Understanding the Environmental and Genetic Influence
on Fluctuating Asymmetry and Developmental Instability in Primates

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by

Ashly N.Romero
California State University, Long Beach
Bachelor of Arts in Anthropology, 2016
University of Arkansas
Master of Arts in Anthropology, 2018

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University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Claire Terhune, PhD
Dissertation Director

_____
Lucas Delezene, PhD
Committee Member

_____
Kathleen Paul, PhD
Committee Member

_____
Katherine Willmore, PhD
Ex-officio Committee Member

Abstract

This dissertation explored the impact of environmental factors on the development and perpetuation of fluctuating asymmetry (FA) and sought to understand the role evolution may play in the FA exhibited in two primate populations: the free-ranging Cayo Santiago rhesus macaques (*Macaca mulatta*) and the Southwest National Primate Research Center olive baboons (*Papio hamadryas anubis*). Demographic, ontogenetic, secular, external, and genetic factors were examined. Specifically, this dissertation investigated FA over all ontogenetic stages, across decades, between sexes, in association with ecological catastrophes, and with tooth pathology to try and tease apart factors that may influence FA and developmental instability. This dissertation also estimated the heritability and evolvability of FA and used FA levels over decades to examine the role of evolutionary mechanisms on FA. In all, results show that the age at which a macaque experiences a hurricane and baboon antemortem tooth loss impact levels of FA. They also show that sex-related differences are present in the population of baboons but not the macaques. Additionally, FA does not seem to change ontogenetically in either the macaque or baboon population, and secular changes were only found in male baboons where FA decreased over time. Lastly, the heritability and evolvability of FA in the macaque and baboon populations were extremely low, though higher in baboons than macaques. This work suggests that FA levels may be sex-specific in species with extreme sexual dimorphism, and FA generally seems not to change over ontogeny in these populations. Secular changes in FA appear possible in primates, although the pattern remains ambiguous. This work also shows that ecological catastrophes such as hurricanes are likely critical determinants of FA later in life if experienced *in utero*. Lastly, FA seems to have some additive genetic variation that is subject to selection, though minimal. Overall, this work offers additional resolution in teasing apart factors contributing to FA and points to minimal genetic influence on FA levels.

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List of published papers

Chapter 2: Romero, A. N., Dickinson, E., Turcotte, C. M., Terhune, C. E. Skeletal age during hurricane impacts fluctuating asymmetry in Cayo Santiago rhesus macaques. In review at *Ecology and Evolution*.

Chapter 3: Romero A. N., Terhune, C. E. Pathology- and secular-related changes in fluctuating asymmetry detected in olive baboons. In preparation for submission to the *International Journal of Primatology*.

Chapter 4: Romero, A. N., Yim, A., Terhune, C. E. Heritability and evolvability of fluctuating asymmetry in rhesus macaques and olive baboons. In preparation for submission to the *American Journal of Biological Anthropology*.

Chapter 1: Introduction and Background

Introduction

All organisms have a genetic code that maps out their growth and development and largely determines their adult form, but genes are only part of what creates an organism; environment also plays an integral role in shaping an organism's adult form. If the adult phenotype (visible characteristics of an organism) is the end result, then genotype and the environment both affect the growth and development, or ontogeny, that generates these phenotypes. Though individuals in a population vary in their genetics, and environment varies between populations, all individuals of a given species share a target phenotype, one aspect of which is bilateral symmetry.

Bilateral symmetry refers to a condition where an organism exhibits exact, but reflected, copies of a form on each side of a central plane. For example, on each side of most human bodies, we have an arm, leg, ear, etc. These are reflections of one another; our left arm looks the same as our right arm, just opposite. Bilateral symmetry is a trait present in most organisms, but its changes lack in-depth investigation. Symmetry has functional advantages that allow organisms to move around their environments more efficiently (Auerbach & Ruff, 2006; Didde & Rivera, 2019) and represents an incredibly conserved and optimal phenotype across taxa (Palmer, 1996a; Rasmuson, 2002). Problems during development result in more asymmetric phenotypes, indicating decreased developmental stability (Klingenberg, 2015). These problems, or fluctuations/perturbations, can be genetic (e.g., heterozygosity and inbreeding depression) or environmental (e.g., habitat change, parasites, disease, or psychosocial stress) in origin. Specifically, the asymmetry arising from developmental instability is fluctuating asymmetry (FA), otherwise defined as random deviations from symmetry.

Though the literature documenting FA in animals is vast and variable, our understanding of what causes FA is limited. Data is particularly lacking regarding changes in FA across ontogeny and especially during adulthood, changes in FA over generations in a population, the

relationship between environmental factors and FA, and the heritability and evolvability of FA in primates. Filling these gaps in knowledge is important for understanding to what degree and in what time range fluctuations and perturbations affect developmental instability. Further, generational changes in FA in a natural population have yet to be reported, and secular changes in FA can provide critical information for the evolution of FA and developmental instability.

Research Questions

Considering the ambiguity in the causes of and changes in fluctuating asymmetry in primates, the broad goal of this dissertation is to investigate the development and perpetuation of fluctuating asymmetry during ontogeny and across generations within a population, including its environmental and genetic influences, to better understand developmental instability in this order. These questions are investigated in two primate species: rhesus macaques (*Macaca mulatta*) and olive baboons (*Papio hamadryas anubis*). The samples for this dissertation come from the free-ranging colony of rhesus macaques on Cayo Santiago and the captive colony of olive baboons from the Southwest National Primate Research Center.

How Do Demographic Variables Influence Fluctuating Asymmetry?

This dissertation investigates two demographic factors and their relationship to FA. Relationships between sex and FA and decade of birth and FA were tested in both macaques and baboons. Studies including sex in their analysis of FA remain without consensus as to the degree to which levels of FA are influenced by sex. Meanwhile, this is the first study including decade of birth, presenting a novel opportunity to investigate the effect birth year may have on FA.

How Does Fluctuating Asymmetry Change Ontogenetically?

Previous work has suggested that FA increases ontogenetically in primates (Hallgrímsson, 1999), though only humans and long-tailed macaques have been investigated. This dissertation investigates this question across the entirety of the lifetime in macaques and specifically throughout adulthood in baboons using an age structured, skeletal dataset in an effort to tease apart changes in FA occurring throughout the lifetime. How FA changes during an individual's lifetime provides insight for the mechanisms behind its development, especially in understanding critical periods that increase or decrease levels of FA.

Are There Secular Changes in Fluctuating Asymmetry?

Long-term changes to FA in animals have only currently been investigated in bees (Arce et al., in press), but investigating secular changes to FA are important for understanding the mechanisms behind FA as well. While the consensus is that environmental stress increases FA, there may be evolutionary mechanisms influencing FA too. This dissertation examines FA over decades of times in both macaques and baboons. The former sample is a free-ranging population in a natural environment and the latter a captive colony. These differences help tease apart the impact of environmental stresses and evolutionary mechanisms on FA.

How Do External Perturbations Influence Fluctuating Asymmetry?

Environmental stresses have been established as influential for increasing levels of FA across animal taxa (see Klingenberg, 2015), this dissertation investigates two unexplored environmentally induced insults to primate lives: hurricanes in macaques and antemortem tooth loss in baboons. Antemortem tooth loss in baboons is suggested to be related to male-male competition (Kirchhoff et al., In review), thus making it an external factor rather than internally controlled like feedback loops for bilaterally symmetric tissue development.

To What Degree is Fluctuating Asymmetry Influenced by Genetic Factors?

The heritability of FA was found to be low in previous studies using linear measurements (Fuller & Houle, 2003), while the evolvability of FA has yet to be estimated. These estimates can tell us the amount of FA that can be explained by the genetic structure of the sample, and the degree to which it is accessible to selection. Further, evolvability can be compared between populations because it does not include environmental variance. This dissertation estimates the heritability of FA and the evolvability of FA in macaques and baboons, which has yet to be done for craniofacial FA calculated using 3D geometric morphometric techniques.

Dissertation Outline

This first chapter describes the overarching research questions for this dissertation, outlines the content included in this dissertation, and provides relevant background about the research topic and sample populations used for these studies.

Chapters 2, 3, and 4 present novel analyses designed to investigate factors contributing to FA. Chapter 2 investigates FA in rhesus macaques (*Macaca mulatta*) from the island of Cayo Santiago in Puerto Rico, where multiple Category 3 hurricanes have occurred. This chapter examines the relationship between FA and age, sex, decade of birth, and hurricane experience to understand the influences on FA and the timing in which environmental stress is most impactful for developing FA. Chapter 3 investigates FA in olive baboons (*Papio hamadryas anubis*) from the captive colony at the Southwest National Primate Research Center in Texas. While remaining undisturbed by environmental catastrophes, this population exhibits high rates of antemortem tooth loss. This chapter examines the relationship between FA and age, sex, decade of birth, and antemortem tooth loss to understand the influences on FA and the degree to which dental pathology may impact the development of FA. Chapter 4 provides heritability and evolvability estimates of FA in both macaques and baboons. These are the first heritability estimates for craniofacial FA estimated using 3D geometric morphometric techniques and the first ever evolvability estimates for FA.

Lastly, Chapter 5 summarizes the findings of the research chapters before, discusses how the research results compare to one another and contribute to our overall understanding of fluctuating asymmetry, and proposes future work planned by the author to fill gaps left in the field of fluctuating asymmetry and developmental instability.

Background

Before introducing asymmetry, it is pertinent to first describe symmetry. Symmetry is defined as repeating structures in different orientations (Klingenberg, 2015). For bilateral symmetry, one structure is reflected across a median axis, and this symmetry can take two forms: matching symmetry and object symmetry (Graham et al., 2010; Klingenberg, 2015). Matching symmetry describes two independent structures on either side of a midline axis that are reflections, or mirror images, of one another (e.g., fly wings). The midline axis in matching symmetry is not part of the structures of interest themselves. Object symmetry describes one structure in which one side is a reflection, or mirror image, of the other (e.g., the human head). With object symmetry, the midline is part of the symmetric object. For example, the right and left sides of the human head are both part of the object of interest and the midline runs through the center of it. The bilateral bauplan is so ubiquitous in biological organisms that it supplies the name for an entire clade: Bilateria (Hausdorf, 2000; Robertis & Sasai, 1996).

Bilateral symmetry is thought to have evolved before the common ancestor of the Bilateria clade, as some species in Cnidaria, an outgroup to Bilateria, exhibit bilateral symmetry as well (Finnerty, 2003). *Hox* genes are suggested to pattern the anterior-posterior axis in Bilateria, while *Dpp* genes are thought to pattern the dorsal-ventral axis. Both genes are expressed in Cnidaria, suggesting that bilateral symmetry is more ancient that the clade Bilateria. Mammalian symmetry was widely viewed as a product of post-zygotic mechanisms, but more recent studies have found that this is probably not the case (Gardner, 2001; Weber et al., 1999). Mammalian models show that cues for bilateral patterning appear before cleavage,

suggesting that there is likely a maternal role in patterning that occurs earlier than implantation (Gardner, 1997; Gardner & Davies, 2003; Weber et al., 1999). Maternal effect is an environmental influence that could play a larger role in the degree of symmetry displayed later in life.

Asymmetry describes any deviations from bilateral symmetry, or right-left differences. These differences can be in size and/or shape, but for organisms or structures under study that exhibit object symmetry, only shape is typically analyzed (Klingenberg, 2015). Further, there are three different types of asymmetry that can be observed and studied in a population: directional asymmetry, antisymmetry, and fluctuating asymmetry. These types of asymmetry are not mutually exclusive. In fact, most times at least two types of asymmetry are present. Additionally, when observing asymmetry in a population or individual, you calculate both signed and unsigned asymmetry. Signed asymmetry includes the direction of the asymmetry (right or left as positive and negative), and unsigned asymmetry is the absolute amount of asymmetry that is focused on magnitude and ignores direction (Klingenberg, 2015; Klingenberg & McIntyre, 1998; Ludoški et al., 2014).

Types of Asymmetry

Directional Asymmetry

Directional asymmetry refers to a difference in shape or size that favors one side of an organism (Van Valen, 1962). At a population level, directional asymmetry can be found as the signed differences between the right and the left sides of all individuals that exhibit a distribution with a non-zero mean (Graham et al., 1998; Klingenberg, 2015). This difference is exhibited in an individual as the non-zero difference in the signed average of the right and left side of the organism. In both a population and an individual, directional asymmetry describes the mean asymmetry.

Directional asymmetry is common, especially so in mammals. Many mammalian organs are asymmetric in one direction (e.g., heart, lungs, spleen, liver), and handedness is common among humans (Klingenberg, 2015; Levin, 2005). Directional asymmetry is not limited to obvious occurrences visible to the naked eye. While older, linear measurements of asymmetry did not always observe directional asymmetry, newer methods of measurement (e.g., geometric morphometrics) have observed directional asymmetry in nearly every organism investigated (Klingenberg et al., 1998; Klingenberg & McIntyre, 1998). Though minor, directional asymmetry appears to be widespread in the natural world; however, only recently has this phenomenon begun to be studied (Budečević et al., 2022)

Antisymmetry

Antisymmetry refers to asymmetry in which one side of an organism differs in shape or size, but variation exists in which side is different within a population (Timoféeff-Ressovsky, 1934; Van Valen, 1962). Importantly, this variation is not structured; in other words, there is no pattern through which one side or the other is more developed, but approximately half of the population will exhibit a different right side and the other will exhibit a different left side (Graham et al., 1993). A population with an antisymmetric trait should exhibit a bimodal distribution of the signed differences between the right and left sides with a mean of zero, or a platykurtic distribution in less extreme cases (Van Valen, 1962). Lewontin and Van Valen suggest that there must be a negative interaction between the developing sides for such a type of asymmetry to exist (Graham et al., 1993; Mather, 1953; Van Valen, 1962). Antisymmetry and fluctuating asymmetry can be difficult to differentiate in analyses (Van Dongen et al., 1999), which will be discussed further in the following section.

Male fiddler crabs are perhaps the most classic example of antisymmetry in the literature today (Klingenberg, 2015). Each male fiddler crab exhibits a major cheliped (limb including arm and claw) and a minor cheliped, and about half of the male individuals in a population have a

right major cheliped while the other half have a left major cheliped (Rosenberg, 1995, 2002). The minor claw shape is related to habitat type, whereas the major claw shape is related to efficiency in competition (Rosenberg, 1995, 2002). Females have right and left chelipeds of the same size, which are closer in size to the male minor cheliped (Rosenberg, 2002).

Fluctuating Asymmetry

Fluctuating asymmetry refers to any random, non-directional deviations from bilateral symmetry. Fluctuating asymmetry is most often subtle and does not usually present as extensive, obvious asymmetry. In a population, fluctuating asymmetry exhibits a normal distribution of signed asymmetries around a mean of zero (Graham et al., 1998; Ludwig, 1932; Mather, 1953; Palmer & Strobeck, 1992; Van Valen, 1962). The amount of fluctuating asymmetry in a population or in an individual is calculated around the mean asymmetry, or directional asymmetry (Graham et al., 1998; Klingenberg, 2015; Palmer & Strobeck, 1986; Van Dongen et al., 1999), but antisymmetry and fluctuating asymmetry are difficult to tease apart. The issue is easier to understand when considering the distributions for antisymmetry and fluctuating asymmetry. Antisymmetry exhibits a bimodal or platykurtic distribution around zero, and fluctuating asymmetry exhibits a normal distribution around zero. Essentially, antisymmetry can hide within the fluctuating asymmetry distribution (Van Valen, 1962). Klingenberg (2015) suggests that tests of kurtosis may be able to distinguish between the two.

