003**	79
Eigenvector	I	0.00	0.500	0.00	0.500	**-0.40 (0.10)****	**0.00007**	**5.81 (1.06)****	**0.0002**	**-0.18 (0.03)****	**<0.0001**	182 (105)	**1.93 (0.96)***	**0.046**	**21.51 (5.96)****	**0.0003**	79
Proximity																	
Strength	D	0.28 (0.24)	0.122	0.07 (0.09)	0.135	0.02 (0.02)	0.497	**1.54 (0.21)****	**0.002**	**-0.02 (0.01)****	**0.002**	185 (107)	**1.18 (0.49)***	**0.016**	-0.28(2.72)	0.920	79
Betweenness	I	0.00	0.500	0.00	0.500	-0.03 (0.02)	0.094	**0.60 (0.18)****	**0.006**	0.002 (0.01)	0.706	180 (102)	0.001 (0.002)	0.560	-0.15xe-4 (0.24xe^{-4})	0.549	79
Eigenvector	I	**0.33 (0.22)**†**	**0.060**	0.03 (0.07)	0.127	-0.10 (0.15)	0.876	**11.87 (1.58)***	**0.011**	**-0.17 (0.04)****	**0.0006**	184 (106)	**3.64 (1.25)****	**0.004**	-10.75 (17.44)	0.538	79

Sociality measures represent direct (D) and indirect (I) interactions. h^2 is variance explained by additive genetic variance, c^2 is variance explained by household effects (matriline for females, natal group for males). Age, sex and rank included as fixed effects. We retained in models environmental effects, household effects > 0, and significant fixed effects. Quantitative genetic analyses performed using sample size N1 (number of data points, number of unique individuals). Quadratic regression for directional (S) and stabilizing/disruptive (C) selection differentials performed using sample size N2. Sex and rank included as fixed effects. Positive C values represent disruptive selection, negative values stabilizing selection. Stabilizing/disruptive selection estimates and errors were multiplied by 2. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.07$.



Supplementary Figure S1: Serotonergic gene profiles and grooming eigenvector (GE). 3x3 punnet square of the gene profiles that result from the interaction between the 5-HTTLPR and *TPH2* polymorphisms, divided by major and minor alleles (a, c). The number of individuals with each gene profile in each year is given within the square (a = 2010, c = 2011). Mean GE (± SE) for individuals with all possible gene profiles (b, d). Data from 2010 are shown in (b), 2011 in (d). These graphs are for illustrative purposes only. Data were analysed by grouping gene profiles according to major and minor allele carriers (see Figure 3 in the main text) due to small numbers of individuals homozygous for minor alleles.

Supplementary Figure S2: Confidence intervals for social network measures. Violin plots based on bootstrapped replicates (1,000 replicates) of each association matrix. Each "violin" represents the network metric and its associated error for an individual. Plots created in the tnet package in R. Violin plots were generated independently for both years of behavioural data (2010 and 2011). This process allowed us to determine that, while errors for the social network metrics of many individuals overlap, many individuals also exhibit significant differences in network position from others.



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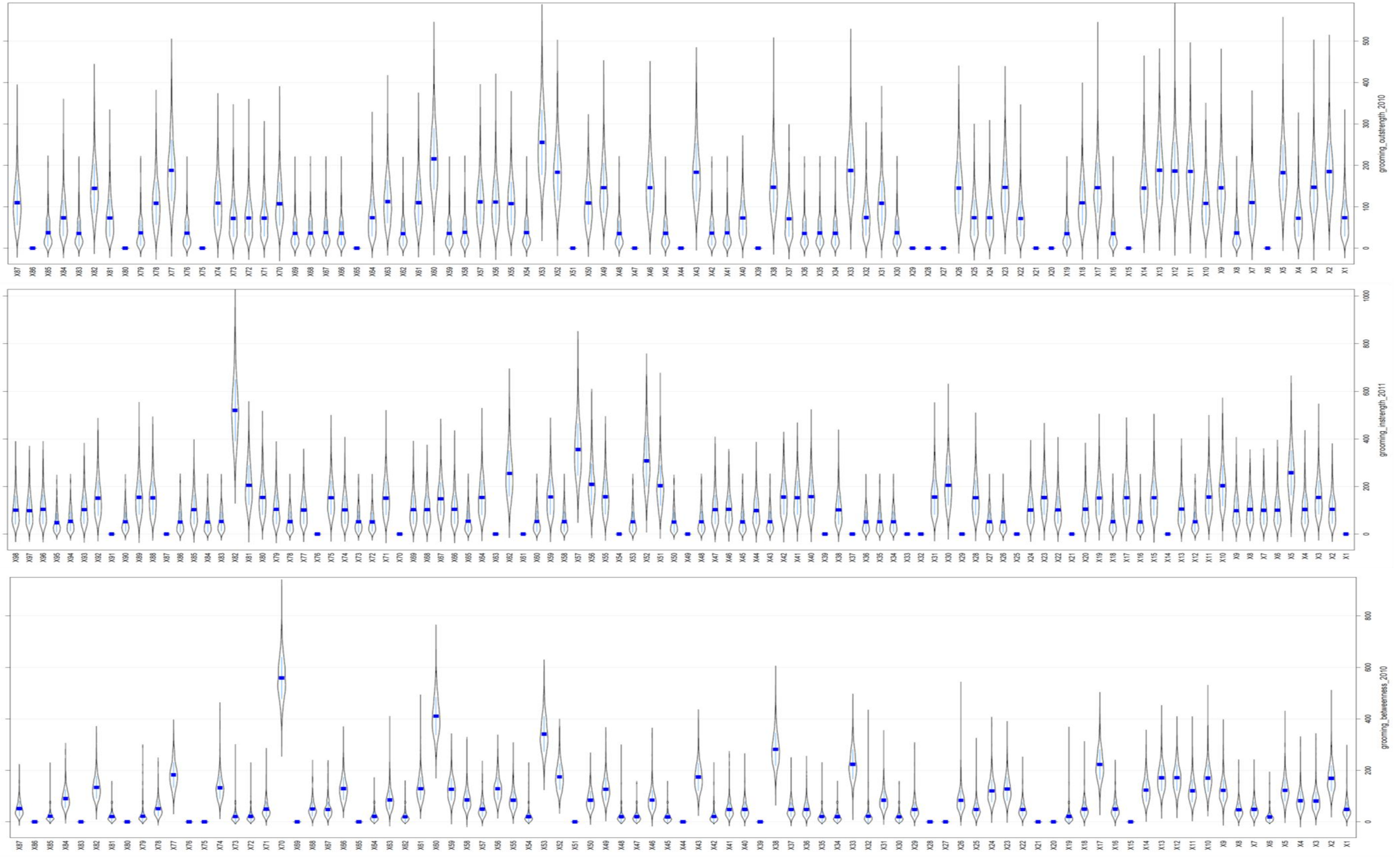

