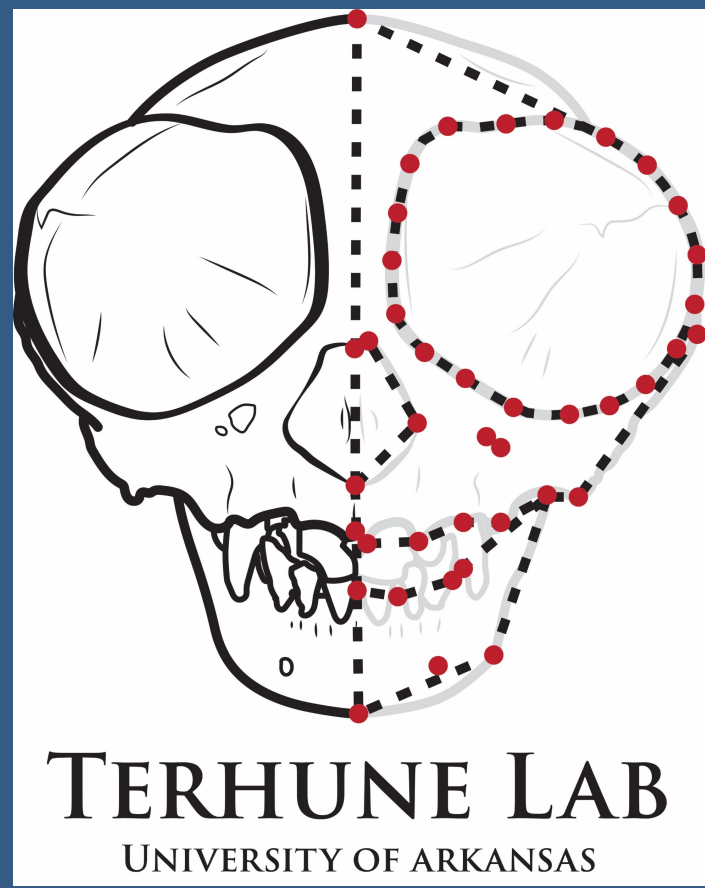


# Fetal individuals that experienced hurricanes exhibit increased craniofacial fluctuating asymmetry

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## Introduction

The right and left side of bilaterally symmetric organisms develop under the same environment and genome and should, therefore, reflect one another exactly. Fluctuating asymmetry (FA), or random deviations from bilateral symmetry, is thought to occur with developmental instability in an organism<sup>1</sup>. Developmental instability can be caused by a number of environmental and genetic factors, one of which is natural disasters<sup>2,3</sup>.

This study investigates the effect of two Category 3 hurricanes (Hugo and Georges) on craniofacial FA in the Cayo Santiago macaques, especially the impact of experiencing such an event during different developmental periods.



Fig. 1: Aerial images of Cayo Santiago, Puerto Rico before (left) and after (right) hurricane Maria (September 2017). Figured modified from Testard et al. (2021).

## Materials and Methods

We tested the effect of demographic and environmental factors on craniofacial FA using a cross-sectional, ontogenetic sample of rhesus macaques (*Macaca mulatta*)<sup>2,3</sup>.

### Materials:

- 275 3D scanned macaque crania ages 9 months to 31 years (Y.O.B. 1951-2005)

Table 1: Sample composition.

Female	154
Male	121
Experienced at least one hurricane	101
Never experienced any hurricane	174
Experienced one hurricane	78
Experienced two hurricanes	23
Experienced hurricane during fetal stage	10
Experienced hurricane during juvenile stage	50
Experienced hurricane during adult stage	41

Fig. 2: Anterior view of landmarks on 3D model in study (CPRCMUS-04439).