Fluctuating asymmetry is proposed to be related to developmental instability, though it has been used as a tool for answering many types of questions (Polak, 2003). Studies have compared various environmental stresses, genetic stresses, and fitness with levels of asymmetry (Klingenberg, 2015). Further, studies have used fluctuating asymmetry to answer questions about developmental processes, origins, modularity, and integration both in natural populations and in clinical settings. Now, fluctuating asymmetry is often regarded as a bioindicator of health in many populations. Fluctuating asymmetry has a long history of

discovery and use over the past 60 years and understanding its changes over time and its relationship to various environmental and genetic factors is the focus of this dissertation.

Individual Asymmetry

Directional asymmetry, antisymmetry, and fluctuating asymmetry are often examined at the population level, though directional asymmetry and fluctuating asymmetry are investigated now at an individual level as well. Many investigations require analysis of asymmetry for each individual in the sample in order to associate various groups or factors with levels of asymmetry. The preferred method of quantifying asymmetry is to use a composite of measurements (Leung et al., 2000). Asymmetry across these measurements can be applied at a populational or individual level. There are two general approaches to calculating individual asymmetry: Procrustes distances and Mahalanobis distances (Klingenberg, 2015; Klingenberg et al., 2002; Klingenberg & Monteiro, 2005). The difference between these two approaches is in the assumption of asymmetry variation.

Procrustes distance, or squared Procrustes distance, between the right and left side of an individual provides a magnitude of asymmetry, though this distance includes both directional and fluctuating asymmetry (Klingenberg, 2015; Klingenberg et al., 2002). Before calculating the Procrustes distance, subtracting the mean asymmetry removes directional asymmetry from this calculation. Procrustes distance gives the absolute magnitude of differences in shape between the right and left side. This measure assumes isotropic differences in asymmetry, where variation is the same in all directions and independent at each point measured. The Procrustes method has been critiqued due to its inconsistency when error is high (i.e., a low signal to noise ratio), but this does not seem to be an issue in most biological samples, especially those that are intraspecific or closely related (Kent & Mardia, 1997; Klingenberg & Monteiro, 2005).

If the differences between the right and left side of an individual are nonisotropic, then Mahalanobis distance is a more appropriate measure of individual asymmetry (Klingenberg,

2015; Klingenberg et al., 2002). This would be the case when particular shape features are more variable than others. The calculation of Mahalanobis distance is the same as the Procrustes distance, using the right-left differences between sides and subtracting the mean asymmetry, except there is additional scaling in each direction so there is an equal amount of asymmetry in each direction. This method requires larger sample sizes to reliably estimate the covariance matrix. Additionally, Mahalanobis distances are difficult to interpret because they are not comparable to other measures of shape variation (Klingenberg, 2015).

Because directional and fluctuating asymmetry can be assessed for an individual, the terminology “individual asymmetry” is not necessary other than to clearly communicate that the study is focused on asymmetry at the individual level rather than the population level, though this can be done other ways as well. This dissertation investigates fluctuating asymmetry in large samples of just two species, so individual fluctuating asymmetry will be the focus here because population level data would only provide two data points.

Measuring Fluctuating Asymmetry Using Geometric Morphometrics

Geometric morphometric techniques are a popular way of analyzing fluctuating asymmetry today. Geometric morphometric methods of analysis were originally developed with length and angle measurements, but now allow the quantification of multivariate two-dimensional (2D) and three-dimensional (3D) shape, which allows the measurement of shape and symmetry/asymmetry in more complex and informative ways. This dissertation utilizes 3D data, so 3D geometric morphometric techniques will be described and applied throughout.

At the most basic level, asymmetry is the difference between the right and left side of an organism, and fluctuating asymmetry is this difference minus the mean, or directional, asymmetry (Klingenberg, 2015). With geometric morphometrics, homologous 3D landmarks are placed on prescribed points of interest for an individual (Graham et al., 2010; Klingenberg, 2015). After digitization of all the individuals in a sample, the landmark configurations undergo a

Procrustes superimposition, or Procrustes fit. In Greek mythology, Procrustes was a son of Poseidon who invited travelers to lie on an iron bed and either stretched these victims or cut their limbs to fit the length of the bed (POWER, 2011; The Editors of Encyclopaedia Britannica, 2011). In a similar vein, a Procrustes superimposition moves all landmark configurations to the same position (translation), rotates all landmark configurations to the same orientation (rotation), and makes each landmark configuration the same size (scaling) to "fit" the configurations like Procrustes did to his victims (Dryden & Mardia, 1998; Goodall, 1991; Gower, 1975; Klingenberg, 2015). These translation, rotation, and scaling steps are important for eliminating location, orientation, and size variables and leave only shape (i.e., everything that remains after superimposition) for analysis. The Procrustes paradigm has become the standard analytical method for geometric morphometric studies (Adams et al., 2013). One type of Procrustes fit, generalized Procrustes analysis (GPA), is an iterative procedure that fits each landmark configuration to the consensus/average configuration using the sum of squared distances between corresponding landmarks and a scaled centroid size of zero (Klingenberg, 2015). First, a target specimen is chosen, and all configurations are fit to that target to create a consensus configuration. Then, the same process is repeated with the consensus configuration as the target and a new consensus is created. This process is repeated until the consensus configuration does not change. This can usually be achieved in two to three iterations. The square root of the sum of squared distances between corresponding landmark configurations is the Procrustes distance between configuration shapes. The landmark coordinates of the superimposed configurations are called Procrustes coordinates and contain all shape variation present (Klingenberg, 2015).

Translating, rotating, and scaling the landmark configurations reduces the dimensionality of the data because the landmarks can no longer vary in every way (Klingenberg, 2015). Dimensionality starts with three times the number of landmarks (*k*) for 3D data, and a dimension is lost for scaling, three dimensions are lost for translating, and three dimensions are lost for

rotating (3*k*-7). The resulting multidimensional shape space that is produced following a GPA contains every possible shape with that number of landmarks and is called Kendall's shape space. Kendall's shape space is extremely complex for configurations with many landmarks, but this shape space can be approximated locally by a linear tangent space for simplicity (Dryden & Mardia, 1998; Goodall, 1991; Rohlf, 1999). This simplification is acceptable because biological data tend to occupy small regions of the shape space, even when comparing large scale taxonomic differences (Marcus et al., 2000). This linear tangent space uses the consensus configuration as the articulation point between Kendall's shape space and the tangent space onto which the data points are projected (Klingenberg, 2015). The tangent space is locally approximated by the Procrustes superimposition.

To measure object symmetry, both median/midline landmarks and bilateral landmarks are needed (Klingenberg, 2015). The entire configuration of landmarks is reflected across the midline, and the bilateral landmarks of the reflected copy are relabeled to match the original shape (Kent & Mardia, 2001; Klingenberg et al., 2002; Mardia et al., 2000). A consensus of the original and reflected/relabeled copy that is perfectly symmetric is created via a Procrustes fit of the original and reflected/relabeled configurations. Then, the original, reflected/relabeled, and consensus configuration undergo a Procrustes fit where the sum of squared deviations of the original and reflected/relabeled copy are minimized from the consensus shape. Both the individual consensus shapes and the overall consensus shape are perfectly symmetric, which allows the midline landmarks to lie in a plane that represents an informed anatomical midline, and the bilateral landmarks are connected by lines that are perpendicular to this plane. The differences between corresponding landmarks in an original and reflected/relabeled configuration represent the asymmetry present. This is the same as the difference between the original configuration and the symmetric consensus or the difference between the reflected/relabeled configuration and the symmetric consensus.

The most widely used method of analyzing fluctuating asymmetry is the Procrustes ANOVA (Klingenberg et al., 2002; Klingenberg & McIntyre, 1998; Palmer & Strobeck, 1986). This ANOVA is a two-factor, mixed-effect model using individuals (specimens) and sides (right, left) as main effects. The individual effect is the variation in the right and left average trait values, while the sides effect is the average difference between the right and left sides (directional asymmetry). The interaction effect of individual by side is the difference in individuals due to their differences in left and right sides, which represents fluctuating asymmetry and/or antisymmetry. This model includes replicate measurements to assess measurement error. The subtlety of fluctuating asymmetry requires large sample sizes and replicate measurements to ensure that the noise of measurement error is not overwhelming the fluctuating asymmetry signal. If the signal to noise ratio is low, then additional specimens or additional replicate measurements should be included.

Developmental Instability

Stable development is extremely important for any organism to reach a target phenotype; any fluctuation (intrinsic) or perturbation (extrinsic) to a developmental system needs to be returned to its original trajectory for typical growth and development toward this target (Graham et al., 2010). Returning to the original trajectory requires stability of development itself but also buffering or resilience mechanisms that minimize perturbations. The converse of developmental stability is developmental instability, or the inability of a developmental system to accommodate fluctuations and buffer perturbations.

The right and left side of an organism grow and develop under the same genome and the same environmental conditions (Klingenberg, 2015; Polak, 2003). Therefore, the right and left sides of a bilaterally symmetric organism should exhibit symmetry, and any residual variation is likely due to developmental instability. Of course, the question arises: What exactly is causing differences in the right and left sides of an organism? This question has generated

many investigations into what can cause variance, or instability, in an organism's optimal, target, bilateral phenotype (Ludwig, 1932; Mather, 1953; Van Valen, 1962). Developmental processes are not entirely deterministic; variation in processes at the molecular and cellular levels add up to small deviations between the left and right sides, and these processes are influenced by the genome and environment of an individual (Emlen et al., 1993; Huh & Paulsson, 2011; Klingenberg, 2015; Raj & van Oudenaarden, 2008). The fluctuating asymmetry that is characteristic of developmental instability is minor. Large scale asymmetries in any given individual are likely of a different origin than developmental instability such as trauma or congenital defects (Debat et al., 2009; Klingenberg, 2015).

Variation during the developmental process is called developmental noise (Palmer, 1996b). Any observable fluctuating asymmetry in an organism is a result of both developmental noise and the mechanisms that buffer it (Klingenberg, 2015). Any mechanism that causes or allows amplification of random fluctuations and perturbations generates developmental noise (Willmore & Hallgrímsson, 2005). However, there are no "genes for" developmental stability (Klingenberg, 2015; Klingenberg & Nijhout, 1999; Leamy & Klingenberg, 2005). Rather, it is likely that nonlinearity of development, dominance and epistatic gene interactions, trait-specific processes, and trait-nonspecific processes are influencing the buffering of developmental noise (Klingenberg & Nijhout, 1999; Leamy & Klingenberg, 2005; Takahashi et al., 2011). Further, stress is an important factor for understanding developmental instability. Stress can be defined as anything that takes energy away from growth and development (Alekseeva et al., 1992; Parsons, 1990, 1992), which then reduces the efficiency of the system and reduces developmental homeostasis (Escós et al., 2000; Graham et al., 2010). The overloading of energy can also be considered stress if it decreases the efficiency of the system (Parsons, 2005). This decrease in efficiency can take place in a number of ways including changes in gene expression (Morgan et al., 2005), dissipating energy from feedback loops (Graham et al.,

2010), increasing heat shock protein production (Parsons, 2005; Sørensen et al., 2003), and changing metabolic pathways (Parsons, 2005).

The timing at which fluctuations and perturbations occur during development is likely important. Vrijenhoek (1985) suggested that there is a window of vulnerability, which relies on system research that states that systems can be changed the most when the energy of the system is at its highest (Hollebone & Hough, 1991). Thus, traits developing earliest might experience the most vulnerability (Alados et al., 1998). This indicates that we can expect to see more developmental instability, or fluctuating asymmetry, in traits with the earliest developmental window. This concept has been brought to the forefront of research today with the more recent focus on plasticity (McPherson, 2021) but applies to developmental instability as well.

Known Influences on Fluctuating Asymmetry

Fluctuating asymmetry has been associated with a variety of factors during an organism's lifetime, all of which can be divided into two major groups: environmental and genetic. Environmental factors are those that arise from the macro- or micro-environment of an organism. Macro-environmental factors arise outside of the organism (e.g., temperature, resource availability), while micro-environmental factors arise within the organism (i.e., developmental noise from mistakes in molecular or cellular processes). Genetic factors are those that are related to the genetic makeup and allele combinations of an organism. Heterozygosity related to species hybridization and inbreeding depression are thought to have an influence on fluctuating asymmetry, and the genetic variation related to fluctuating asymmetry (or the developmental system) appears to influence levels of fluctuating asymmetry as well. While some studies have found no association with particular environmental (Allen & Leamy, 2001; López-Romero et al., 2012; Pound et al., 2014; Radwan, 2003) and genetic

factors (Mikula & Macholán, 2008; Windhager et al., 2014), the scale tips in favor of those that find a relationship between these factors and fluctuating asymmetry.

Further, maturational spans and functional constraints are shown to be important factors for fluctuating asymmetry as well. Fluctuating asymmetry increases with maturational span in mammalian species, suggesting that longer development comes at the cost of developmental stability (Hallgrímsson, 1995). In her dissertation, Martin (2013) found a similar result for dental fluctuating asymmetry across primate species, where primates with longer maturational spans exhibited higher levels of fluctuating asymmetry. The ratio of bone turnover to bone growth is suggested to determine the fluctuating asymmetry that accumulates, so faster growth with less background bone turnover and more directed bone growth likely results in higher levels of fluctuating asymmetry (Hallgrímsson, 1993, 1998). Regarding functional constraints, a number of studies have found results that suggest there is lower fluctuating asymmetry in areas of the body that are important for mastication and locomotion. High performance cyclists exhibit less fluctuating asymmetry than those with lower performance (Rauter & Simenko, 2021). In bats, parts of the cranium with important masticatory function had lower levels of fluctuating asymmetry than non-masticatory regions (López-Aguirre & Pérez-Torres, 2015). However, a study in Japanese macaques (*Macaca fuscata*) found that there was no difference in fluctuating asymmetry in the mandibles of captive and wild macaques with different diets (Landi et al., 2021). Likewise, levels of fluctuating asymmetry did not affect bite force performance in mice (Ginot et al., 2018). In all, it is important to be careful when choosing traits in studies of fluctuating asymmetry because there is some evidence to support functional constraints affecting the amount of fluctuating asymmetry present in a given region.

Environmentally-related Fluctuating Asymmetry

Fluctuations in various environmental factors have been shown to be associated with changing levels of fluctuating asymmetry, and therefore developmental instability, in a range of

organisms. Temperature (Debat et al., 2009; Gerard et al., 2018; Hosken et al., 2000; Siegel et al., 1977), environmental toxins (Amarena et al., 1994; Coda et al., 2016; Costa & Nomura, 2016; Ding et al., 2022; J. M. Keller et al., 2007; Kristensen et al., 2004; Lens et al., 2002; Nunes et al., 2001; Oleksyk et al., 2004; Romero et al., 2017), habitat disturbance (Badyaev et al., 2000; Castilheiro et al., 2022; Frota et al., 2019; Hopton et al., 2009; Lazić et al., 2013, 2015; Maestri et al., 2015; Teixeira et al., 2006; Wauters et al., 1996; Wójcik et al., 2007), population density (Sheftel et al., 2020; Tuyttens et al., 2005; Zakharov et al., 1991), disease (Jung & von Cramon-Taubadel, 2018; Kohn & Bennett, 1986; O'Donnell & Moes, 2020; Weisensee, 2013), nutritional stress (DeLeon, 2007; Rusk et al., 2021), prenatal stress (Planas et al., 2018), psychosocial stress (Newell-Morris et al., 1989; Özener, 2010; Zurawiecka et al., 2019), and parasites (Agnew & Koella, 1997; Folstad et al., 1996; Jojić et al., 2021; Polak, 1997) have all been associated with higher fluctuating asymmetry levels. Environmental toxins include heavy metals, radiation, PCBs (polychlorinated biphenyls), and pesticides, while habitat disturbance is comprised of both anthropogenic and natural components and includes deforestation, habitat fragmentation, increased human traffic, and urbanization. Study taxa have included flies, springtails, bees, various rodents, primates, and humans. While some studies do not find associations between environmental factors and fluctuating asymmetry (Allen & Leamy, 2001; Bushell et al., 2021; Gonzalez et al., 2014), those that do find that fluctuating asymmetry increases with the degree of environmental stressor.