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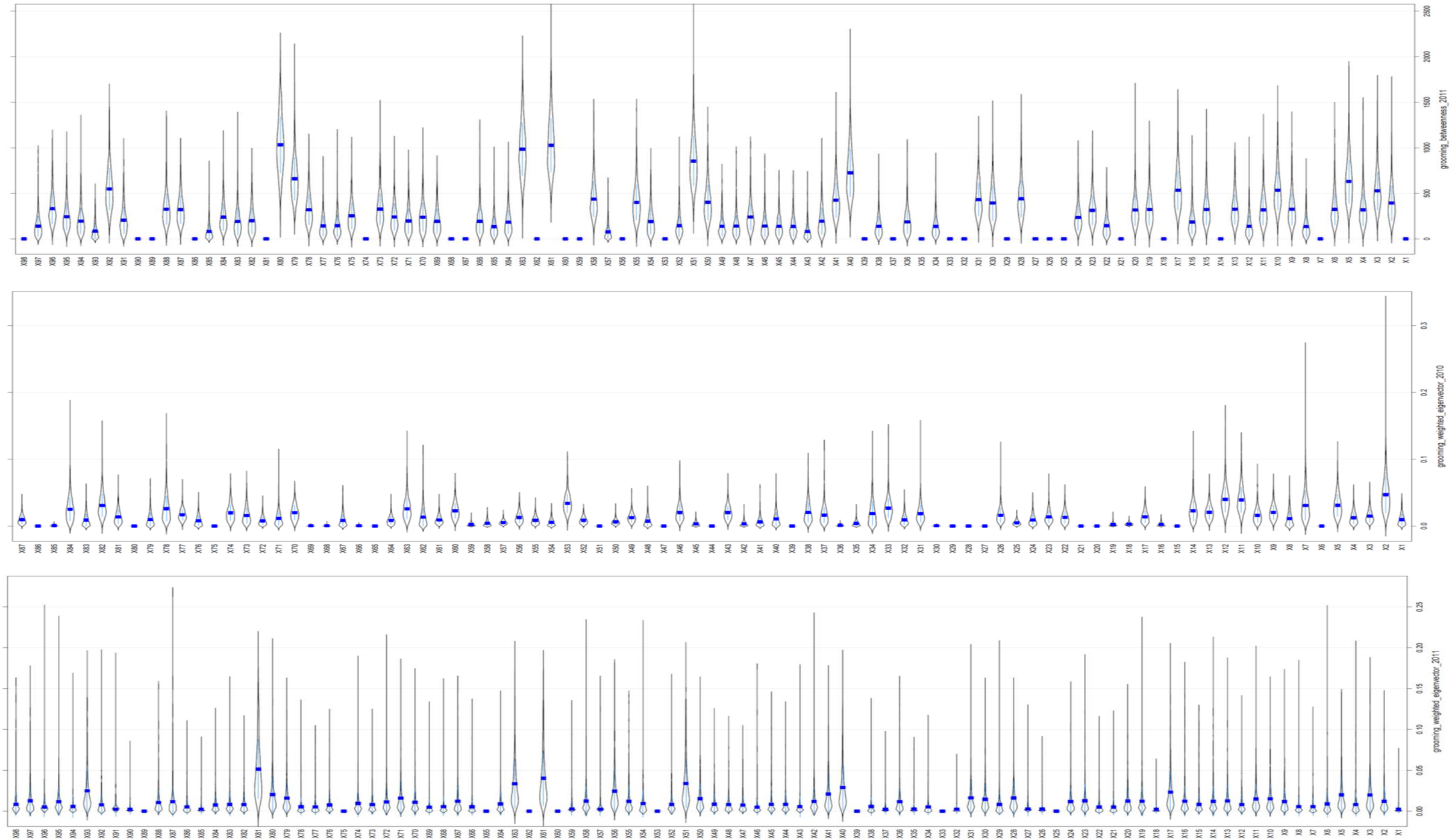

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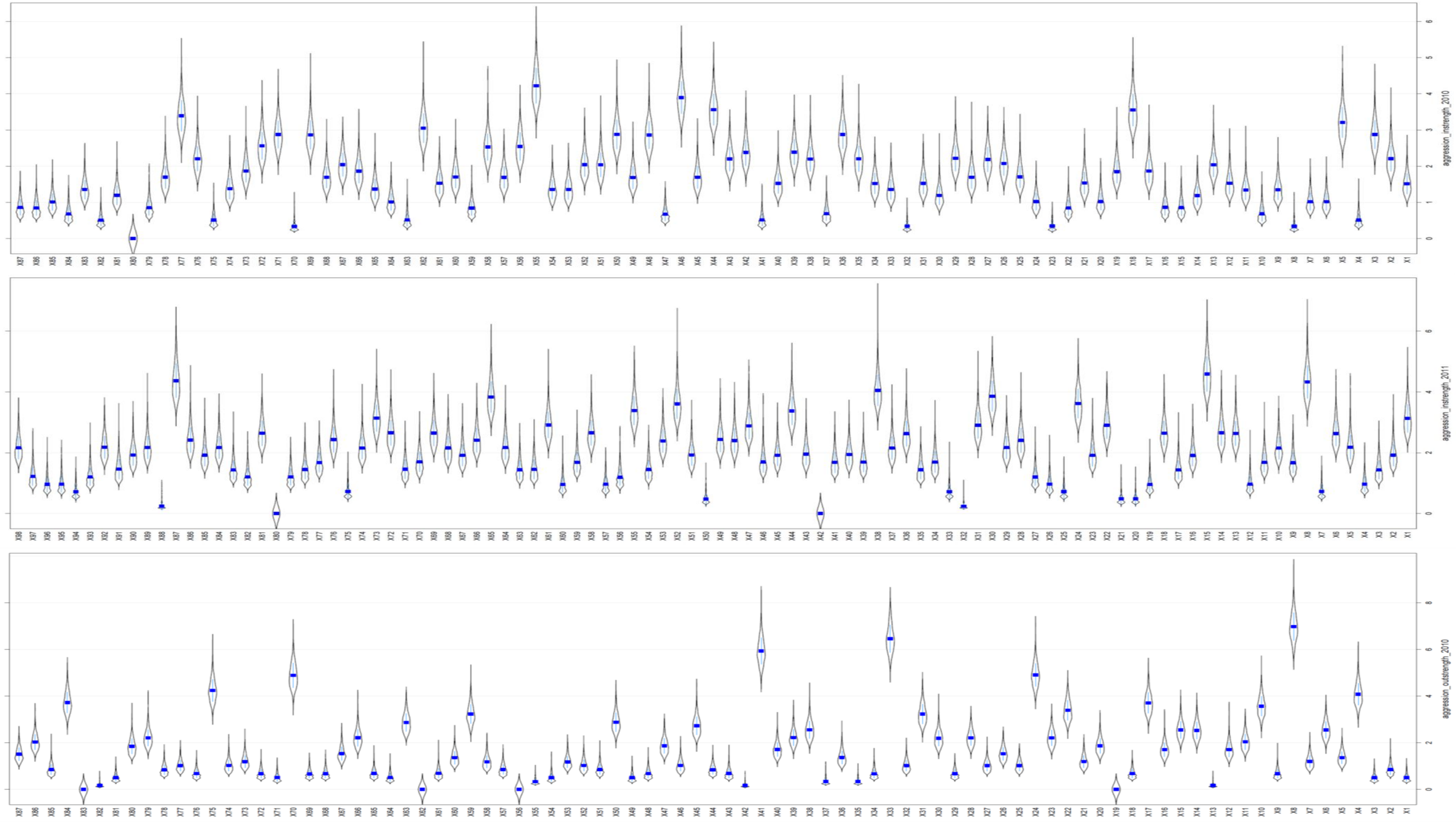