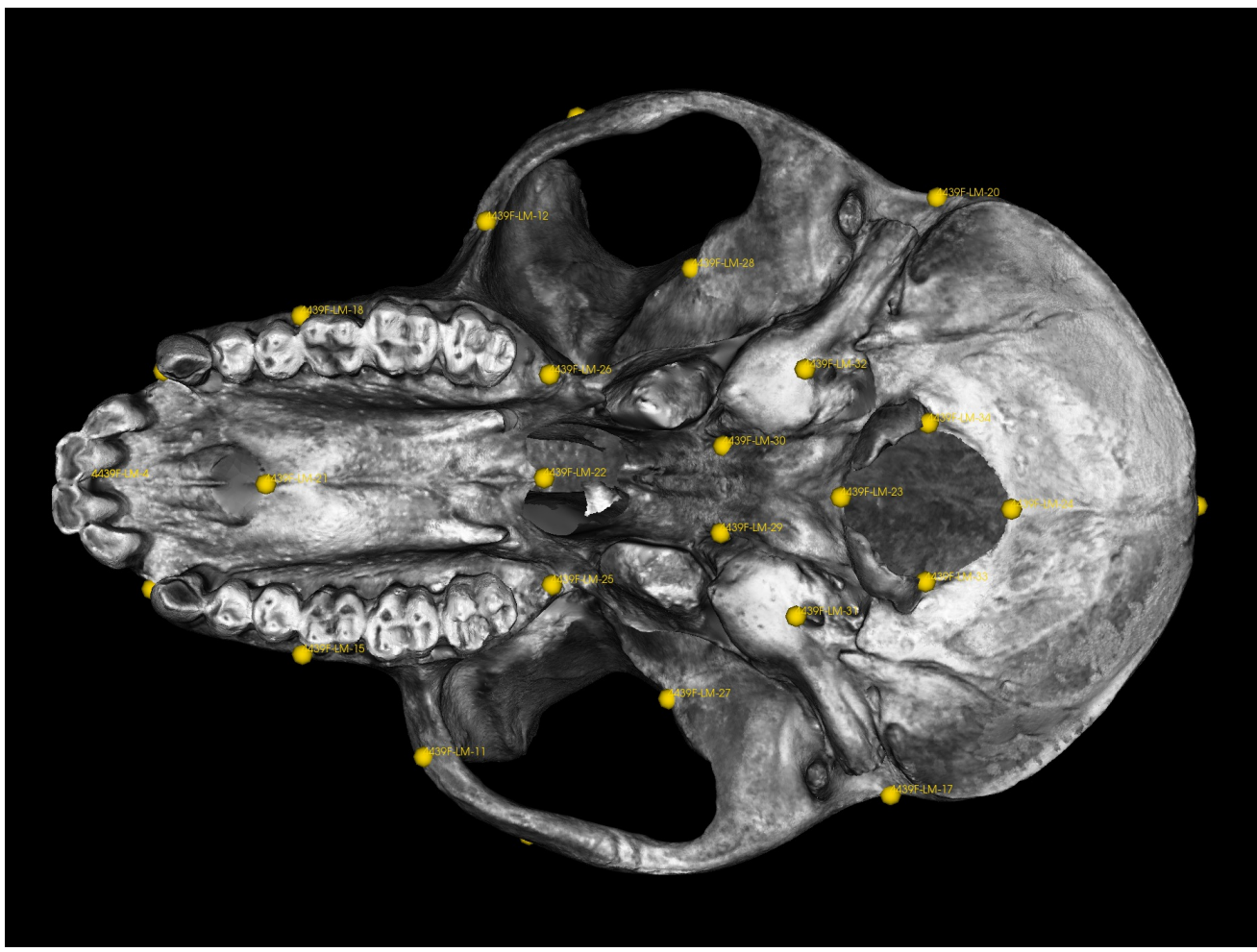