Fluctuating asymmetry studies in primates often include many uncontrollable variables because few studies have been possible in controlled, captive environments. The primate studies on fluctuating asymmetry to date remain useful due to their proposed associations with various environmental stressors, and studies of both skeletal and soft-tissue traits have been conducted. In a cross-species study with 12 catarrhine and platyrrhine primate genera, growth duration was associated with increased dental fluctuating asymmetry, and males in sexually dimorphic species exhibited more fluctuating asymmetry than females (Martin, 2013). Further,

apes had the greatest fluctuating asymmetry out of any primate in the sample. Chimpanzees (*Pan troglodytes*) exhibited the greatest dental fluctuating asymmetry overall, which is the opposite trend as seen in Romero et al. (2022) where chimpanzees (*Pan troglodytes troglodytes*) exhibited the lowest craniofacial fluctuating asymmetry when compared with gorillas (*Gorilla gorilla gorilla*) and macaques (*Macaca fascicularis fascicularis*). Romero et al. (2022) also found lower variation in fluctuating asymmetry levels in chimpanzees compared to the other two taxa. Additionally, dental fluctuating asymmetry in baboons (*Papio anubis*) was greater in males than females (Hoover et al., 2021). A comparison of chimpanzee and human brain shape found higher contribution of fluctuating asymmetry to overall variation in humans than chimpanzees (Gómez-Robles et al., 2013), and fluctuating asymmetry was also found in crania across ape taxa (*Pan*, *Gorilla*, *Pongo*, and *Homo*) where fluctuating asymmetry contributed to the overall cranial variation the most in humans (13%) followed by bonobos (11%), chimpanzees (10%), gorillas (9%), and orangutans (7%; Singh et al., 2012). In a study of baboons (*Papio anubis*), gorillas (*Gorilla beringei graueri*), and chimpanzees (*Pan troglodytes*), Van Dongen (2015) found no association in skull masculinity/femininity as measured by percentage of difference in size between each sex and the mean skull size of the pooled sample and individual scores of the canonical variate of shape sexual dimorphism. This finding suggests that fluctuating asymmetry does not differ between males and females or the amount of distinguishability as a male or female in these primate species. Limb measurements also exhibit fluctuating asymmetry in primates. In cotton-top tamarins (*Saguinus oedipus*), fluctuating asymmetry in limb lengths and diaphyseal breadths matched that found in humans, with the diaphyses exhibiting a greater degree of fluctuating asymmetry than the long bone lengths (Reeves et al., 2016).

In southern or Sunda pig-tailed macaques (*Macaca nemestrina*), psychological stress in pregnant female macaques is associated with increased dermatoglyphic asymmetry in their offspring. Dermatoglyphs form in weeks 14-22 of fetal development, a time period that is also

characterized by rapid brain growth and has been thought to be associated with issues in brain development (Bushell et al., 2021; Stiles & Jernigan, 2010). A study using the crab-eating or long-tailed macaque (*Macaca fascicularis*) found that cranial fluctuating asymmetry increased over developmental time in this species and that juveniles tended to have more fluctuating asymmetry than would be expected if this were a linear relationship (Hallgrímsson, 1993). The author of this study suggests that fluctuating asymmetry increases as a result of the accumulation of noise over developmental time and that fluctuating asymmetry may not be environmentally derived. In rhesus macaques (*Macaca mulatta*), higher levels of dental fluctuating asymmetry were found in fetuses whose mothers had diabetes mellitus than in those with unaffected mothers (Kohn & Bennett, 1986). Further, greater asymmetry deviations have been associated with greater environmental variance in rhesus macaques (Willmore et al., 2005). This relationship had a low, but significant correlation, suggesting that there is much variance unaccounted for. The authors attribute these results to overlapping regulatory mechanisms for canalization and developmental stability in rhesus macaques. In a study comparing rhesus macaques and humans, Hallgrímsson (1999) found that variance in fluctuating asymmetry increased over ontogeny, which when coupled with the finding that fluctuating asymmetry variance accumulates to higher levels in slower growing mammals suggests that fluctuating asymmetry is a result of accumulation of asymmetric mechanical factors, variation in growth regulation, and the tendency for morphological drift during bone remodeling.

In humans, low quality diets (Rusk et al., 2021) and other nutritional stress (DeLeon, 2007) have been linked to higher levels of craniofacial fluctuating asymmetry. Further, lower socioeconomic status seems to be associated with higher facial fluctuating asymmetry, especially in males (Jandová & Urbanová, 2021; Özener, 2010; Zurawiecka et al., 2019). This finding suggests that there may be discrepancies in the effect of stressful conditions on males and females. Manning et al. (1996) and Longman et al. (2021) found that resting metabolic rate

in males is associated with fluctuating asymmetry levels in anthropometric measurements. Males with lower metabolic rates had lower levels of fluctuating asymmetry. In a study on dermatoglyphics, prenatal alcohol exposure was linked to higher levels of fluctuating asymmetry as well (Planas et al., 2018). Prenatal exposure to amniotic cortisol, however, does not appear to influence levels of fluctuating asymmetry in finger length (Bushell et al., 2021).

Human skeletal studies have found that individuals with degenerative diseases such as heart disease, nephritis, and diabetes have higher levels of fluctuating asymmetry than those with infectious diseases such as tuberculosis, influenza, and pneumonia (Weisensee, 2013). Additionally, individuals with active cribra orbitalia and porotic hyperostosis, which are porous lesions in the orbital roof and cranial vault that typically signify anemia, exhibited higher levels of fluctuating asymmetry when compared with individuals with healed lesions (O'Donnell & Moes, 2020). Further, in a Thai sample, sub-adults with developmental disorders exhibited higher levels of fluctuating asymmetry than their nonpathological counterparts (Jung & von Cramon-Taubadel, 2018). This study also found that fluctuating asymmetry was not confined to the cranial region where the pathology was present, refuting the idea that modularity contributes to differing fluctuating asymmetry levels between cranial regions.

Overall, fluctuating asymmetry magnitude seems to increase with age in humans (Quinto-Sánchez et al., 2015). In anthropometric measurements, levels of fluctuating asymmetry in males gradually increase until age 9, increase rapidly from 9-13, and then level off or even decrease slightly. In females, levels of fluctuating asymmetry gradually increase until around age 13 and then level off after that (Palestis & Trivers, 2016). Higher levels of fluctuating asymmetry in the hard palate were found in early life (Oxilia et al., 2021), and anthropometric measurements show that higher growth rates early in the developmental window seem to be associated with higher levels of fluctuating asymmetry later in life (Wells et al., 2006). However, studies have found that individual bones in the hand exhibit more fluctuating asymmetry than the total finger length in an individual (Livshits & Kobyliansky, 1989), and there does not appear

to be any correlation between fluctuating asymmetry in various human skeletal regions (Eriksen, 2020). These findings indicate that variation in fluctuating asymmetry in the skeleton is relatively unknown and researchers should be careful when deciding which regions to measure. Further, no relationship has been found to date between fluctuating asymmetry and linear enamel hypoplasias in any species (Eriksen, 2020; Martin, 2013), suggesting that the period of development when enamel is laid down is not necessarily the same window in which stressors are particularly important for developmental instability.

Micro-environmental factors cannot be discounted in the measure of fluctuating asymmetry and developmental instability. Fluctuations in developmental processes are guaranteed to be frequent at the molecular and cellular levels in complex organisms based on the sheer amount of production (Graham et al., 2010; Klingenberg, 2015; Willmore et al., 2007). This randomness in areas such as chemical gradients, transcription, translation, and cell division is termed stochasticity (Harmansa & Lecuit, 2021; Huh & Paulsson, 2011; Losick & Desplan, 2008; Raj & van Oudenaarden, 2008). These fluctuations may or may not occur in the same number or same way on both the right and left side of any bilateral organism, resulting in random asymmetry. The nonlinearity of developmental processes and nonlinearity of feedback occurring between body parts both contribute to and dampen fluctuating asymmetry at an organism-wide scale (Emlen et al., 1993; Graham et al., 1993). In an estimate of the amount of random developmental variation using human twins, Graham (2021) found that between 5 and 26% of the variance in digit length and ear width can be attributed to a stochastic component of variance. This finding suggests that up to one quarter of the variance in a trait could be attributable to stochastic processes at the molecular and cellular level.

While environmental factors have certainly been linked to levels of fluctuating asymmetry, the relationship is tenuous at best. Especially in primates, there are just a few studies that have extensively studied the relationship between fluctuating asymmetry and known environmental factors or the changes that might occur over a lifespan. No study has examined

how fluctuating asymmetry changes temporally from generation to generation because the populations needed for these types of studies are few and far between.

Genetically-related Fluctuating Asymmetry

Fluctuating asymmetry has been found to increase under a few different genetic conditions, and researchers have also pointed to the genetic origin of developmental instability and fluctuating asymmetry. Heterozygosity has been shown to be a major influence on developmental instability and fluctuating asymmetry in two ways. First, heterozygosity of alleles from within-species crosses is associated with decreased fluctuating asymmetry (Hutchison & Cheverud, 1995; Lacy & Alaks, 2012; Leamy, 1984; Quinto-Sánchez et al., 2015; Zachos et al., 2007). This is often attributed to “heterozygote advantage” or overdominance theory, where heterozygous allele combinations are thought to convey a fitness advantage because a diverse genetic background allows broader resistance to a wide variety of perturbations (Mitton & Grant, 1984; Parsons, 1990; Wright et al., 2007). Second, heterozygosity due to species hybridization, or outbreeding, is associated with increases in fluctuating asymmetry (Alibert & Auffray, 2003; Graham, 1992; Schneider et al., 2003). This is attributed to the disruption of genetic coadaptations between and within loci that cause fitness decreases (Clarke, 1993). Inbreeding or inbreeding depression, on the other hand, has also been associated with increases in fluctuating asymmetry (Gomendio et al., 2000; Loy et al., 2021; Ludoški et al., 2014; McGrath et al., 2022). The overexpression of deleterious alleles in homozygotes causes decreases in fitness (Keller & Waller, 2002; Parsons, 1990; Wright et al., 2007).

While some loci linked to fluctuating asymmetry or developmental instability have been proposed (Klingenberg et al., 2001; Leamy et al., 2005, 2015; Miller et al., 2014), most researchers agree that there is no gene for developmental stability (Green et al., 2017; Klingenberg, 2015; Leamy & Klingenberg, 2005; Willmore et al., 2007), and neither is there control of developmental stability at a trait-specific, genetic level (Windhager et al., 2014).

Rather, developmental stability, or lack thereof, is likely a property of the developmental system as a whole. There are no loci that increase or decrease the level of fluctuating asymmetry, but the developmental system is complex and nonlinear, which allows perturbations to be buffered but also amplified depending on their entry point. Moreover, the heritability of fluctuating asymmetry has been shown to consistently quite low when quantified with linear measurements (Fuller & Houle, 2003), but further investigation is needed with methods that capture 3D information about symmetry.

Heritability and Evolvability

Interindividual differences in an observed trait result from the conditions in which they develop and the underlying genome that produces them. The degree to which genetic factors contribute to fluctuating asymmetry remains largely ambiguous, though the consensus is that there is no organism-wide gene for developmental stability, developmental instability, or fluctuating asymmetry (Leamy & Klingenberg, 2005). Heritability estimates the amount of phenotypic variation in a sample that can be explained by its genetic structure (Falconer & Mackay, 1996; Vitzthum, 2003). Total phenotypic variation is made up of genetic variance (σ_G^2), the additive, dominance, and epistatic effects, and environmental variance (σ_E^2) such as diet, behavior, temperature (Falconer & Mackay, 1996; Hardin, 2019). Further, heritability estimates can be divided into broad sense heritability (H^2) and narrow sense heritability (h^2). Broad sense heritability (H^2) estimates the proportion of phenotypic variance that is due to the overall genetic variance in the sample, including dominance and epistatic variance ($H^2 = \sigma_G^2 / \sigma_P^2$). Because dominance and epistatic contributions are inconsistent from generation to generation, narrow sense heritability (h^2) is widely used in evolutionary quantitative genetics and includes only the additive genetic variance (σ_A^2) rather than the total genetic variance ($h^2 = \sigma_A^2 / \sigma_P^2$). Heritability estimates range from 0 to 1 because this estimate is the slope of a regression of phenotypic

values of offspring on the phenotypic values of parents. Heritability estimates of 0 represent either little additive genetic variation or a lot of phenotypic variation and 1 represent a lot of additive genetic variation or very little phenotypic variation. In general, heritability estimates for traits fall between 0.2 and 0.6 and represent the effects of multiple loci seeing as continuous traits are typically polygenic (Arnold, 2023).

Additive genetic variance is the variation in a population due to the known effect of particular alleles (Falconer & Mackay, 1996). For example, allele **A** adds ½ meter to plant height potential and allele **a** adds ¼ meter to plant height potential. Plants that are homozygous dominant (**AA**) will then have the potential to be 1 meter tall, plants that are heterozygous (**Aa**) will have the potential to be ¾ of a meter tall, and plants that are homozygous recessive will have the potential to be ½ of a meter tall. The height effect of each of these alleles is known and their effects are additive. In quantitative genetic studies, additive genetic variance can be theoretically approximated from pedigree data (i.e., relatedness coefficients). Heritability estimates range from 0 to 1, where 0 represents either little additive genetic variation or a lot of phenotypic variation and 1 represents a lot of additive genetic variation or very little phenotypic variation.

Most estimates of the narrow sense heritability of fluctuating asymmetry are low and nonsignificant (Fuller & Houle, 2003; Leamy, 1997; Leamy & Klingenberg, 2005). Across a variety of traits and a variety of animals, the average h^2 of FA was found to be only about 0.026 (Fuller & Houle, 2003). Other meta-analyses since then have found similar results (Van Dongen, 2000). Many studies of the heritability of fluctuating asymmetry recognize that this is an imperfect measure of developmental instability and stability, so researchers have developed methods for estimating the heritability of developmental instability as well (Whitlock, 1996). To estimate the heritability of developmental stability, the h^2 of fluctuating asymmetry is divided by the repeatability of the fluctuating asymmetry measure (Whitlock, 1996, 1998). The heritability of

developmental instability is generally higher than the heritability of fluctuating asymmetry (Carter & Houle, 2011; Fuller & Houle, 2003; Whitlock, 1996).

The heritability of fluctuating asymmetry and developmental instability is important for the evolutionary implications of these traits. In general, natural selection is likely reducing fluctuating asymmetry in a population, which then reduces developmental instability (Carter & Houle, 2011). The ability to reduce developmental instability may still exist because the costs of developmental precision are countered by the selection for precision, which could be due to the susceptibility of epigenetic systems to external influences. High sensitivity allows for imprecision in development, while low sensitivity makes developmental regulation difficult (Carter & Houle, 2011). Additionally, epistatic interactions may be important in the evolution of fluctuating asymmetry. While a population might respond to an environmental stress with high fluctuating asymmetry initially, epistatic interactions can develop that reduce developmental instability over time (Cheverud & Routman, 1996; Leamy & Klingenberg, 2005; McKenzie, 1997). Nevertheless, some researchers suggest that heritability is not the appropriate measure for evolutionary potential in natural populations because the selection differential (i.e., natural selection in wild populations) cannot be controlled by investigators like in artificial selection experiments (Hansen et al., 2011).

Evolvability is a measure proposed to scale additive genetic variation (σ_A^2) by the trait mean ($\bar{X}$) rather than the phenotypic variance (σ_P^2) in a population (Houle, 1992). Evolvability (I_A), therefore, can be calculated as the additive genetic variation (σ_A^2) divided by the trait mean ($I_A = \sigma_A^2 / \bar{X}$). This measure is thought to be a better estimate of a trait's potential for evolution than heritability, and it has the advantage of being comparable across populations (Hansen et al., 2011; Hardin, 2019; Houle, 1992). An example of the difference between heritability and evolvability is found in life history traits and morphological traits (Mousseau & Roff, 1987; Roff & Mousseau, 1987). Heritability estimates for life history traits are quite low when compared with

those for morphological traits, but it is hard to imagine that life history traits have less potential for evolutionary change than morphological traits. The evolvability of life history traits, however, is much higher than for morphological traits. These differences can be attributed to inclusion of environmental variance in total phenotypic variance for heritability estimates, which is not included in measures of evolvability (Hansen et al., 2011; Hardin, 2019).