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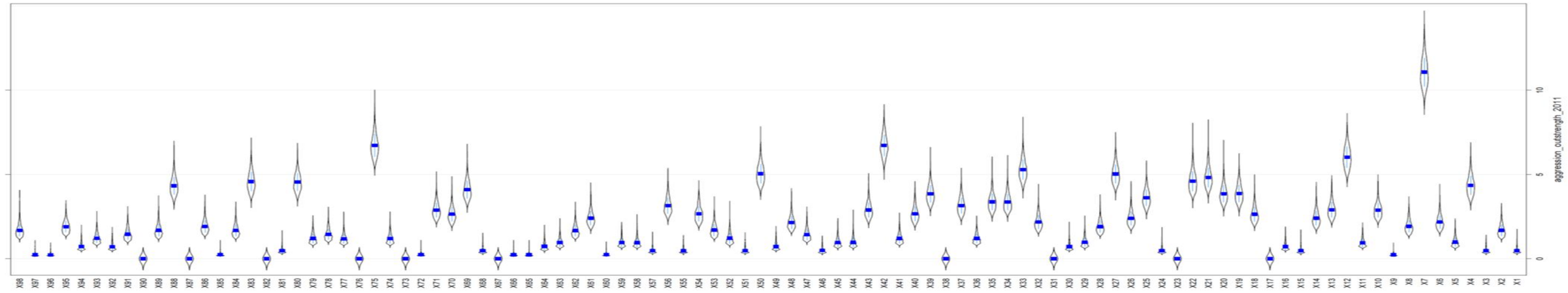