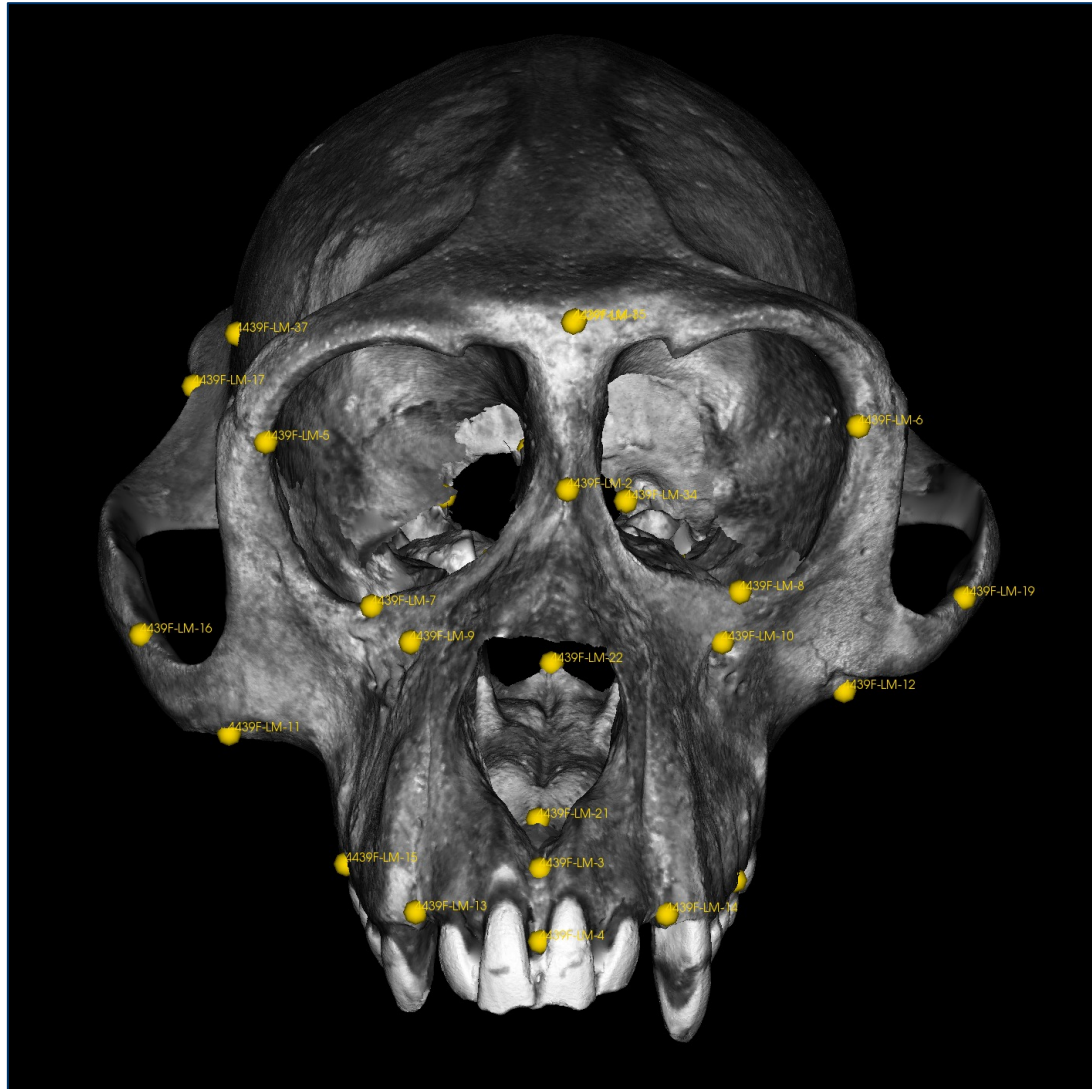