Cayo Santiago Rhesus Macaques

This section provides a general overview of the research colony of rhesus macaques established on Cayo Santiago in Puerto Rico with a focus on skeletal growth and development. For a thorough review of the *Macaca mulatta* species, see Cooper et al. (2022).

Cayo Santiago Research Colony

The Cayo Santiago research colony started with the introduction of 409 founding rhesus macaques (*Macaca mulatta*) on the island of Cayo Santiago in December 1938 (Dunbar, 2012; Kessler & Rawlins, 2016). Cayo Santiago is a 15.5 ha island about 1 km off the coast of Punta Santiago, which is located on the eastern side of the island of Puerto Rico in the Caribbean Sea (Dunbar, 2012). The rhesus macaques introduced on Cayo Santiago were trapped in the 12 districts around Lucknow, India (Kessler & Rawlins, 2016). This region is in the northern part of India, east of New Delhi and south of the Nepalese border. These trapped macaques were brought to Cayo Santiago from India by boat. Approximately 7-14 gibbons (*Hylobates sp.*) from eastern Asia were also introduced on Cayo Santiago at this time, but they were removed by 1941 because they attacked human observers and the first gibbon infant born on the island was killed by a rhesus macaque (Dunbar, 2012; Kessler & Rawlins, 2016). Additionally, three pig-tailed macaques (*Macaca nemestrina*) were introduced on the island, but the last of these was gone from the island by 1956 (Dunbar, 2012).

The rhesus macaques on Cayo Santiago are free-ranging with no human intervention save provisioning with fresh water and monkey chow daily (Dunbar, 2012; Kessler & Rawlins, 2016). Historically, the animals have been fed in one of two ways: monkey chow is distributed within a feeding/trapping corral or monkey chow is spread around at various sites on the island. Each animal is tattooed and given ear notches for identification, and vaccination for particular diseases is commonplace (Kessler & Rawlins, 2016). Between 1941 and 1944, all but 200 rhesus macaques were removed from Cayo Santiago to keep the colony afloat financially and help with the war effort, a significant population decrease (Dunbar, 2012; Kessler & Rawlins, 2016). Almost all the macaques on Cayo Santiago today are descendants of 15 females alive in 1956 (McMillan & Duggleby, 1981). In 1971, a researcher named Donald Sade started the systematic collection and maceration of the deceased rhesus macaques found on Cayo Santiago for a skeletal collection under the Caribbean Primate Research Center. Since its inception, periodic culling of individuals from Cayo Santiago has occurred to aid in biomedical research and maintain a sustainable population size for the island (Kessler & Rawlins, 2016).

Since the start of the colony in 1938, three hurricanes have had significant impact on the island of Cayo Santiago: Hugo (1989), Georges (1998), and Maria (2017) (Historical Hurricane Tracks, 2022). Four tropical storms have crossed the island as well (Frederic in 1979, Gert in 1981, Klaus in 1984, and Irene in 2011). No individuals appear to have died during these hurricanes, though the devastation to the island has had significant impact on the behavior and biology of the macaques (Kessler & Rawlins, 2016; Morcillo et al., 2020; Testard et al., 2021; Watowich et al., 2022).

Rhesus Macaque Life History

Rhesus macaques are cercopithecid primates native to Asia that easily survive in most habitats (Maestripieri & Hoffman, 2012). They live in linearly hierarchical social groups with a few adult males and many adult females with their offspring consisting of multiple matrilines.

Female siblings have dominance ranks reverse of their age, so the youngest sister is the highest ranking. Though the youngest female sibling is initially subordinate to the older sisters, they will outrank them by the pubertal stage with the help of the mother. Males disperse from their social group and must re-establish their dominance rank at puberty due to increased aggression from other adult males and adult females, while females remain in their natal group and maintain their dominance rank for their entire life. Therefore, females within a social group are typically related, and males are not related to other adults in the group. In rhesus macaques generally, males spend time alone or in small all-male groups before joining a larger social group and may leave and join yet another group later (Maestripieri & Hoffman, 2012).

Estrus in rhesus macaques typically lasts 5-10 days, gestation lasts 5.5 months, and females give birth, at most, once a year (Maestripieri & Hoffman, 2012). The birthing season is approximately November to March on Cayo Santiago, when 80% of births occur (Hoffman & Maestripieri, 2012; Rawlins & Kessler, 1985). This period coincides with the onset of the spring rainy season, and, therefore, has shifted over time as the climate has changed. When an infant is born, mothers resume normal menstruation, mating, and conception after 6 months (Maestripieri & Hoffman, 2012). Infant mortality is high, but if an infant survives the first year mortality drops considerably. Infants start eating solid food within the first few months of life and are weaned by the end of their first year. Females typically reach puberty around 3-4 years old, and males reach puberty 6-12 months after that. Sexual maturity occurs between 2.5-3 years in females and 4.5-7 years in males (Nowak & Walker, 1999), and age of first reproduction for females is between 3-6 years with a mean of 4.27 years (Blomquist, 2012). The age at first reproduction is lower in higher ranking females and increases as rank decreases. In other words, lower ranking females have their first offspring later than higher ranking females (Blomquist, 2012).

Adult body size in rhesus macaques is reached between 5-6 years old (Maestripieri & Hoffman, 2012), and male rhesus macaques have a larger body size than females at all ages

(Turcotte et al., 2022). Male body size reaches a maximum at 9 years old and female body size is at a maximum at 12 years old. Sexual dimorphism peaks in body size when males are 6-12 years old and females are 6-17 years, where males are 1.5 times bigger than females. This body size sexual dimorphism is achieved through bimaturism and faster growth rates in males (Turcotte et al., 2022). The rhesus macaque maximum lifespan is about 35-40 years of age in captivity, though free-ranging and wild populations do not live that long (Maestripieri & Hoffman, 2012). The oldest intact male macaque in the Cayo Santiago skeletal collection was 29 years old at the time of death, and the oldest female was 31 years old (personal observation). Males have higher mortality rates than females at all ages, but males die more frequently during mating season and females die more frequently during birthing season (Higham & Maestripieri, 2014; Maestripieri & Hoffman, 2012).

While a large portion of the rhesus macaque life history literature is a result of studies conducted on Cayo Santiago, these macaques differ in a few marked ways from other rhesus macaque populations. Three main differences exist between the Cayo Santiago rhesus macaque colony and other populations: food provisioning, absence of predators, and restricted home range (Maestripieri & Hoffman, 2012). Food provisioning on the island could result in less affiliation among males due to increased competition over food resources. The lack of predators reduces mortality risk generally, but especially among senescent males who typically spend more and more time alone as they age. Further, the limited opportunity for male dispersal due to restrictions on home range size mean that social groups tend to have some males who stay in their natal group or return to their natal group later in life, which influences the dominance hierarchy because their rank status is maintained and results in more males per social group than is typical in wild populations (Maestripieri & Hoffman, 2012).

Rhesus Macaque Skeletal Growth

Crania

The anterior aspect of the cranium increases in size more than the posterior aspect of the cranium throughout growth in rhesus macaques (Wang et al., 2007). The neurocranium and basicranium cease growth at eight years of age in both males and females, and the facial skeleton ceases growth at this age in males as well. In females, the facial skeleton does not cease growth until 15 years of age. In both males and females, the facial skeleton grows faster than the posterior cranial skeleton. In males, years 1-4 show accelerated facial growth, years 2-5 show the most intensive growth, and then growth decelerates in years 4-8 (Schneiderman, 1992; Wang et al., 2007). In females, years 1-2 show very intensive facial growth, years 2-3 decelerate, years 3-5 show very intensive growth again, years 4-8 decelerate, and then years 8-15 exhibit consistent growth.

In infancy, male rhesus macaques exhibit slower growth rates than females, but male growth rates are higher than females in the pre-adult and young adult stages (Wang et al., 2007). After the young adult stage, growth ceases in males but continues until 15 years of age in females. At ages 1-2, females are slightly larger than males, but by ages 2-3 the adult sexual size dimorphism pattern is present. From ages 2-8, sexual size dimorphism increases substantially and consistently and peaks at age 8. After age 8, sexual size dimorphism decreases, especially in the face, and results in a less than 10% difference in male and female craniofacial size on average. Generally, the palate and face are 20% larger in males than females, while the neuro- and basicranium are only 10% larger. Further, it is important to note that male and female macaques are not scaled versions of one another; there are significant shape changes between sexes as well (Simons & Frost, 2016). Overall, males tend to exhibit faster growth rates, but females exhibit longer growth of the craniofacial skeleton. Though males are generally larger than females post-infancy, females do not cease growth until significantly later. This “negative bimaturism” reduces sexual size dimorphism but does not counteract the effect of a faster growth rate in males at younger ages (Wang et al., 2007). This is in direct contrast with analyses of body size data that show that bimaturism and faster growth rates are

responsible for body size sexual dimorphism in rhesus macaques (Turcotte et al., 2022). Wang et al. (2007) suggest that this long growth period may be related to the ecological risk associated with intrasexual competition in macaque social structure and reproductive behavior, which are like chimpanzees that also exhibit longer female growth periods.

Sutural fusion in the cranium is patterned by sex and region in the rhesus macaque (Wang et al., 2006). Males exhibit more fusion in cranial sutures than females across cranial regions, and sutures fuse in males at earlier ages than in females. This is most pronounced in the facial sutures, where facial growth and sutural fusion are greater in males than females. This has led researchers to employ functional explanations for the differences seen in sutural fusion in the face such as the increased biomechanical strain of having greater muscle and bite forces that can cause sutural failure at higher rates in patent, or open, sutures. The fusion of facial sutures in males despite the greater growth could maintain the structural integrity of the facial skeleton. In general, the dentofacial complex matures faster in females than males before five years of age, but after five years, males have a more mature dentofacial complex (Wang, 2012). Facial skeletal maturity in male macaques occurs at about 8 years of age, while females mature around 15 years old. The face is the last region of the cranium to exhibit sutural fusion, whereas the neurocranium is first, and then the basicranium and palate (Wang et al., 2006). This regional pattern is the same in male and female macaques. Males reach 50% sutural fusion in the neurocranium around 7 years old, basicranium around 10 years old, palate around 11 years old, and face around 16 years old. Females reach 50% sutural fusion in the neurocranium around 9 years old, basicranium around 16 years old, palate around 19 years old, and never in the face. It is rare for any individual to have full sutural fusion in the Cayo Santiago rhesus macaques, though non-zero in males.

Post-crania

Epiphyseal fusion in the Cayo Santiago macaques follows a similar pattern in males and females, though fusion timing is different (Cheverud, 1981). At the elbow, knee, and shoulder joints, females exhibit epiphyseal fusion about one year earlier than males. At most other joints, epiphyseal fusion is about four to six months earlier in females, and males fall increasingly further behind in epiphyseal fusion as age increases. Broadly, epiphyseal fusion occurs at a similar time in each joint and follows the general primate condition: elbow, hip, ankle, knee, wrist, shoulder (Brimacombe, 2017; Cheverud, 1981; Washburn, 1943). In the upper limb, the elbow fuses first, and then the wrist and then shoulder, while the lower limb has a hip-ankle-knee sequence (Brimacombe, 2017). A notable exception to this trend is the distal humerus, which fuses earlier than any other part of the elbow joint (Cheverud, 1981).

Rhesus Macaque Dental Eruption and Dental Health

In the Cayo Santiago macaques, dental eruption sequence and timing is meticulously documented (Cheverud, 1981; Wang et al., 2016). Typically, deciduous incisors appear between three days to one month after birth, and all deciduous dentition is erupted by the end of the first year of life. In early life, tooth eruption in males is a bit ahead of that in females. By age two, M1 (1st molar) is erupted, all permanent incisors are erupted by age three, and then sex-based differences in eruption timing start to become more pronounced. Female premolars tend to emerge before male premolars. By age four, M2 (2nd molar) is erupted, and all females have P3 (3rd premolar) and P4 (4th premolar) though only some males have premolars at this age. At this same stage, the lower C (canine) is emerging before the upper C in males. By age 5, P3 and P4 and the female Cs are erupted while some males exhibit emergence of M3. By age 6, the permanent dentition has erupted in most males, but most females still lack M3. By age 7, the permanent dentition has erupted in males while M3 is still emerging in females. By age 8, males have all permanent dentition but only two-thirds of females do. By age 9, all permanent dentition is in place in both sexes (Cheverud, 1981; Wang et al., 2016).

In all, M3 generally erupts earlier in males than females, but P3, P4, and C erupt later in males than females (Wang et al., 2016). In terms of dental maturity, males reach material maturity, where all teeth are functionally erupted and signifies the end of dental ontogeny, before females (Wang, 2012). Functional maturity, where all premolars and the first and second molars are erupted, occurs in females before males, and canine maturity, where canines are fully erupted, follows this same trend (Wang, 2012). The median age of M3 occlusion is 6.5 years old in females and 6 years old in males (Wang et al., 2007). This differs from cranial skeletal maturity, which is reached at 8 years in males and 15 years in females, though sexual maturity is achieved between 4.5 and 7 years in males and 2 and 3 years in females (Nowak & Walker, 1999; Wang et al., 2007).

The Cayo Santiago macaques exhibit good oral and dental health (Wang, 2016). Females tend to have better dental health than males with only 3.13% of females but 11.2% of males exhibiting some sort of dental pathology or abnormality. Only a small percentage of individuals exhibit caries, and broken or missing teeth only occurred at high rates in adult males. On a scale of slight (1) to extreme (5), tooth wear is light to moderate in younger animals but mild to severe in aged animals.

Southwest National Primate Research Center Baboons

Southwest National Primate Research Center Research Colony

The Southwest National Primate Research Center (SNPRC), which is hosted by the Texas Biomedical Research Institute, is located in San Antonio, Texas and houses captive colonies of baboons, chimpanzees, marmosets, and rhesus macaques (Southwest National Primate Research Center, 2022a). Baboons were the first primate to be imported to SNPRC from Darajani, Kenya in 1960 (VandeBerg, 2009). The founders of this population are mostly of the subspecies *Papio hamadryas anubis* (olive baboon) with some *Papio hamadryas cynocephalus* (yellow baboons) (VandeBerg, 2009). It is important to note that as of 2009,

common baboons were a polytypic species assigned to *Papio hamadryas* with five subspecies, though these subspecies are recognized as separate species in Mittermeier et al. (2013). Today, there are over 1000 baboons at SNPRC (Southwest National Primate Research Center, 2022b). Most baboon species are represented at SNPRC today, and many individuals in this research colony are hybrids of multiple species (Kenneth A Sayers, personal communication, January 24, 2022).

The baboons at SNPRC are housed in groups except when specific protocols require paired or single housing. Feeding has occurred in a number of ways over the years, but the baboons are currently fed 5LEO brand monkey chow that is supplemented with foods for foraging on weekdays (fruits, vegetables, nuts, seeds, etc.; Kenneth A Sayers, personal communication, January 24, 2022). Breeding management has occurred in two controlled forms: targeted, single-male, multi-female groups and multi-male, multi-female groups. Corral breeding was discontinued in 2005. The female to male sex ratio at SNPRC is 2:1 (Hlusko, 2006). From the 1980s-1990s, canine teeth were blunted in male baboons, and after that period, male canines have been filed on the tips and lingual side of the tooth (Kenneth A Sayers, personal communication, May 3, 2022; Sharon Price, personal communication, May 3, 2022). Further, canines can be broken during conflict, and veterinarians may shorten or remove the canines if deemed necessary (Sharon Price, personal communication, May 3, 2022).