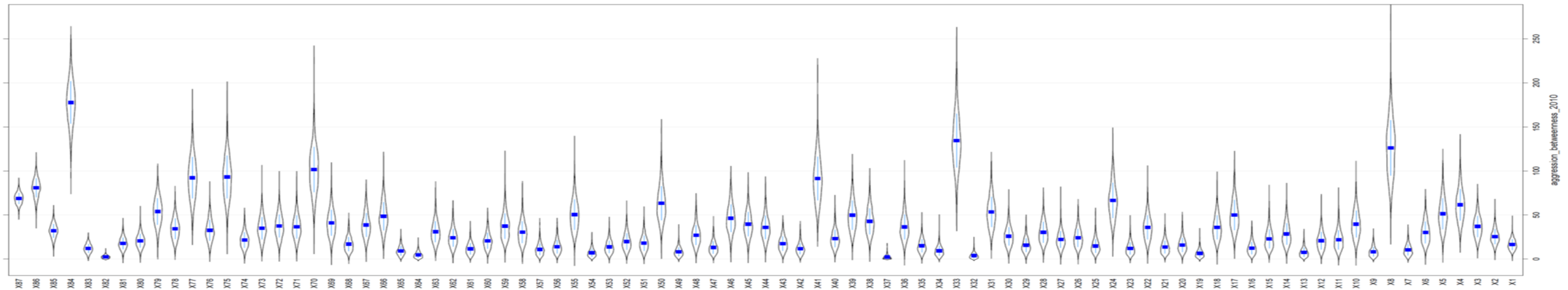

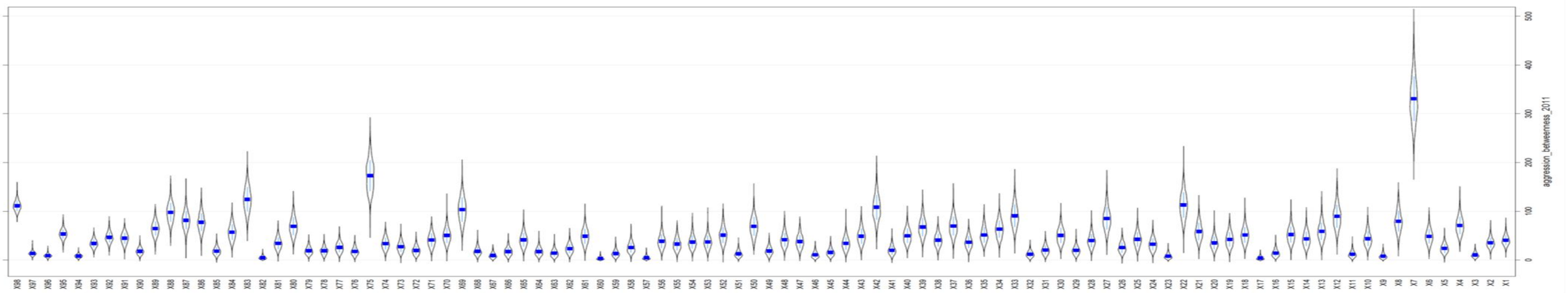

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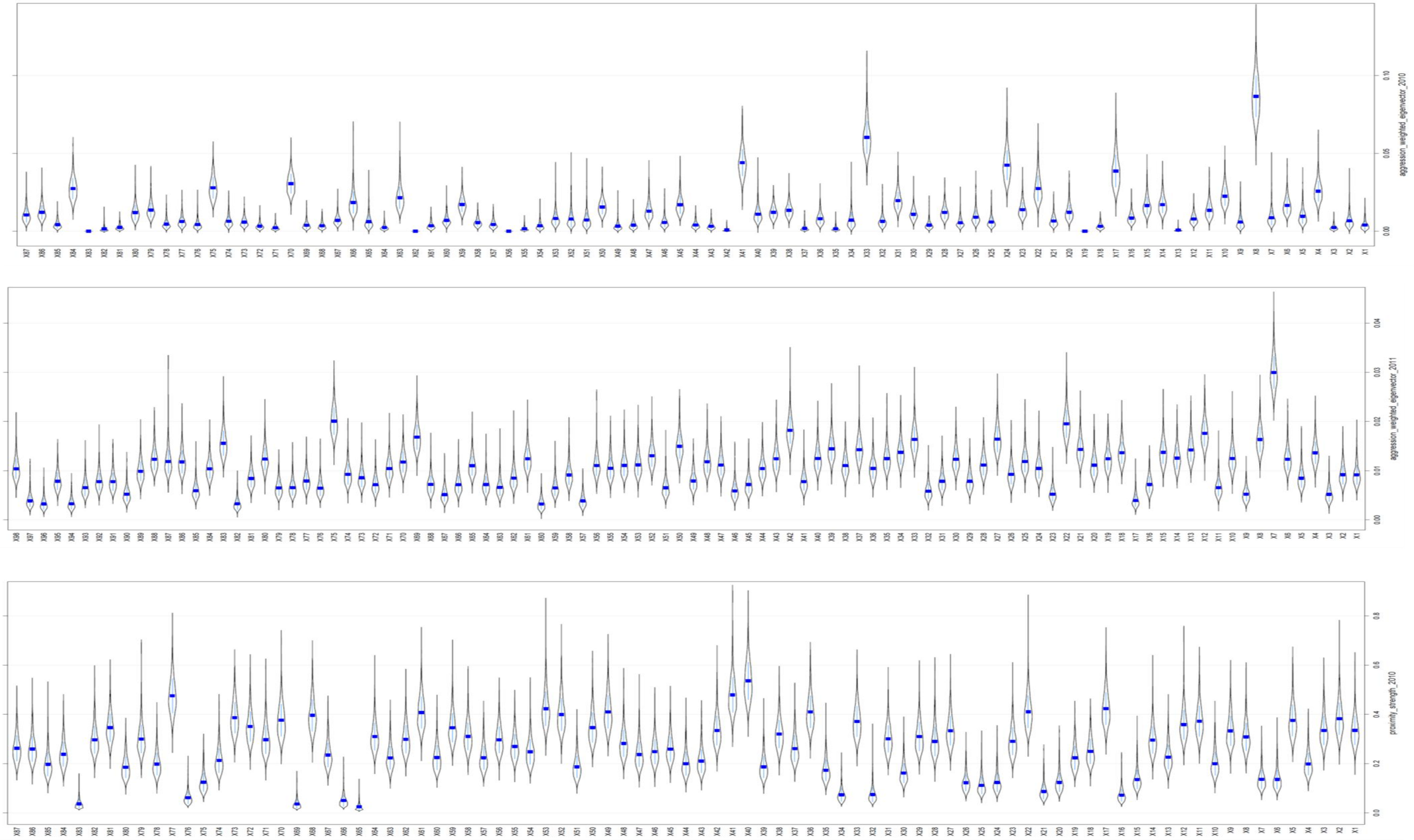

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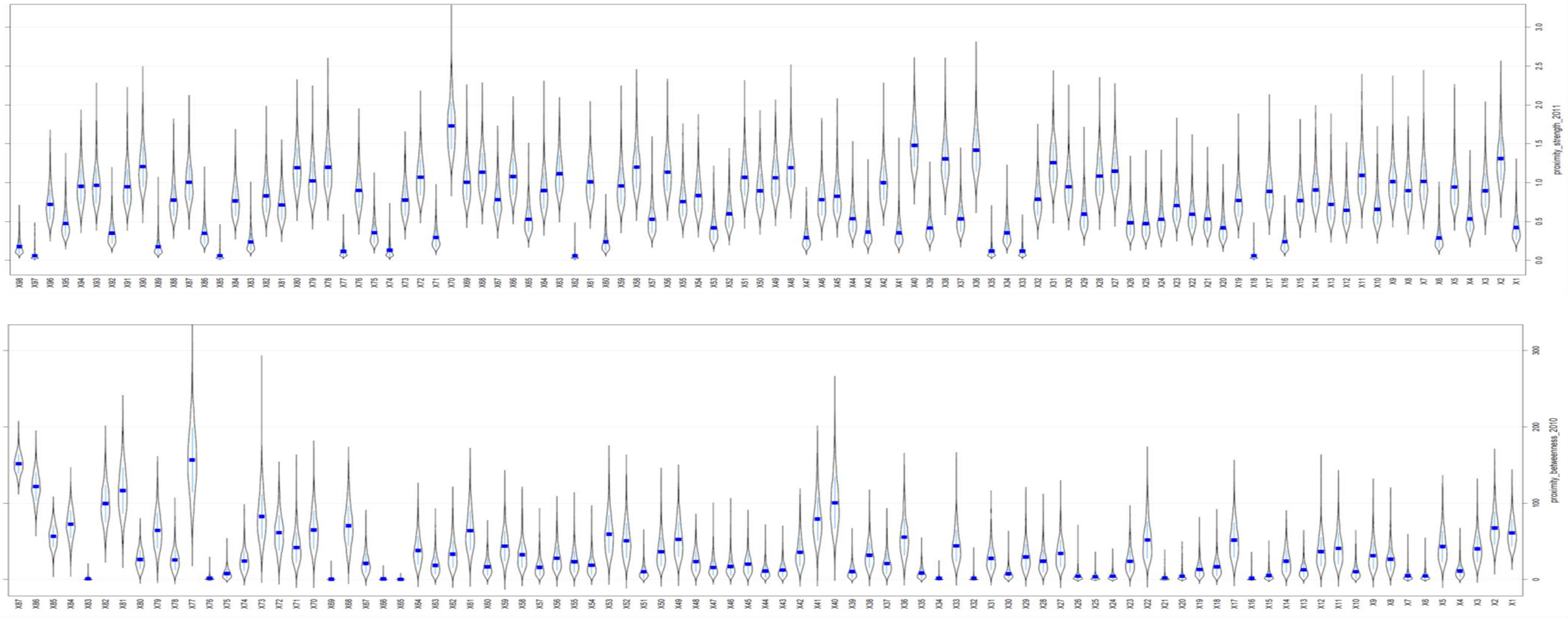