Fig. 3: Inferior view of landmarks on 3D model in study (CPRCMUS-04439).

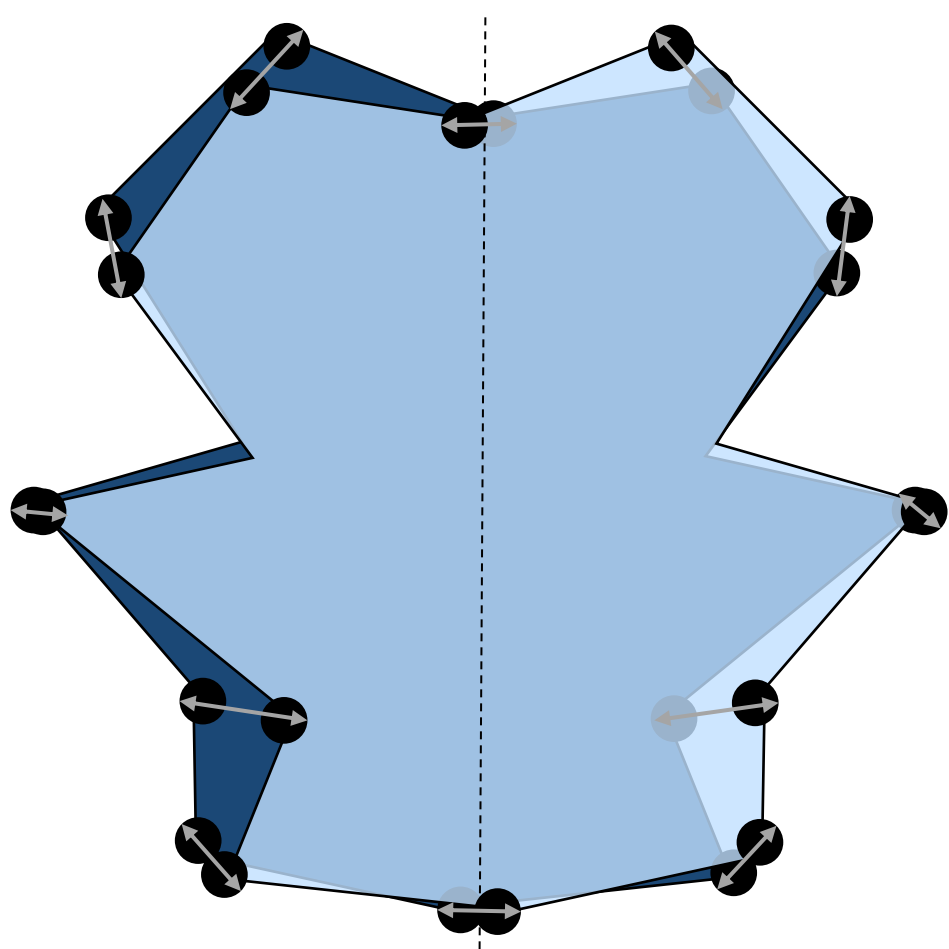


Fig. 4: Hypothetical shapes represent the original (dark blue) and reflected (light blue) copy of an organism, while the arrows (gray) represent distances measured and added to calculate FA level for each individual.

### Methods:

- Placed 35 3D landmarks in 3D Slicer<sup>4</sup> across crania (twice) to quantify FA
- Procrustes ANOVA in MorphoJ<sup>5</sup> provides FA score for each individual
- Linear mixed-effect models in R<sup>6,7,8</sup>
  - Age + sex + decade of birth | matriline on all and then just those that never experienced hurricane
  - Age + sex + decade of birth + hurricane yes/no | matriline on all individuals
  - Age + sex + decade of birth + # of hurricanes | matriline on all individuals
  - Age + sex + decade of birth + age at hurricane | matriline on all individuals that experienced a hurricane
- Posthoc Tukey's HSD tests with Bonferroni corrections<sup>9</sup>

## Results

Table 2: Statistical parameters derived from linear mixed-effect models demonstrating the importance of various fixed effects (age, sex, decade of birth, hurricane Yes/No, # of hurricanes, and age at hurricane) on FA score while controlling for matriline as a random effect. Reference variable for sex = Female; reference variable for decade of birth = 1950s; reference variable for # of hurricanes = 0; reference variable for age at hurricane = fetal.

Model	Fixed Effect	p-value
Model 1 (Hurricane Y/N)	Age	0.669
	Sex	0.381
	Decade of Birth	0.729
	Hurricane (Y/N)	0.411
Model 2 (only Hurricane N)	Age	0.209
	Sex	0.395
	Decade of Birth	0.825
Model 3 (all animals, # of hurricanes experienced)	Age	0.631
	Sex	0.396
	Decade of Birth	0.711
	# of Hurricanes (1)	0.400
	# of Hurricanes (2)	0.765
Model 4 (only hurricane Y, age at hurricane)	Age	0.094
	Sex	0.115
	Decade of Birth	0.419
	Age at Hurricane (1)	<0.001
	Age at Hurricane (2)	0.001

Table 3: Results of Tukey's HSD tests of FA level between age groups experiencing hurricanes.

Fetal-Juvenile	p<0.001
Fetal-Adult	p=0.002
Juvenile-Adult	p=0.534

Age at hurricane significantly affects level of FA, specifically for the fetal stage.