Baboon Life History

Baboons are cercopithecid primates native to eastern Africa, and olive and yellow baboons live in hierarchical, matrilineal, multi-male, multi-female groups (Brent, 2009). Baboons exhibit female dispersal from the natal unit in the wild (Honoré & Tardif, 2009). Therefore, females in any given unit tend to have low degrees of relatedness. Further, female baboons tend to mate exclusively with a single male but may mate with multiple males. Higher ranking males mate more frequently but for a short period of time while lower ranking males mate less

frequently but over a longer period. If spatial constraints are not present, such as in wild populations, male baboons do not increase aggression in the presence of a receptive female. Female baboons exhibit the most aggression during the birthing season. Captive olive baboons exhibit more hierarchical groups than wild baboons (Brent, 2009). Additionally, captive conditions lead to more tension, aggression, and behavioral disturbance in olive baboons when compared with their wild counterparts. Research has shown that enrichment significantly reduces abnormal behaviors in baboons, so this tactic is employed by most research colony managers.

The menstrual cycle of a baboon is around 33 days, and estrus lasts about 11-13 days during which time the perineal sex skin remains turgescent (Honoré & Tardif, 2009; VandeBerg, 2009). Menarche is reported to be at about 4-5.5 years of age in wild *Papio hamadryas anubis* but 3-4 in the captive baboons at SNPRC, and females typically conceive about a year after menarche (Honoré & Tardif, 2009). Gestation is typically about 175 days, or approximately 6 months. Wild baboons do not exhibit breeding seasonality, but there is a relationship between conception and resource availability where conception is most frequent at the end of the rainy season. Birth rates, then, are most frequent during the dry season. The more concentrated and synchronous a birthing season, the more female-female aggression, which can even lead to infanticide of lower ranking offspring. Lower ranking females birth less offspring than those at higher ranks due to reproductive suppression, longer nursing periods that cause fewer reproductive cycles, and lower diet quality. Weaning typically occurs around the start of the next rainy season. The interbirth interval for baboons is longer in the wild than in captivity; 21-24 months in wild baboons but only about 13 months in captive baboons. In both wild and captive baboons, the interbirth interval is 11 months after a stillborn infant or infant that dies soon after birth. Postpartum amenorrhea is significantly shorter in captive baboons as well: 5.5 months after birth compared to 14 in the wild. Puberty occurs around 3.5 years of age in male and female baboons, but males do not usually reproduce until about 5-6 years of age (VandeBerg,

2009). Male and female infants grow at approximately the same rate until about 2.5 years of age, and then male growth rates increase significantly until around 6-8 years of age. The age at first reproduction is between 3.85 and 13.11 years with a mean of 6.32 years of age (Williams-Blangero & Blangero, 1995). In captivity, baboons live around 20-30 years, and females begin to experience irregular menstrual cycles around 18-19 years of age (VandeBerg, 2009). Female baboons cease to cycle by age 26 but can live years longer, meaning that baboons exhibit menopause (Honoré & Tardif, 2009).

Body mass size dimorphism in baboons is quite low from about 1-4 years of age (Leigh, 2009). Females tend to be slightly larger in torso and limb measurements than males up to about 1 year, after which high male growth rates cause increases in size dimorphism. In general, males exhibit longer growth periods than females for all aspects of growth. At birth, males weigh 3.6% of adult size and females weigh 5.8% of adult size. Males have a general increase in growth rate until before 3 years of age when there is a large increase in growth rate, while females have a constant rate of growth for the first few years of life with a peak in growth rate at age 4. The male growth spurt starts around 2-2.5 years of age and peaks around 5 years when a drop in growth rate occurs and becomes trivial around 8-9 years of age. Variance in body mass increases with age in both male and female baboons. This increase in variance occurs largely around age 3 in males but increases consistently in females until about age 12. Male body mass growth ceases in males around 8 years and females around 5-6 years. Generally, body mass grows for a longer period than the skeleton (Leigh, 2009).

Baboon Skeletal Growth

Crania

There are not many changes in head length (measured as nasion to inion) postnatally in baboons, with length about 67% of the adult size in males at birth and 76% in females (Leigh, 2009). The neurocranium exhibits the highest growth rate in the first year of life but ceases

growth soon after. Upper facial height (measured as prosthion to nasion) exhibits a different growth pattern than the broader head length. The upper facial height is smaller in males than females at birth (28% of adult size and 33% of adult size, respectively). Female facial growth exceeds male facial growth in early life with a growth spurt around 2 years of age (Leigh, 2009), and an earlier report suggests that female craniofacial growth slows around 3 years of age (Leigh & Cheverud, 1991). The male facial growth spurt starts around 3 years of age and peaks in velocity with the eruption of the canine teeth. A previous report suggests that this increase in growth happens around 4.5 years, but the sample is limited (Leigh & Cheverud, 1991). Female facial height increases until 5 years of age, while male facial height increases until 7 years of age (Leigh, 2009). The face of male and female baboons largely follows the same ontogenetic trajectory, but at different scales (Leigh & Cheverud, 1991). In the cranium, it appears to be a longer duration of growth, rather than increased growth rates, that causes the extreme size dimorphism seen in baboons (Leigh & Cheverud, 1991), though cranial sutures are reported to never completely close in either sex (Zuckerman, 1926). When compared to rhesus macaques, baboons grow faster for shorter periods of time, which could indicate a highly integrated developmental pattern (Leigh, 2009).

Post-crania

Baboon skeletal dimensions triple in size postnatally (Leigh, 2009). As found in the cranium, female skeletal growth ceases around 5 years and male skeletal growth ceases around 7 years. The fusion pattern of baboons follows that of other cercopithecid primates: elbow, hip, hand and foot, ankle, wrist, knee, and shoulder (Bramblett, 1969). Typically, growth in the arms stops around 5-6.5 years of age in both male and female baboons, though other elements exhibit differences by sex (Leigh, 2009). The hands cease growth relatively early, 4 years old in females and 6 years old in males. The foot follows this same trend of early cessation and does not differ by sex. The femur ceases growth around 5 years old in females

and 6.5 years old in males. Male growth rate falls behind female growth rate in the first years, but then speeds up after this period. Growth spurts are largely non-existent in post-cranial skeletal elements, though males might exhibit growth spurts in some post-cranial skeletal elements (crown-rump length) while females do not. Further, growth seems to be more synchronized in skeletal dimensions – between the axial and appendicular sections – in baboons than in macaques (Leigh, 2009).

Baboon Dental Eruption and Dental Health

The rate of tooth eruption in baboons is about twice that of humans (Hlusko & Mahaney, 2009), and there does not seem to be any dimorphism in size or wear pattern (Leigh, 2009). Baboon deciduous teeth erupt entirely within the first year of life, potentially earlier in males than in females, and females tend to lose their deciduous teeth before 5 years of age while males may keep them past this time (Leigh, 2009). Adult teeth typically start erupting around age 4 (Leigh, 2009). Canine teeth are typically trimmed in the SNPRC colony, so adult canine height and thereby full canine eruption, is difficult to determine. Dental eruption in captive populations is shown to occur 1-1.5 years before eruption in wild populations (Hlusko & Mahaney, 2009; Kahumbu & Eley, 1991). For both wild and captive populations, the permanent first molar erupts between 5.5 and 7 years of age, the central incisor between 6 and 7 years, the lateral incisors between 7 and 8 years, the canines between 9.5 and 11.5 years, the third premolars between 10 and 11.5 years, the fourth premolars between 11.5 and 12 years, and the second molar between 11 and 12.5 years. Fourth molars are present in a handful of individuals in many baboon samples, including those at SNPRC, and some suggest that this may extend the dental life of the individual (Bramblett, 1969). The supernumerary molars may exist due to the cleavage of a developing tooth bud or the replication of some genetic component of the tooth (Bramblett, 1969). Data on dental health has not been reported in the SNPRC colony.

Summary

This dissertation aims to tease apart some of the environmental and genetic factors contributing to the development and perpetuation of FA in two primate species using a 3D geometric morphometric approach to quantifying FA. We examine FA in rhesus macaques and olive baboons and investigate its relationship with natural disaster experience, pathology, and various demographic factors. This work provides a unique look into the impact of hurricanes on stress and development in primates species and investigates some factors that have never been included in previous studies of FA (pathology). Results of this work can highlight the vulnerability of particular age groups to stress, examine the influence of biomechanical factors in the development of FA, and help us understand the primate physiological response to natural disasters that are more and more frequent with increased climate change.

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Chapter 2: Skeletal age during hurricane impacts fluctuating asymmetry in Cayo Santiago rhesus macaques

Ashly N. Romero[1], Edwin Dickinson[2], Cassandra M. Turcotte[2], Claire E. Terhune[1]

[1]Department of Anthropology, University of Arkansas, [2]College of Osteopathic Medicine, New York Institute of Technology

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Abstract:

As natural disasters become more frequent with climate change, understanding the biological impact of these ecological catastrophes on wild populations becomes increasingly pertinent. Fluctuating asymmetry (FA), or random deviations from bilateral symmetry, is reflective of developmental instability and has long been positively associated with increases in environmental stress. This study investigates craniofacial FA in a population of free-ranging rhesus macaques (*Macaca mulatta*) that has experienced multiple Category 3 hurricanes since the colony's inception on Cayo Santiago, including 275 individuals from ages 9 months to 31 years (F=154; M=121). Using geometric morphometrics to quantify FA and a linear mixed-effect model for analysis, we found that sex, age, and decade of birth did not influence the amount of FA in the individuals included in the study, but the developmental stage at which individuals experienced these catastrophic events greatly impacted the amount of FA exhibited (p=0.001). Individuals that experienced these hurricanes during fetal life exhibited greater FA than any other post-natal developmental period. These results indicate that natural disasters can be associated with developmental disruption that results in long-term effects if occurring during the pre-natal period, possibly due to increases in maternal stress-related hormones.

Introduction

Fluctuating asymmetry (FA) – defined as random deviations from symmetry in traits that are otherwise bilaterally symmetrical – has been repeatedly demonstrated to reflect a morphological proxy for the frequency and/or magnitude of stress events experienced by an individual (Badyaev et al., 2000; Lens et al., 1999; Polak, 2003; Sherman et al., 2009; Weller & Ganzhorn 2004). As bilateral traits share a common genome (Klingenberg, 2015; Polak, 2003), the presence of FA is a manifestation of developmental instabilities that disrupt typical developmental patterns, resulting in the phenomenon of asymmetry (Møller, 1991; Palmer & Strobeck, 1986; Waddington, 1957). Though literature documenting FA is vast and variable, our understanding of how demographic factors such as age and sex influence FA – as well as when individuals may be most susceptible to developmental disruptions – remains ambiguous, particularly within the context of broad-scale ecological catastrophes. This study investigates FA in a cross-sectional, ontogenetic sample of free-ranging rhesus macaques (*Macaca mulatta*) on the island of Cayo Santiago. The macaques in this sample span multiple generations and include individuals that experienced one or more Category 3 hurricanes during their lifetime on Cayo Santiago. We examine how FA changes across ontogeny, with demographic factors such as age and decade of birth and examine the impact of experiencing such a major natural disaster on FA levels.

Both anthropogenic and natural disruptions have been shown to impose stress on individuals, leading to the use of FA as an indicator of environmental stress levels (Clarke, 1992; Manning & Chamberlain, 1994; Soderman et al., 2007). For example, levels of FA in the mandibles of immature common shrews (*Sorex cinereus*) are significantly greater in populations subjected to environmental disturbance via industrial logging activity (Badyaev et al., 2000). Higher levels of FA were further associated with decreases in general fitness, measured via each individual's body mass (Badyaev et al., 2000). Similarly, habitat disturbance has been inferred to drive temporal increases in FA between historical and modern populations of

endangered bird species, with levels of asymmetry reaching a sevenfold increase in highly degraded (i.e., deforested) localities (Lens et al., 1999). Further, young mice in deforested environments with higher food scarcity exhibit higher levels of FA than adults (Díaz & Morán-López, 2023). In addition to anthropogenic destruction, environmental change following a 1999 hurricane in Ohio, USA was shown to increase levels of FA within populations of forest-dwelling deer mice (*Peromyscus maniculatus*; Hopton et al., 2009). Indeed, hurricane and tornado events are well-documented in driving changes in mortality profile, community structure, and fitness in both vertebrates (e.g., Gannon & Willig, 1994; Weidenfeld & Weidenfeld, 1993; Woolbright, 1996) and invertebrates (e.g., Willig & Camilo, 1991) by increasing food scarcity, altering local microclimates, or destratifying habitats through the loss of shrubbery and canopy coverage (Bellingham et al., 1995; Ney-Nifle & Mangel, 2000; Wunderle, 1995).

Other Potential Contributors to Fluctuating Asymmetry

Extrinsic disturbances are not the only mechanisms by which asymmetry is accumulated within the skeleton. As described by Hallgrimsson (1999), magnitudes of FA increase over ontogeny in both humans and nonhuman primates; this phenomenon was ascribed to the additive accumulation of asymmetrical mechanical factors (e.g., stresses placed on bones during locomotion or mastication) and undirected bone remodeling (e.g., drift) throughout an individual's life. While the first of these processes may arguably reflect directional asymmetry (as opposed to fluctuating asymmetry), morphological drift via a linearly increasing quantity of random deviations over time would predict an increase in FA within older individuals (Hallgrimsson, 1999). Bone remodeling is a maintenance process, involving the coordinated action of osteoclasts and osteoblasts to iteratively remove and replace skeletal tissue over time. As osteoblastic and osteoclastic activity are occurring at the same site, the potential for tangible morphological changes to be manifested is minimal. However, during bone modeling – wherein bone deposition may occur independent of, or spatially separated from, bone resorption – the

opportunity for morphological variation to be incurred is increased. As most bone modeling occurs prior to skeletal maturity, it is reasonable to infer that opportunities for FA to manifest via this mechanism are increased within developing individuals. To this end, the impact of developmental instabilities on FA is thought to be magnified during ontogeny, such that the impact of early-life adversity may contribute more strongly to FA than hardship experienced when the skeleton has already been formed (Gluckman & Hanson, 2006; Halgrimsson, 1999). This theory is substantiated by recent work into the human cranium, which highlights a window of vulnerability to developmental instability occurring between 1 and 5.5 years of age, with a uniquely sensitive time between 4 and 5.5 years (Moes et al., 2022).

In addition to age, other demographic variables – most notably sex – have been hypothesized to impact skeletal FA, with varying degrees of support. Measurements of cranial FA in humans and nonhuman primates have largely yielded no sex-specific patterns (e.g., Hallgrimsson, 1993; Hallgrimsson, 1999; Van Dongen, 2015), with two notable exceptions: an increase in osseous nasal FA in male humans from multiple populations as compared to females (Schlager and Rüdell, 2015) and a similar increase in overall cranial FA in male gorillas relative to females (Romero et al., 2022). Beyond primates, sex was not observed to drive differences in FA within either hurricane-affected or control-group deer mice (Hopton et al., 2009), nor in red squirrels occupying either disturbed or undisturbed woodland habitats (Wauters et al, 1996). Sex is similarly reported as a non-significant factor upon FA within South American water rats (Caccavo et al., 2021), long-tailed spiny rats, hairy-tailed akodonts, woolly mouse opossums, or Amazonian red-sided opossums (Castilheiro et al., 2022). Finally, among Italian wall lizards, sex-based differences in FA are observed in femoral pore distribution, but not in head shape (Simbula et al., 2021), and sex-based differences in FA are found in the mandible of common shrews exposed to habitat disturbance (Badyaev et al., 2000).

Study Aims

In this study, we use a free-ranging sample of rhesus macaques (*Macaca mulatta*) from Cayo Santiago, Puerto Rico, to assess three distinct aims: 1) clarify the relationship between FA and age within a model primate taxon; 2) quantify the potential role of other demographic variables – specifically sex and decade of birth – in driving FA; and 3) assess the impact of a catastrophic natural event (namely the landfall of two devastating hurricanes in 1989 and 1998, respectively) upon FA levels in a free-ranging primate population.