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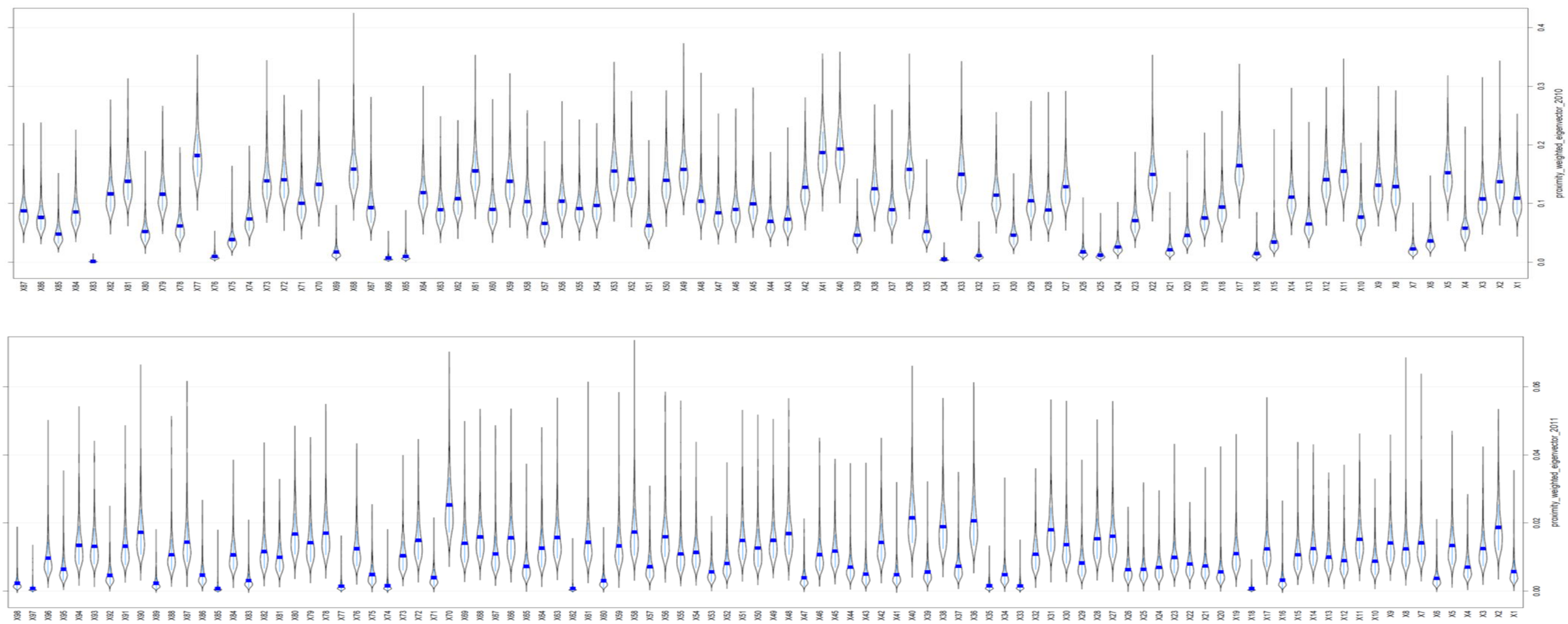

Supplementary Table S4: Additive genetic variance of bootstrapped social network measures

	Proportion of variance					
	h^2 (error)	c^2 (error)	age (error)	sex (error)	rank (error)	N
Grooming						
Instrength	0.00 (0.50)	0.00 (0.50)	-0.70 (0.58)	-10.63 (6.07)	0.36 (0.18)*	185 (107)
Outstrength	0.00 (0.50)	0.00 (0.50)	0.77 (0.38)	8.93 (3.95)	-0.23 (0.11)*	185 (107)
Betweenness	0.00 (0.50)	0.00 (0.50)	3.54 (1.12)*	24.18 (11.63)	-0.63 (0.34)	185 (107)
Eigenvector	0.00 (0.50)	0.00 (0.50)	0.33 (0.20)	3.86 (2.04)	-0.12 (0.06)*	185 (107)
Aggression						
Instrength	0.00 (0.50)	0.00 (0.50)	-0.70 (0.58)	-10.63 (6.07)*	0.36 (0.18)*	185 (107)
Outstrength	0.00 (0.50)	0.00 (0.50)	11.15 (6.70)*	128.33 (69.64)	-3.94 (2.02)*	185 (107)
Betweenness	0.00 (0.50)	0.00 (0.50)	2.22 (0.60)*	-7.36 (5.96)	0.13 (0.18)	180 (103)
Eigenvector	0.00 (0.50)	0.00 (0.50)	1.05 (0.64)	12.18 (6.63)	-0.36 (0.19)	185 (107)
Proximity						
Strength	0.00 (0.50)	0.00 (0.50)	14.93 (8.87)	170.26 (92.21)	-5.07 (2.68)*	185 (107)
Betweenness	0.00 (0.50)	0.00 (0.50)	-2.11 (2.12)	-43.61 (22.07)	1.27 (0.64)*	185 (107)
Eigenvector	0.00 (0.50)	0.00 (0.50)	1.27 (0.76)	14.54 (7.91)	-0.44 (0.23)*	185 (107)

To confirm the results of our quantitative genetic analyses based on observed networks, we re-ran heritability analyses using mean network measures generated from random networks that had the same number of individuals and probability of association as our observed networks (1,000 random networks generated in the tnet package in R (Opsahl 2009). We calculated social network metrics for each individual for each of the 1,000 bootstrapped networks, and ran the mean values of these metrics in quantitative genetic analyses. As would be expected, metrics based on random networks did not demonstrate additive genetic variance. h^2 is variance explained by additive genetic differences, c^2 is variance explained by household effects (matriline for females, natal group for males). Age, sex and dominance rank were included as fixed effects. Quantitative genetic analyses were performed in SOLAR (Almasy & Blangero 1998) using sample size N: number of data points (number of unique individuals). * $P \leq 0.05$.

References:

Almasy, L. and Blangero, J. 1998. Multipoint quantitative-trait linkage analysis. Am J Hum Genet. 62:1198-1211.

Opsahl, T. 2009. Structure and Evolution of Weighted Networks. University of London, London.

Supplementary Methods

Pedigree data and genetic parentage assignment

We obtained pedigree data from the Caribbean Primate Research Center (CPRC) long-term database. This database contains maternal assignments based on census information (i.e. based on behaviours of putative mothers, such as lactation) for all animals from the founding population onwards, as well as maternity and paternity based on analysis of 29 microsatellite markers for most animals born since 1990 (~2,886 monkeys) (details on parentage analysis are below). Maternity based on genetic data was known for 104 animals in our study (97.2 %), paternity for 89 animals (83.2%). We used census information to determine maternal identity when genetic maternity was unknown (*n*= 3). Missing paternity links could result in underestimation of additive genetic variance, although rates of paternity errors of 20 % or less introduce few biases in genetic analyses (Charmentier et al. 2011). There is little evidence for high rates of inbreeding on Cayo Santiago, with little difference in blood polymorphism or mitochondrial haplotype diversity between this population and wild Indian rhesus macaques (Blomquist 2009).