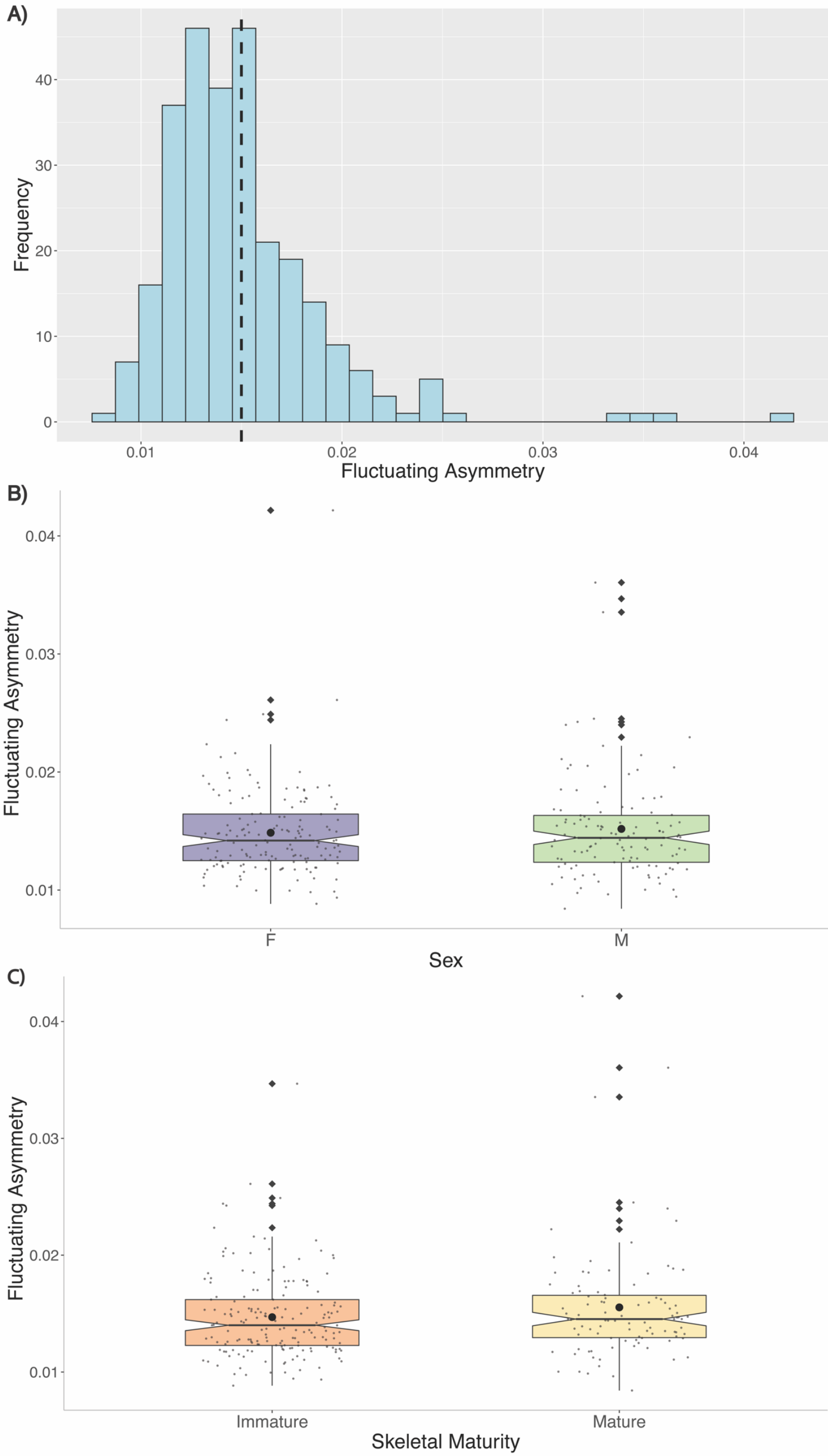


Fig. 5: Plots illustrating the distribution of data in a A) histogram showing the frequency and mean (dashed black line) of FA values, B) boxplot of FA values separated by sex showing the mean (solid round point) value of males and females, and C) boxplot of FA values separated by skeletal maturity showing the mean (solid round point) value of skeletally mature and immature individuals.

## Discussion and Conclusion

What impacts craniofacial FA level in the Cayo Santiago rhesus macaques?

- Age at death
- Sex
- Number of hurricanes experienced
- Age at which hurricanes were experienced

Prenatal vulnerability to stress could be causing the elevated levels of FA in individuals that experienced a hurricane during fetal life. This vulnerability could be a result of increased maternal stress-related hormones such as cortisol and glucocorticoids that disrupt fetal development during such a critical period<sup>10</sup>.

Prenatal individuals are impacted more than any other developmental stage by hurricane events.



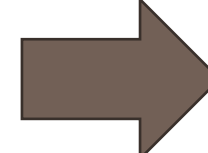
Fig. 6: Free-ranging rhesus macaque mother and infant (*Macaca mulatta*) on the island of Cayo Santiago.

## Acknowledgements and Contact

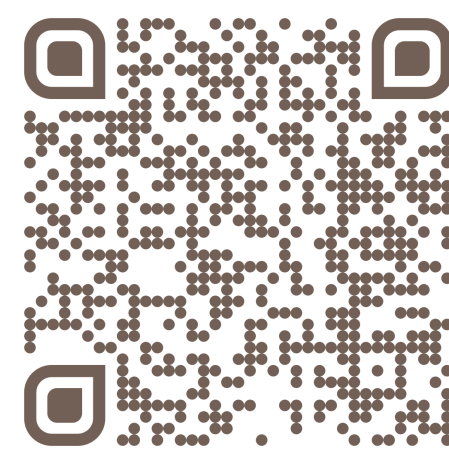
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