Materials and Methods

Sample Composition

Our sample derives from the free-ranging rhesus macaque colony of Cayo Santiago, where a group of 409 rhesus macaques were originally transported to the island in 1938 from point of capture in India (Carpenter, 1971). Over the past century, the population grew to its current level of 1800 individuals. After death, the bodies of all animals are collected, macerated, and stored long-term at the University of Puerto Rico Recinto de Ciencias Médicas.

Cayo Santiago is an 18.2 hectare island off the coast of Puerto Rico, characterized by a tropical environment with no predators. From 1950 to 2012, the island has experienced two named hurricanes: Hurricane Hugo in 1989 and Hurricane Georges in 1998. Both Hugo and Georges were Category 3 hurricanes at landfall. In each case, the island experienced little loss of primate life but suffered significant ecological damage in the form of vegetation and infrastructural loss.

The Caribbean Primate Research Center (CPRC) oversees the health and maintenance of the colony, which is otherwise free-ranging. Once commercial primate diets were produced in the United States, the CPRC began provisioning the macaques with fresh water and monkey chow, and have increased supplementation plans in recent years due to hurricane-related environmental instability (Kessler and Rawlins, 2016).

We analyzed crania from 275 individuals of both sexes (female=154; male=121). This sample is cross-sectional, containing individuals aged from 9 months to 31 years (Table 2.1; Table 2.S1), and represents animals born across six decades (1951-2005). The sample is further subset into animals that did not experience a hurricane (n=174; F=90, M=84) and animals that experienced at least one named hurricane in their lifetime (n=101; F=64, M=37). Of the latter group, 78 animals experienced just one hurricane and 23 animals experienced two. To better understand the effect of adversity on the ontogeny of fluctuating asymmetry, the individuals who experienced a hurricane were further divided into groups on the basis of age at which the hurricane was experienced: fetal (n=10) individuals who experienced the hurricane prenatally; juvenile (n=50) individuals who experienced the hurricane prior to skeletal maturity; and adult (n=41) individuals who were skeletally mature during the hurricane event.

Data Collection and Processing

Crania were 3D-scanned in Puerto Rico using an HDI 120 blue LED scanner (LMI Technologies). After scanning, the 3D surface models were processed in Geomagic Studio (3D Systems) using the “fill holes” and “mesh doctor” functions. After processing, the 3D models were imported into 3D Slicer (Version 4.11.20210226; Fedorov et al. 2012) for landmarking. For better visualization of anatomically-based landmarks on the 3D models (e.g., sutural intersections, foramina), the “display” settings in the “models” module were adjusted to make the “scalars” visible, the “active scalar” RGB, and the “scalar range mode” direct color mapping. This overlays the 3D model with surface images collected during the scanning process. A total of 34 fixed landmarks (13 bilateral landmark pairs, plus 8 midline points) were placed on the cranium using the “fiducial markups” function in the “markups” module of 3D Slicer (Table 2.2; Figure 2.1). These landmark configurations were then exported as .fcsv files, imported into R (R Core Team, 2020) and collated, and then saved as .tps files for analysis in MorphoJ

(Klingenberg, 2011). Landmarks were placed twice on each of the 275 individuals in the sample to include an error effect during data analysis.

Quantification of Fluctuating Asymmetry

A Procrustes superimposition or Procrustes fit was performed on all the landmark configurations in MorphoJ to translate, rotate, and scale the configurations to the same position, orientation, and size using a least squares approach (Dryden & Mardia, 1998; Goodall, 1991; Gower, 1975; Klingenberg, 2015). Then, object symmetry was assessed by reflecting the bilateral landmarks across the midline (Kent & Mardia, 2001; Klingenberg et al., 2002; Mardia et al., 2000). This process calculates the equivalent of the distance between the right and left landmark pairs using the sum of squared distances.

A Procrustes ANOVA (analysis of variance) was then performed in MorphoJ to determine the levels of FA present in each individual's cranium. This analysis includes individuals (specimens) and sides (right/left) as main effects, as well as an interaction term between individual and side (individual*side). The average difference between the right and left sides represents directional asymmetry and the individual-by-side term represents FA (Klingenberg et al., 2002; Klingenberg & McIntyre, 1998; Palmer & Strobeck, 1986). This model also includes the replicate configurations to quantify measurement error and assess error in relation to FA signal. Ideally, measurement error should be low for studies of FA to optimize the signal to noise ratio. Terms were considered statistically significant at α=0.05 or below. The mean squares in the Procrustes ANOVA were used to calculate the percent of variation that each term in the model contributed to overall variation in the sample (Gómez-Robles et al., 2013). The output of the Procrustes ANOVA from MorphoJ includes Procrustes FA scores that were used for further analysis. Procrustes FA scores rather than Mahalanobis FA scores were used because the latter metric requires large sample sizes to reliably estimate the covariance matrix and are difficult to interpret due to their lack of comparability to other measures of shape

variation (Klingenberg & Monteiro, 2005; Klingenberg, 2015). After extracting the Procrustes FA scores for each individual in the dataset, all further analyses were performed in R.

Statistical Analysis

To assess drivers of FA, several iterations of a linear mixed-effect model were constructed using *R* (R Core Team, 2020) with the packages 'lmerTest' (Kuznetsova et al., 2017) and 'lme4' (Bates et al., 2014). To first assess the potential relationship of age and demography to FA (Aims 1 and 2), we constructed a model containing age, sex and decade of birth as fixed effects, while accounting for the potential confounding influence of matriline as a random effect, following Winter (2013) and Bates et al. (2014). This model was run on two subsets of the FA scores: first the full dataset including all individuals (n = 275) and then a second subset of 174 individuals that had never experienced a hurricane to mitigate any potential influence of environmentally-driven FA upon these results. Post-hoc Tukey tests utilizing Bonferroni-Holm correction were subsequently applied using the *R* package 'comptest' (Hothorn et al., 2016). Terms were considered statistically significant at α=0.05 or below for these and all further analyses.

To assess Aim 3, we constructed three separate linear mixed effect models using the previously mentioned packages to investigate how experiencing a hurricane may alter FA. The first model was run on the entire dataset (n = 275) and included age, sex, decade of birth and hurricane yes/no (a Boolean summary of whether an individual had, or had not, experienced a hurricane in its lifetime; Yes=101; No=174) as fixed effects, and matriline as a random effect. The second model sought to investigate whether experiencing multiple hurricanes had an additive effect, and included age, sex, decade of birth and number of hurricanes experienced in an animal's lifetime (0: n=174; 1: n=78; 2: n=23) as fixed effects, and matriline as a random effect. Finally, we explored whether experiencing a hurricane at different periods of ontogeny influenced the development of FA. This model was run only on animals that had experienced a

hurricane (n=101) and included age, sex, decade of birth and age at hurricane (fetal, juvenile, adult) as fixed effects, and matriline as a random effect.

Results

Both directional asymmetry and FA are present in the sample ($p<0.001$ for all; Table 2.3). Most shape variation in the sample comes from variation between individuals (91.06%; Table 2.3). This high level of individual variation can be attributed to variation between the left and right averages of landmark positions for the individuals in the sample (Klingenberg, 2015). The Procrustes FA scores extracted from MorphoJ had a mean of 0.015, median of 0.014, variance of 0.00001727, and a standard error of 0.000251. Distribution of the data can be observed in Figure 2.2, where the frequency of FA scores is shown in a histogram (A) and the distribution of FA scores is illustrated by sex (B) and skeletal maturity (C). While mean FA is not comparable to other studies (because each Procrustes superimposition is unique), the variance and standard error here are slightly lower than those reported for *Macaca fascicularis* in Romero et al. (2022). This could be because the sample size in our study is much larger and thus provides a more accurate reflection of species-level variation.

Fluctuating Asymmetry as a Product of Age or Demography

No significant effect of age at death on FA is observed in either our full dataset ($p = 0.282$) or subset of macaques that did not experience a hurricane ($p=0.203$). Sex ($p = 0.371$ for full sample; $p = 0.399$ in subset model) and decade of birth ($p = 0.339$ in full sample; $p = 0.824$ in subset model) are similarly non-significant throughout. All results from the linear mixed-effect models are reported in Table 2.4.

The Impact of Natural Disasters on the Development of Fluctuating Asymmetry

Modeling hurricane experience as a binary effect, where an animal either did or did not experience an event, has no significant effect on FA ($p=0.429$). Further, no differences are found between individuals that had experienced 0 vs 1 vs 2 hurricanes ($p=0.710$).

However, among animals that had experienced hurricanes, age at hurricane yields a significant effect on FA ($p=0.001$). A post-hoc, Bonferroni-Holm adjusted Tukey's test demonstrated that fetal individuals during a hurricane event exhibit significantly greater FA than those that were either juveniles ($p<0.001$) or adults ($p=0.002$) during the hurricane; however, no differences are observed between individuals that experienced a hurricane as juveniles *vs.* adults ($p=0.534$).

Discussion

In an assessment of the influence of age (Aim 1), sex and decade of birth (Aim 2), and natural disaster experience on FA (Aim 3), our results indicate that age, sex, and decade of birth have no statistical influence on FA in the population of rhesus macaques living on Cayo Santiago. While a binary hurricane experience factor did not appear to influence levels of FA, the developmental period in which an individual experienced a hurricane had a significant impact on FA levels. Specifically, individuals that experienced hurricanes during fetal development exhibit significantly higher levels of FA than those that experienced a hurricane during either the juvenile or adult postnatal periods.

Sex and Fluctuating Asymmetry

These results support earlier findings that sex has little influence on FA in the Cayo Santiago macaque population (Hallgrímsson, 1999), which aligns with many studies on FA across animal clades (Caccavo et al., 2021; Castilheiro et al., 2022; Hallgrimsson, 1993; Hopton et al., 2009; Van Dongen, 2015; Wauters et al, 1996) but does not align with a handful of studies in humans (Schlager & Rüdell, 2015), gorillas (Romero et al., 2022), olive baboons

(Romero et al., unpublished data), lizards (Simbula et al., 2021), and shrews (Badyaev et al., 2000). These studies used a variety of data collection methods (e.g., caliper measurements, 2D photographs, 3D landmark patches) and measured different body components (e.g., mandibles, crania, femoral pores), making consistency impossible and comparisons relatively difficult. It is possible that FA is more prevalent in particular traits, causing the range of results on sex-specific FA. For example, traits that exhibit high levels of sexual dimorphism may also exhibit greater FA. Further, this study accounts for many factors that are not known in most populations (exact age at death, decade of birth, natural disaster experience, matriline, social group, etc.). Any subtle signal for sex-specific FA may be overwhelmed by the inclusion of other, more strongly correlated factors.

Aging and Fluctuating Asymmetry

Unlike the previous study of FA in this population (Hallgrímsson, 1999), our results indicate that there are no age-associated increases in FA in the Cayo Santiago rhesus macaques. This phenomenon was ascribed by Hallgrimsson (1999) to multiple potential factors, including the cumulative effects of asymmetrical mechanical factors such as a side preference in chewing, and a tendency for bone form to drift through undirected remodeling, though chewing side preference has since been shown to be a minor contributor to FA levels (McGrath et al., 2022). Disagreements between this study and our own are potentially attributable to differences in methods or sample composition. To address this first point, it is important to note that the data presented by Hallgrímsson (1999) used linear measurements to quantify FA, as this study predated readily accessible 3D technology for geometric morphometric analyses. Linear measurements include less information than 3D landmarks in terms of position and, therefore, are potentially less accurate in quantifying FA. In terms of sample, meanwhile, this previous study did not include individuals that had experienced both hurricane Hugo and Georges, and further did not account for any potential impact of hurricane experience during

analysis. Finally, from an analytical perspective, the original study did not attempt to account for inter-relatedness of individuals by controlling for matriline, a method used within this study. In all, while an important first step in quantifying FA in the macaque and human skeleton, the present study includes additional information unavailable to Hallgrímsson (1999) and updated techniques that we feel render it a more accurate representation of FA in macaques than the previous work.

Age-related increases in fluctuating asymmetry are also variably supported outside of macaques. Within moose, FA of the antlers is reported to be lowest in young calves (1-2 years of age); however, no significant differences were observed between age classes older than 2 years (Solberg et al., 1993). This suggests that magnitudes of FA do increase after birth, but may plateau relatively early in life. The authors also observe that, for a given antler size, larger bulls exhibited less FA than relatively smaller bulls, suggesting that the ability to buffer environmental stress is improved in larger body sized individuals, an oft-cited measurement of individual fitness. Within developing humans, meanwhile, both cranial and postcranial FA reduce with age until ~10 years of age, then increase during adolescence to peak at 13-14 years, before subsequently reducing until 18 years of age (Wilson & Manning, 1996). Similarly, Hope et al. (2013) observed that manual asymmetry decreased between the ages of 4-8, plateaued during early adolescence, and further decreased after 13 years of age. The disruption to a general trend of reducing FA with age that occurs during adolescence is attributed in both studies to hormonal changes and rapid growth coincident with the onset of puberty. Alternatively, however, both Kobyliansky & Livshits (1989) and Penke et al. (2009) report that extreme senescence (>80 years of age) was associated with elevated FA in human populations. Thus, it is possible that age-related increases in FA may be associated only with the extremes of old age, as opposed to a linear accumulation of asymmetry throughout life. This hypothesis could be tested in more diverse populations of nonhuman primates to further explore the nature of any potential relationship.

Prenatal Vulnerability to Natural Disasters

Prenatal growth is characterized by the greatest velocity of bone growth, as the template for adult skeletal morphology is quickly laid down. Accordingly, perturbations - such as the stress experienced during and immediately following natural disasters - can have major consequences for the physical formation of bony structures (Liu et al., 2012). Notably, maternal stress - either nutritional or psychological - can be transmitted to the gestating fetus. For example, the hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine mechanism through which the body regulates psychological stress, such as the experience of a hurricane (Smith and Vale, 2006). The end product of the HPA axis is production of cortisol, a hormonal biomarker commonly used as a proxy for stress (Bergman et al., 2010; Davis & Sandman, 2010; Rothenberger et al., 2011). Approximately 3% of maternal cortisol is transferred to fetal circulation (Stirrat et al., 2018) via the placenta (Argyraki et al., 2019), and excessive fetal exposure has been demonstrated to dysregulate the fetal HPA axis and disrupt tissue development (Argyraki et al., 2019; Provencal & Binder 2015).

Additionally, high levels of maternal glucocorticoids can degrade the integrity of the placenta itself, disrupting placental transport of key histone modifiers and altering the landscape of fetal methyl bioavailability (Myatt, 2006; Hogg et al., 2012; Argyraki et al. 2019). In this way, maternal stress can have a life-long impact on the skeleton of the offspring (Bateson, 2001; Morgan et al., 2005; Gluckman et al., 2008). Specifically, prenatal glucocorticoid overexposure alters histone acetylation and DNA methylation (Weaver et al., 2004). Methylation of the regulatory regions involved in the WNT/β-catenin signaling pathway dysregulate osteoclast formation as well as the process of osteoblast differentiation (Bocheva & Boyadjieva, 2011). Meanwhile, disruption to the RANKL/RANK/OPG signaling pathway has been linked to deleterious changes in bone mineral density, which negatively impact fetal bone development and may predispose individuals to senescent disorders such as osteoporosis (Bocheva & Boyadjieva, 2011). Such mechanisms likely explain the role of catastrophe-induced maternal

stress in driving prenatal morphological disruptions such as those manifested as fluctuating asymmetry. Previous studies have shown that habitat destruction impacts FA levels in a variety of animals (e.g., Badyaev et al., 2000; Hopton et al., 2009; Lens et al., 1999), and understanding the timing of these major environmental changes is a step closer to understanding the mechanisms by which this occurs.