Genetic parentage assignment was done by analysis of microsatellite markers carried out by the Veterinary Genetics Laboratory (VGL), University of California, Davis. Multiplex PCR reactions were used to amplify 29 markers distributed in 19 chromosomes (D1S548, D2S1333, D3S1768, D4S413, D4S2365, D5S1457, D6S276, D6S291, D6S501, D6S1691, D7S794, D7S513, D8S1106, D9S921, D10S1412, D11S925, D11S2002, D12S67, D12S364, D13S765, D15S823, D16S403, D17S1300, D18S72, D18S537, D22S685, DXS22685, MFGT21 and MFGT22). This same marker panel is used for parentage analysis of rhesus macaques at other National Primate Centers. Multiplex PCRs were set up in 25µl reactions containing 30-60ng of DNA extracted from whole blood samples, 2.5mM $MgCl_2$, 200µM dNTPs, 1X PCR buffer II, 0.5 U Amplitaq (Applied Biosystems, Foster City) and fluorescence-labelled primers in concentrations ranging from 0.06 to 0.9 µM. Cycling conditions consisted of 4 cycles of 1min at 94°C, 30sec at 58°C, 30 sec at 72°C, followed by 25 cycles of 45sec at 94°C, 30sec at 58°C, 30sec at 72°C and a final extension at 72°C for 30min. PCR products were separated by capillary electrophoresis on ABI 3730 DNA Analyzer (Applied Biosystems, Foster City) according to manufacturer instructions. Fragment size analysis and genotyping was done with the computer software STRand (available at http://www.vgl.ucdavis.edu/informatics/Strand/). Loci identified by letter "D" prefix were amplified using heterologous human primers. Parentage analysis was performed using software developed by the VGL. Individuals are assigned as parents if genotypes are determined to be fully compatible with the offspring, with allowance made for single-locus mismatches that can be explained by a mutation event or by a null allele (failure to amplify sequence) known to be present in some loci (e.g. D16S403, D7S513).

Quantitative Genetic Analyses

We retained in quantitative genetic models used to estimate heritability and functional gene effects the following: environmental effects, household effects greater than zero, and significant fixed effects. Inclusion of non-genetic terms such as household effects is critical as phenotypic similarities are likely to be the result of shared environments in addition to, or instead of, being the result of shared genetic variation, allowing us to be confident our estimates of additive genetic variance represent genetic effects (Lea et al. 2010, Kruuk and Hadfield 2007).

We did not consider infant presence to be a potential confound in our analyses as we have shown previously in this group that females with infants do not experience higher levels of affiliation compared to females without infants (Brent et al. in review). Additionally, variance in this factor was low, with the majority of females having an infant in both years of study (2010: 40/58 females, 2011: 55/66 females).

Female rhesus macaques bias their affiliative interactions toward close kin (Widdig et al. 2001). The relationship between sociality and female reproductive output may therefore be conflated as females with adult daughters present in the group may be more likely to have both higher reproductive outputs as well as higher sociality scores. Only 8 of 58 (13.8 %) females had an adult daughter present in the study group in 2010. There was no significant difference in reproductive output for females with adult daughters present compared to females without (non-parametric t-test, $P = 0.335$), nor where there differences in the social network positions for females with adult daughters compared to those without (non-parametric t-test, $p > 0.05$ for all comparisons). Kin-biased interactions do not appear to substantially impact the relationship between sociality and reproductive output in the current study.

References

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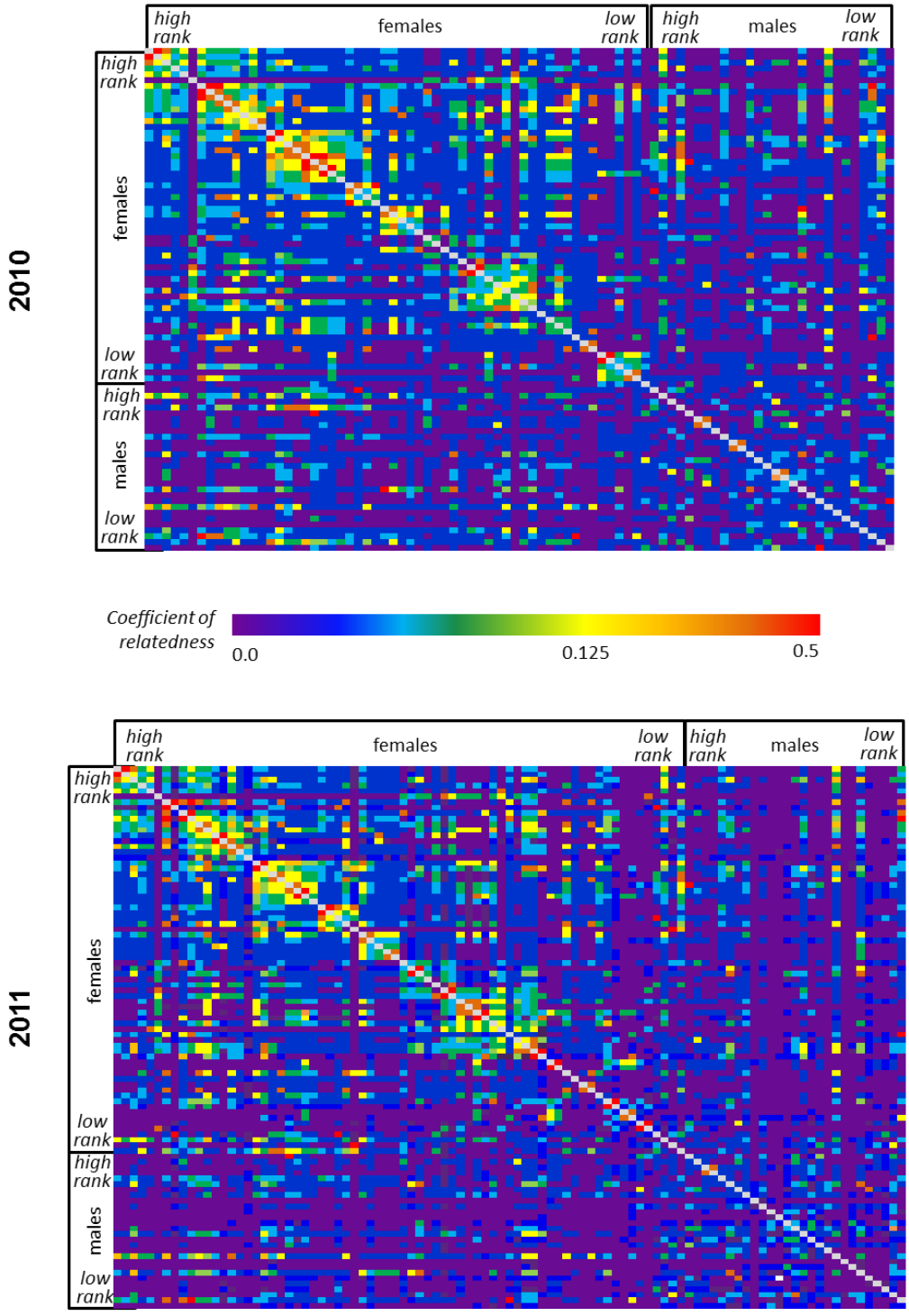
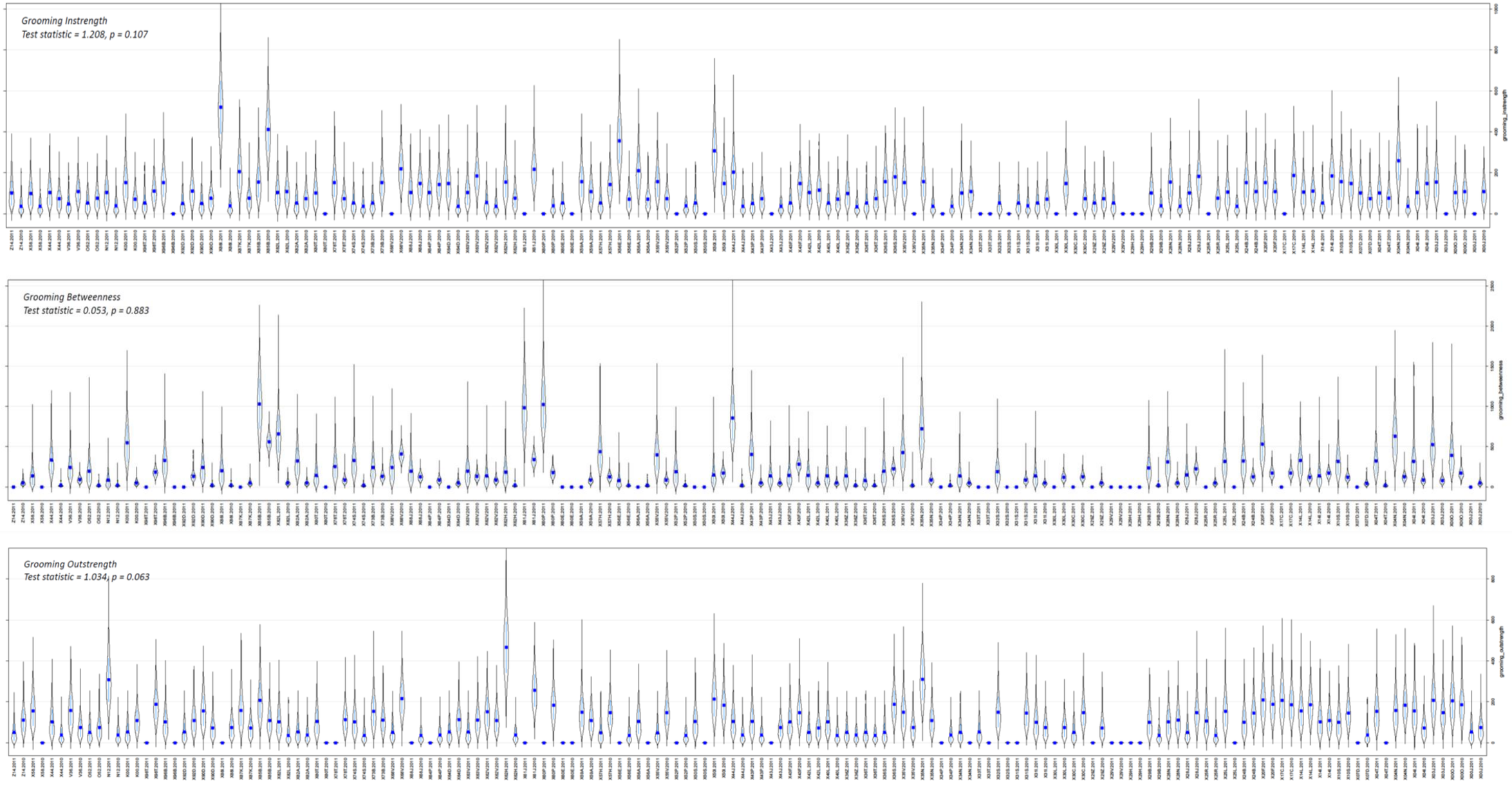


Supplementary Figure S3: Relatedness matrix. Cells represent the coefficient of relatedness between all pairs of adult rhesus macaques in study group F (n = 87 in 2010, n = 98 in 2011). Coefficients of relatedness were determined based on assessment of 29 microsatellite marker loci in combination with behavioural census data. Individuals are arranged by sex and by decreasing dominance rank. As would be expected based on rhesus macaque social structure and rank ascendency, females who are close in rank are closely related. Moreover, males, the dispersing sex, are not very closely related to females, nor to one another. The average (SD) coefficient of relatedness in this group in 2010 was r = 0.022 (0.055). There were 12 parent-offspring pairs (4 father-offspring, 8 mother-offspring), but no full siblings. Pedigree information was included in all quantitative genetic analyses.

Supplementary Figure S4: Consistency in social network measures over time. Graphs represent different social network measures. On each graph, individuals (n = 78) are represented by two violin plots, one for their social network position in 2010, the other for their social network position in 2011. Overlapping whiskers within violin plots indicate a lack of significant difference between years. Results of pair-wise statistical comparisons are presented in the corner of each graph