Developmental Instabilities in a Changing Environment

Our data demonstrate that natural disasters are associated with long-term developmental disruptions that are most acutely experienced by prenatal individuals. The magnitude of such disruption is evidenced by the elevated levels of FA that persist in individuals more than a decade after the hurricane event they experienced. Thus, the impacts of such disasters are not transient, but instead manifest as lifelong deviations from the normal level of FA observed within the population. Though most individuals that experienced a prenatal hurricane were of a similar gestational age (~8-10 weeks gestational age, owing to the relatively consistent annual cycle of both macaque breeding and the tropical hurricane season), individuals who experienced the hurricane both at earlier and later periods of prenatal development exhibit similar levels of FA (Table 2.S1). Such data demonstrate the vulnerability of fetal individuals (and potentially neonatal individuals, though this hypothesis should be explored in future studies with greater numbers of neonates) to developmental instability, and the far-reaching effects of such disturbances throughout an individual's life.

Hurricane disturbances are complex, dynamic events that can change in both size and intensity while traveling thousands of miles. As hurricane formation is linked – among other external factors – to ocean surface temperatures, both the frequency and magnitude of hurricanes have been tied to global climate change, particularly within tropical oceans between latitudes of 40°S and 40°N (Lugo, 2000; but see Bengtsson et al., 1997), a region referred to as the global hurricane belt. Specifically, global warming has been linked to an increase in the

maximum speed of hurricanes, but not the area encapsulated by the hurricane itself (Emanuel, 1997). Similarly, through the use of the Anthropogenic Climate Change Index (ACCI), Holland & Bruyere (2014) demonstrate that the proportion of Category 4 and 5 hurricanes has increased at a rate of ~25–30 % per °C of global warming – a global signal reproduced in all ocean basins. Thus, as sea surface temperatures continue to rise, it seems reasonable to project an increase in high-magnitude hurricanes in the coming decades: both within the global hurricane belt and potentially beyond. This phenomenon could have a variety of consequences in that it could 1) expose new populations, previously at low risk of habitat disturbance, to the catastrophic consequences of hurricane events, and 2) subject currently at-risk populations to the risk of higher magnitude hurricane events. For instance, the macaques of Cayo Santiago recently experienced a third major hurricane event (Hurricane Maria) in 2019, which made landfall as a Category 4 event in September 2017: the most intense strike experienced by the island since 1928 (Zorilla, 2017). A 63% decrease in vegetation was observed on the island following this hurricane event, resulting in resource scarcity indicated by a peak in adult death rate one month after the storm (Testard et al., 2021). Several behavioral and physiological changes were observed as well, especially an increase in the number of social connections (Testard et al., 2021) and an increase in immunological aging in individuals that experienced hurricane Maria (Watowich et al., 2022). This potential danger underscores the need to better understand the vulnerabilities of populations to natural disasters and better understand the long-term morphological and fitness implications of catastrophe-induced environmental stressors.

Conclusion

This study provides evidence that stress from natural disasters during the prenatal period exhibits lasting effects on the primate skeleton, possibly due to increases in maternal stress-related hormones such as cortisol and glucocorticoids that cause disruptions to typical fetal development. The macaques living on Cayo Santiago are an ideal sample for investigating

the effect of hurricane disturbances as most major hurricanes arriving in Puerto Rico have a drastic effect on this island and its inhabitants. Further research in this population is warranted and can provide a clearer picture of the impact of natural disasters on skeletal development, including insights into the effect of social connectedness and nutrition. As climate change continues to create more instability in climatic events, natural disasters are becoming more frequent and severe. These macaques provide a window into the effect such catastrophes can have on both human and non-human populations around the world.

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Data Availability

All 275 cranial 3D surface scans used in this study are on Morphosource.org along with their associated mandibles under the project “Romero Dissertation Scans – CPRC Macaques.” These scans are free for use with the acknowledgement of the Caribbean Primate Research Center that is provided in the project description on Morphosource. The demographic data and

Procrustes FA scores associated with these rhesus macaques are available in the supplementary information of this publication.

Competing Interests

Funding for this research was provided by P.E.O. International, the University of Arkansas Fulbright College of Arts and Sciences, and the University of Arkansas Department of Anthropology. All authors declare no competing interests.

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Tables and Figures

Table 2.1. Number of female and male individuals in the skeletally mature and skeletally immature groups included in the sample.

	Female	Male	Total
All	154	121	275
Skeletally immature	117 (<15y)	60 (<8y)	177
Skeletally mature	37 (>15y)	61 (>8y)	98

Table 2.2. Description of the 34 landmarks used in this study.

Landmark	Midline/Bilateral	Location	Description
1	Midline	Face	Nasion (point where two nasal bones and frontal bone intersect)
2	Midline	Face	Premaxillary midline suture (superior point)
3	Midline	Face	Nasospinale (midpoint on lower border of nasal aperture)
4	Midline	Face	Alveolare (inferior tip of bone between upper central incisors)
5, 6	Bilateral	Face	Frontozygomatic suture at orbital rim
7, 8	Bilateral	Face	Zygomaxillare superior
9, 10	Bilateral	Face	Infraorbital foramen (most medial and superior point)
11, 12	Bilateral	Face	Zygomaxillare inferior
13, 14	Bilateral	Face	Premaxilla-maxilla junction at alveolus
15, 18	Bilateral	Face	Midpoint on alveolus between the 4th premolar and the first molar
16, 19	Bilateral	Face	Temporozygomatic suture (superior point)
17, 20	Bilateral	Face	External auditory meatus (most superior point)
21	Midline	Face	Incisive fossa (most posterior and inferior point on the incisive fossa; between incisive foramina when there are two)
22	Midline	Face	Interpalatine suture (posterior point)
23	Midline	Base	Basion (anterior margin of foramen magnum)
24	Midline	Base	Opisthion (posterior margin of foramen magnum)
25, 26	Bilateral	Face	Maxillary tuberosity (intersection of maxilla and palatine)
27, 28	Bilateral	Face	Sphenosquamosal suture along infratemporal crest
29, 30	Bilateral	Base	Lateral joining of spheno-occipital suture
31, 32	Bilateral	Base	Carotid canal (anterior point)
33, 34	Bilateral	Base	Posteromedial junction of occipital condyle and foramen magnum

Table 2.3. Results of the Procrustes ANOVA performed on all landmark configurations after a Procrustes fit. The side effect represents the directions asymmetry (DA) in the sample, and the individual*side effect represents fluctuating asymmetry (FA). The percent variation that each effect contributes to the sample is calculated in the last column (% var). Asterisk notes statistically significant relationships at the α=0.05 level.

Effect	**df**	**SS**	**MS**	**F**	**p**	**% var**
Individual	13974	3.38149449	0.0002419847	21.91	<0.001*	91.06%
Side (DA)	44	0.01183859	0.0002690589	24.36	<0.001*	0.32%
Individual*Side (FA)	12056	0.13313906	0.0000110434	1.54	<0.001*	3.59%
Error	26125	0.18699523	0.0000071577			5.04%

Table 2.4. 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	**Response**	**Fixed Effect**	**Estimate**	**Standard Error**	**df**	***t-value***	***p-value***
Model 1 (only Hurricane N)	FA Score	Age	-7.900e-5	6.270e-5	1.740e+2	-1.26	0.209
		Sex	4.517e-4	5.297e-4	1.740e+2	0.853	0.395
		Decade of Birth	4.223e-6	1.908e-5	1.740e+2	0.221	0.825
Model 2 (all animals, Hurricane Y/N)	FA Score	Age	2.249e-5	1.718e-3	2.750e+2	0.428	0.669
		Sex	4.489e-4	5.257e-5	2.748e+2	0.877	0.381
		Decade of Birth	7.474e-6	2.155e-5	2.455e+2	0.347	0.729
		Hurricane (Y/N)	5.819e-4	7.067e-4	2.5540e+2	0.823	0.411
Model 3 (all animals, # of hurricanes experienced)	FA Score	Age	2.73e-5	5.670e-5	2.750e+2	0.481	0.631
		Sex	4.374e-4	5.146e-4	2.749e+2	0.850	0.396
		Decade of Birth	8.049e-6	2.171e-5	2.424e+2	0.371	0.711
		# of Hurricanes (1)	6.004e-4	7.121e-4	2.586e-2	0.843	0.400
		# of Hurricanes (2)	3.617e-4	1.280e-3	2.676e-2	0.299	0.765
Model 4 (only hurricane Y, age at hurricane)	FA Score	Age	1.894e-4	1.120e-4	9.941e+1	1.691	0.094
		Sex	1.978e-3	1.244e-3	9.742e+1	1.591	0.115
		Decade of Birth	-6.051e-5	7.459e-5	9.587e+1	-0.811	0.419
		Age at Hurricane (1)	-6.e00e-3	1.716e-3	1.005e+2	-3.671	**<0.001**
		Age at Hurricane (2)	-7.032e-3	2.150e-3	9.899e+1	-3.271	**0.001**

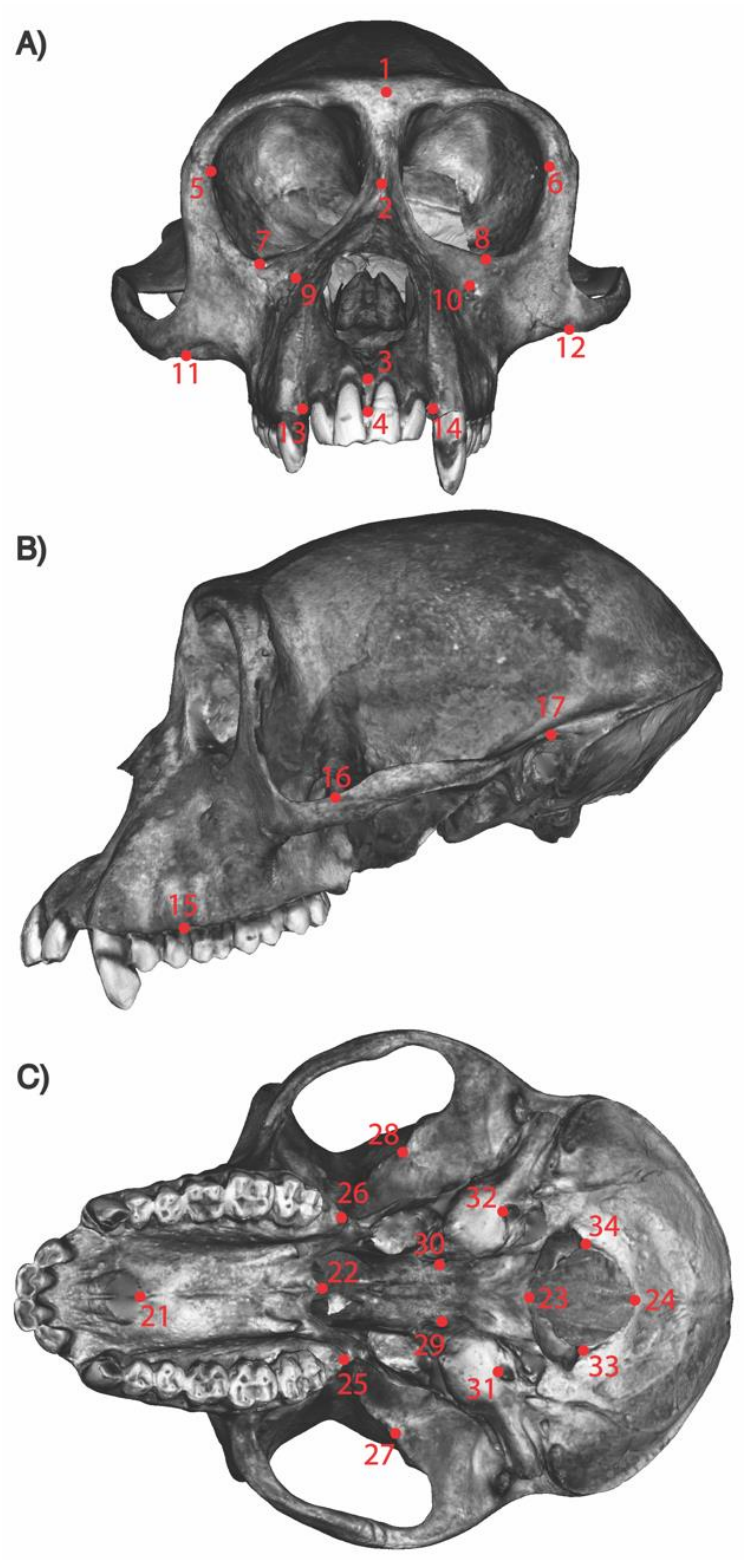

Figure 2.1. Landmarks used in this study on the A) anterior view, B) left lateral view, and C) inferior view of a female rhesus macaque (CPRCMUS-04439). Landmark definitions can be found in Table 2.2.

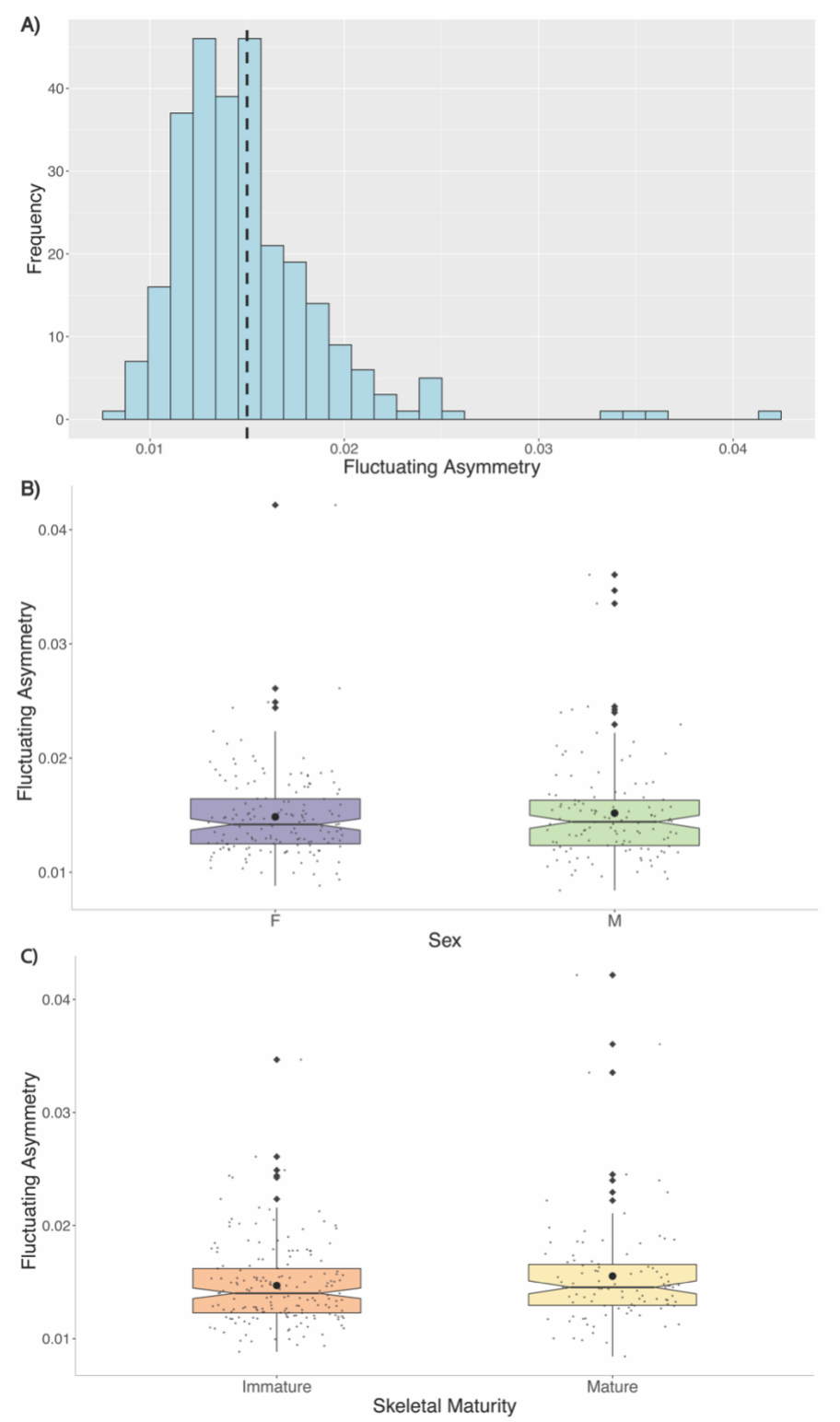

Figure 2.2. Plots illustrating the distribution of data in a A) histogram showing the frequency and mean (dashed black line) of FA values, B) boxplot of FA values separated by sex showing the mean (solid round point) value of males and females, and C) boxplot of FA values separated by skeletal maturity showing the mean (solid round point) value of skeletally mature and immature individuals.