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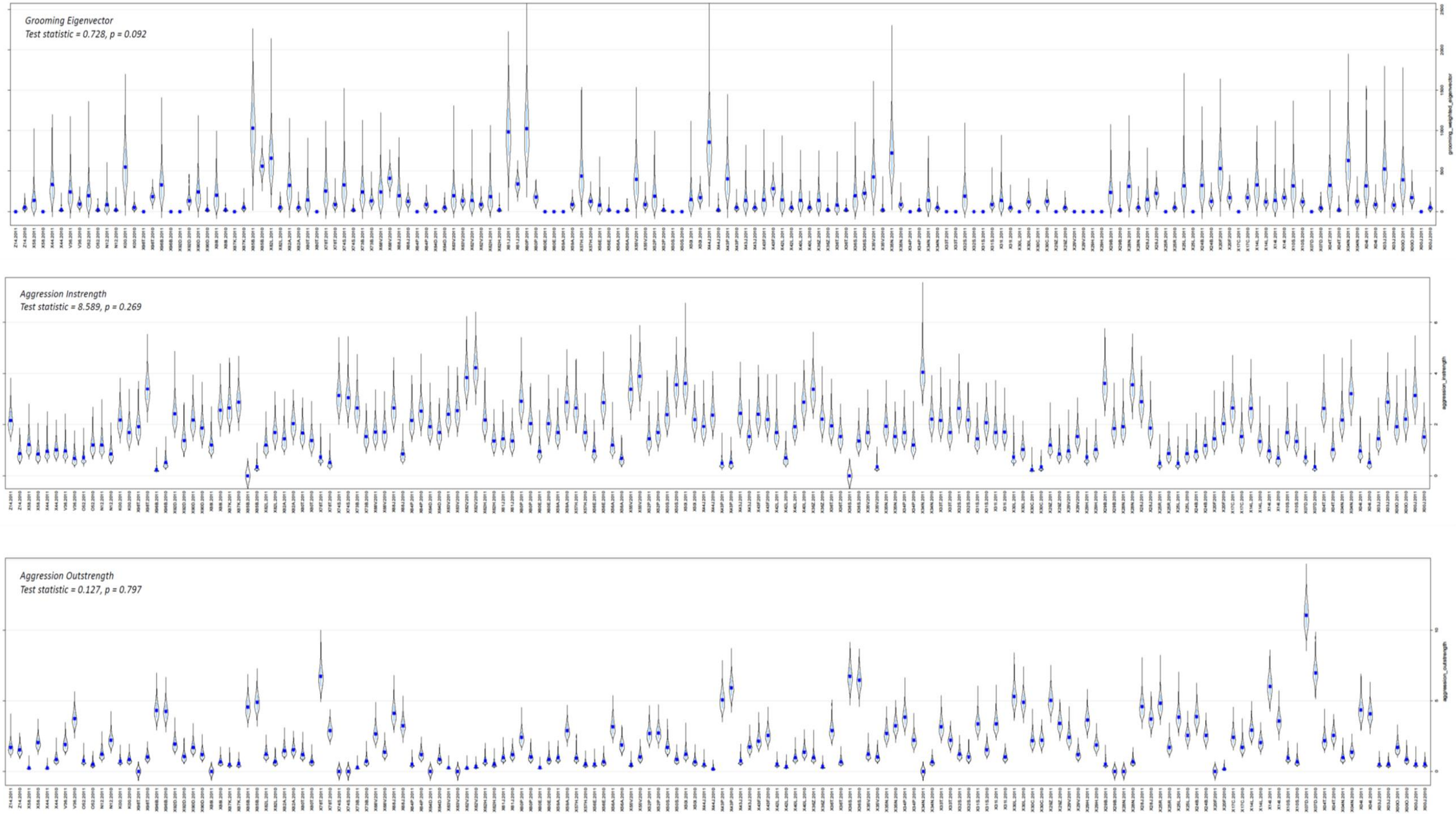

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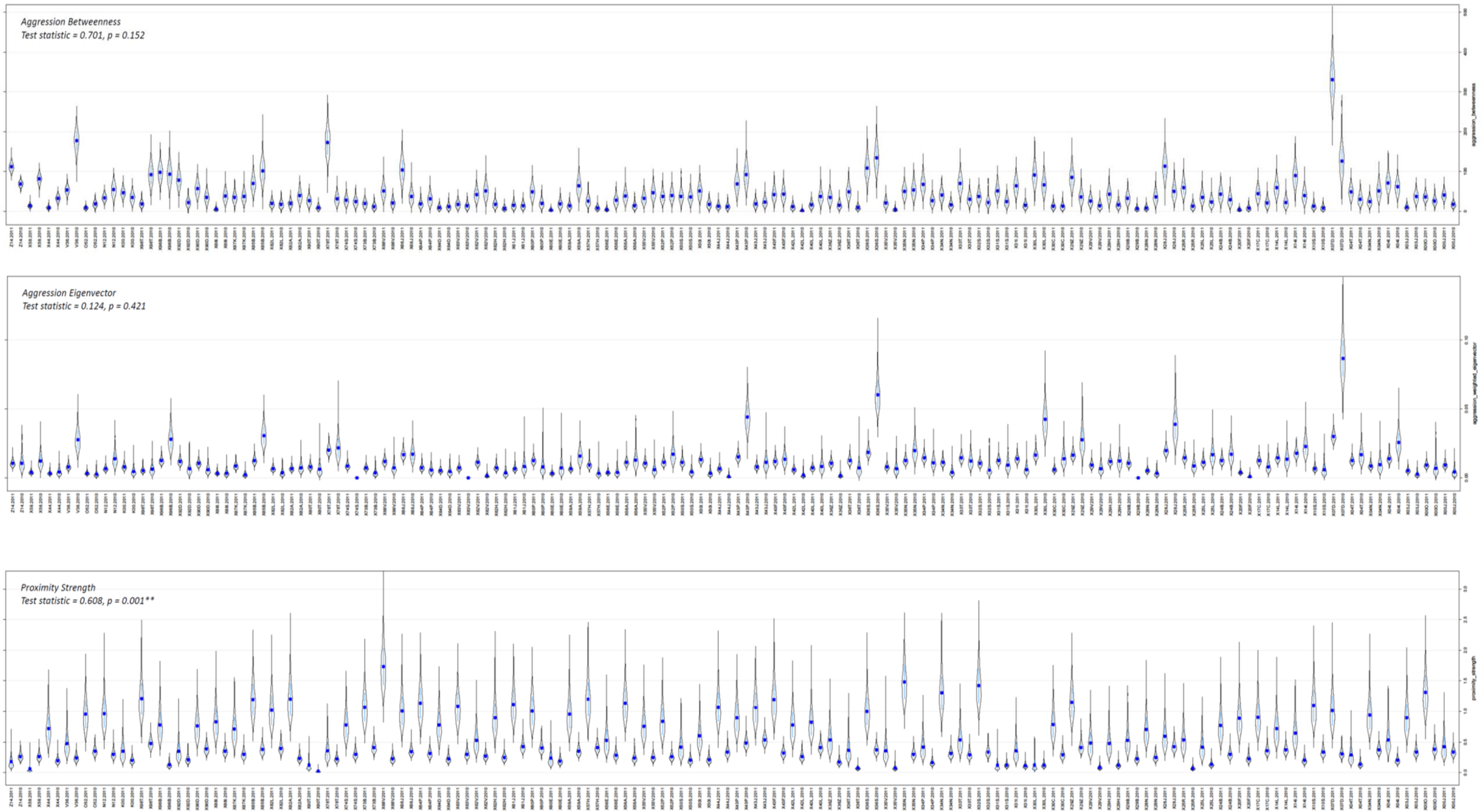

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