Supplementary Information

Table 2.S1. Individuals used in this study that are housed in the Laboratory of Primate Morphology at the University of Puerto Rico Recinto de Ciencias Medicas. Catalog number at LPM, individual id tattoo, exact age at death (in years), and Procrustes FA score are included.

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-00008	EP	M	6.49	0.013367004
CPRCMUS-00011	FR	F	0.87	0.022349203
CPRCMUS-00019	129	F	14.17	0.010890405
CPRCMUS-00024	BV	F	10.28	0.014668791
CPRCMUS-00025	OZ	M	1.90	0.011753388
CPRCMUS-00031	NL	M	7.85	0.017900907
CPRCMUS-00032	IW	F	6.53	0.015035026
CPRCMUS-00037	CD	F	12.26	0.011770711
CPRCMUS-00038	BP	F	9.94	0.012899127
CPRCMUS-00040	LV	F	7.14	0.015361101
CPRCMUS-00045	GP	F	3.66	0.018429749
CPRCMUS-00047	HP	F	8.57	0.012971885
CPRCMUS-00052	TK	F	1.61	0.01295964
CPRCMUS-00053	011	F	12.90	0.017790057
CPRCMUS-00054	YK	F	2.51	0.00996115
CPRCMUS-00059	T	M	2.90	0.01106533
CPRCMUS-00060	EJ	M	6.46	0.011976978
CPRCMUS-00061	KU	M	4.70	0.010712334
CPRCMUS-00062	HH	F	4.02	0.012473262
CPRCMUS-00065	R011	F	8.20	0.014561016
CPRCMUS-00068	BM	M	4.87	0.017780572
CPRCMUS-00072	LQ	F	1.58	0.018031538
CPRCMUS-00079	HA	F	2.77	0.014372127
CPRCMUS-00088	BT	F	3.08	0.015525268
CPRCMUS-00096	BX INF 1962	F	1.88	0.016046454
CPRCMUS-00104	RB INF 1959	M	1.17	0.012664266
CPRCMUS-00109	078	F	11.07	0.02159338
CPRCMUS-00112	BL	F	7.53	0.01463098
CPRCMUS-00114	R003	F	10.21	0.012272454
CPRCMUS-00116	XZ	M	2.18	0.01561476
CPRCMUS-00118	9	M	3.79	0.015386578
CPRCMUS-00120	Z	F	5.53	0.012507864
CPRCMUS-00127	010	F	9.15	0.01264758

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-00131	FX	M	5.49	0.009433778
CPRCMUS-00133	024	F	15.53	0.014405662
CPRCMUS-00147	UG	F	9.53	0.010987812
CPRCMUS-00148	393	M	1.54	0.020520108
CPRCMUS-00153	OJ	M	7.62	0.011974755
CPRCMUS-00154	W3	M	5.69	0.015325055
CPRCMUS-00155	GQ	M	7.54	0.010915061
CPRCMUS-00160	8L	F	3.94	0.014209482
CPRCMUS-00163	GN	M	7.93	0.015137733
CPRCMUS-00164	FA	M	10.51	0.014470746
CPRCMUS-00174	KZ	F	14.24	0.009949127
CPRCMUS-00196	GK	F	8.03	0.014810395
CPRCMUS-00202	106	F	17.12	0.018504774
CPRCMUS-00219	XK	F	9.11	0.008834357
CPRCMUS-00220	H6	F	7.16	0.012783785
CPRCMUS-00221	E3	F	7.06	0.01352445
CPRCMUS-00225	XP	F	9.11	0.014035421
CPRCMUS-00226	LG	M	11.04	0.016591855
CPRCMUS-00236	UI	F	9.98	0.01399345
CPRCMUS-00238	HJ	F	13.04	0.012768234
CPRCMUS-00244	DK	M	13.03	0.013026294
CPRCMUS-00245	OY	M	7.03	0.014333076
CPRCMUS-00247	XA	M	10.03	0.015686726
CPRCMUS-00258	Z9	M	5.09	0.011831418
CPRCMUS-00271	ZQ	F	8.04	0.014234407
CPRCMUS-00281	TB	M	9.91	0.016016573
CPRCMUS-00289	HC	M	12.05	0.015001534
CPRCMUS-00300	EG	M	12.21	0.014667162
CPRCMUS-00314	9U	M	4.69	0.015442058
CPRCMUS-00320	ZR	M	8.74	0.016918543
CPRCMUS-00321	3U	M	5.70	0.012350153
CPRCMUS-00324	8N	M	4.79	0.020178916
CPRCMUS-00326	K	F	12.53	0.014959207
CPRCMUS-00333	391	M	4.01	0.021426327
CPRCMUS-00337	ZK	M	9.38	0.014239032
CPRCMUS-00338	031	F	18.25	0.011781241

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-00339	519	M	1.11	0.013414805
CPRCMUS-00345	AH	F	14.99	0.012543485
CPRCMUS-00346	286	F	3.12	0.016198167
CPRCMUS-00349	382	F	3.43	0.018682357
CPRCMUS-00352	290	F	3.39	0.015118887
CPRCMUS-00353	253	F	4.08	0.026100887
CPRCMUS-00354	022	F	18.64	0.014529247
CPRCMUS-00358	S017	F	16.42	0.015056478
CPRCMUS-00361	E2	M	8.34	0.015948294
CPRCMUS-00364	DS	M	14.47	0.010501039
CPRCMUS-00366	559	F	1.21	0.01778617
CPRCMUS-00368	318	M	3.61	0.011877415
CPRCMUS-00371	201	M	5.45	0.018517616
CPRCMUS-00374	XQ	F	10.39	0.012918293
CPRCMUS-00379	F7	M	8.50	0.016358766
CPRCMUS-00380	F8	M	8.50	0.009634998
CPRCMUS-00381	FJ	M	9.63	0.014688066
CPRCMUS-00382	TD	M	11.58	0.013911209
CPRCMUS-00383	XC	F	10.44	0.011736225
CPRCMUS-00385	7J	M	6.24	0.013611381
CPRCMUS-00398	9Z	M	7.91	0.02030072
CPRCMUS-00402	JX	M	13.90	0.014421994
CPRCMUS-00406	OS	F	9.53	0.015450767
CPRCMUS-00417	2C	M	7.53	0.024246441
CPRCMUS-00422	JI	F	14.74	0.013589156
CPRCMUS-00426	489	M	3.85	0.020593506
CPRCMUS-00427	434	M	3.11	0.017767957
CPRCMUS-00434	ZB	M	11.69	0.012081041
CPRCMUS-00440	YB	F	12.04	0.013513306
CPRCMUS-00442	ZH	M	11.66	0.01675941
CPRCMUS-00447	9T	F	7.96	0.009353646
CPRCMUS-00471	730	F	1.79	0.014997439
CPRCMUS-00477	737	F	0.95	0.015211228
CPRCMUS-00478	359	F	8.51	0.00982092
CPRCMUS-00479	706	M	1.05	0.015324666
CPRCMUS-00543	A05	M	0.95	0.015003182

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-00562	949	M	2.81	0.01703194
CPRCMUS-00574	258 INF 1980	M	0.77	0.018456009
CPRCMUS-00582	495	M	9.38	0.033535791
CPRCMUS-00593	B15	M	1.75	0.012787146
CPRCMUS-00596	283	F	10.97	0.012367505
CPRCMUS-00597	306	F	11.07	0.01774536
CPRCMUS-00616	C36	M	0.93	0.009753086
CPRCMUS-00617	A6	F	16.97	0.018511927
CPRCMUS-00620	G8	F	16.91	0.012914771
CPRCMUS-00637	500	F	10.64	0.010326309
CPRCMUS-00644	703	M	8.16	0.012672132
CPRCMUS-00672	258	F	14.20	0.01400371
CPRCMUS-00684	V7	F	17.94	0.013297711
CPRCMUS-00799	5D	M	16.49	0.013522309
CPRCMUS-00801	648	M	8.18	0.016835408
CPRCMUS-00806	599	M	10.47	0.010026183
CPRCMUS-00841	287	F	15.04	0.019498109
CPRCMUS-00842	606	F	10.07	0.012555671
CPRCMUS-00848	569	M	10.50	0.011062162
CPRCMUS-00852	615	F	9.78	0.016427653
CPRCMUS-00853	643	F	8.47	0.015168751
CPRCMUS-01213	A20	M	7.64	0.01016217
CPRCMUS-01216	4T	M	18.73	0.014735844
CPRCMUS-01231	619	M	12.10	0.013710497
CPRCMUS-01232	604	F	11.84	0.014766653
CPRCMUS-01233	438	F	12.35	0.012648259
CPRCMUS-01243	B67	M	7.23	0.015703467
CPRCMUS-01246	B23	M	7.55	0.020389381
CPRCMUS-01252	962	M	9.46	0.01455131
CPRCMUS-01570	9L	M	19.64	0.012745899
CPRCMUS-01571	B06	M	7.53	0.012608427
CPRCMUS-01573	B61	M	7.37	0.013326985
CPRCMUS-01574	894	M	9.52	0.016318173
CPRCMUS-01575	564	M	14.64	0.024512947
CPRCMUS-01579	996	M	7.94	0.012289452
CPRCMUS-01580	935	M	9.70	0.014812385

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-01587	B65	F	7.63	0.011896158
CPRCMUS-02029	B74	F	7.90	0.017009446
CPRCMUS-02031	676	F	12.51	0.013762115
CPRCMUS-02032	795	M	11.50	0.018752461
CPRCMUS-02039	818	M	10.90	0.011256605
CPRCMUS-02092	D13	M	8.11	0.014391824
CPRCMUS-02961	436	M	18.39	0.020164868
CPRCMUS-02965	504	M	18.66	0.012780984
CPRCMUS-02968	405	F	21.92	0.019924961
CPRCMUS-02971	WK	M	29.10	0.017420707
CPRCMUS-03007	348	F	20.10	0.011900383
CPRCMUS-03015	568	M	18.38	0.013703612
CPRCMUS-03017	976	F	10.59	0.012171821
CPRCMUS-03023	941	F	12.97	0.010821029
CPRCMUS-03024	787	M	14.37	0.016560131
CPRCMUS-03028	C28	M	8.89	0.017256252
CPRCMUS-03029	974	F	12.02	0.010547408
CPRCMUS-03034	D53	M	8.65	0.018993334
CPRCMUS-03044	D84	M	8.52	0.011854031
CPRCMUS-03060	798	F	14.42	0.011164568
CPRCMUS-03129	H50	M	7.71	0.016442575
CPRCMUS-03196	G24	F	7.03	0.019998767
CPRCMUS-03214	K12	M	7.27	0.01548637
CPRCMUS-03260	I07	F	8.73	0.014684494
CPRCMUS-03287	D68	M	13.32	0.017659374
CPRCMUS-03291	971	F	15.61	0.013659002
CPRCMUS-03307	FB	F	31.42	0.011517633
CPRCMUS-03308	F19	F	13.19	0.010364663
CPRCMUS-03317	O78	F	7.62	0.012055998
CPRCMUS-03319	E67	F	11.25	0.022217672
CPRCMUS-03341	K82	M	7.82	0.01216616
CPRCMUS-03348	B03	F	13.01	0.012027313
CPRCMUS-03450	H42	M	11.50	0.011753782
CPRCMUS-03453	S11	F	7.44	0.019820857
CPRCMUS-03477	S09	M	4.86	0.013255979
CPRCMUS-03531	725	F	17.41	0.013278241

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-03533	J43	M	10.24	0.015784153
CPRCMUS-03600	J61	F	9.58	0.011363093
CPRCMUS-03602	439	M	23.89	0.01106301
CPRCMUS-03603	I95	F	9.71	0.018698302
CPRCMUS-03630	L93	F	7.09	0.009868347
CPRCMUS-03637	V61	F	6.36	0.017355576
CPRCMUS-03646	D10	M	15.16	0.0188552
CPRCMUS-03689	V97	F	0.98	0.01248337
CPRCMUS-03694	R52	M	2.97	0.013505994
CPRCMUS-03697	X62	F	2.22	0.042164742
CPRCMUS-03745	87B	F	1.11	0.012910114
CPRCMUS-03766	Z09	M	1.66	0.011536106
CPRCMUS-03787	26B	F	2.09	0.014166141
CPRCMUS-03811	T93	F	2.84	0.012052053
CPRCMUS-03853	10K	M	2.28	0.013440044
CPRCMUS-03906	H66	M	10.72	0.015090152
CPRCMUS-03909	J88	M	17.34	0.013789214
CPRCMUS-03910	86A	M	7.69	0.015921142
CPRCMUS-03911	H47	M	12.33	0.012291091
CPRCMUS-03913	845	F	23.47	0.013049958
CPRCMUS-03917	E04	F	18.69	0.034677137
CPRCMUS-03919	H57	F	8.28	0.012471204
CPRCMUS-03921	08I	F	5.08	0.011924043
CPRCMUS-03929	O50	F	4.06	0.013647602
CPRCMUS-03930	L43	M	14.35	0.012892915
CPRCMUS-03931	J95	F	17.41	0.012673017
CPRCMUS-04078	84O	M	1.27	0.015381109
CPRCMUS-04112	08A	M	11.31	0.015739067
CPRCMUS-04147	S37	F	14.87	0.014534706
CPRCMUS-04164	59B	M	9.83	0.013789383
CPRCMUS-04168	39C	M	10.07	0.013594749
CPRCMUS-04176	H71	F	19.86	0.01491666
CPRCMUS-04181	679	F	28.93	0.011044323
CPRCMUS-04187	X67	F	13.55	0.012380091
CPRCMUS-04248	56O	F	3.59	0.016229665
CPRCMUS-04276	21I	M	5.99	0.019755542

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-04300	84F	M	9.74	0.014640524
CPRCMUS-04306	33A	M	10.52	0.016200135
CPRCMUS-04310	H45	F	20.94	0.012884548
CPRCMUS-04314	23H	M	7.63	0.011955276
CPRCMUS-04316	B60	F	23.39	0.012861147
CPRCMUS-04318	O53	F	17.51	0.024407693
CPRCMUS-04328	J99	F	18.81	0.008418087
CPRCMUS-04332	03G	M	6.60	0.011780228
CPRCMUS-04335	85H	M	5.84	0.016878771
CPRCMUS-04341	47K	F	5.53	0.017876095
CPRCMUS-04343	28E	M	8.74	0.021264279
CPRCMUS-04409	81L	F	3.97	0.015942977
CPRCMUS-04423	08N	M	6.82	0.01510583
CPRCMUS-04426	31A	F	14.81	0.014949547
CPRCMUS-04431	25O	F	6.86	0.018428265
CPRCMUS-04432	48B	F	13.70	0.013401117
CPRCMUS-04433	K81	F	18.81	0.015424828
CPRCMUS-04439	T98	F	15.88	0.02108866
CPRCMUS-04458	74E	F	7.15	0.018592975
CPRCMUS-04459	J79	F	17.27	0.036047244
CPRCMUS-04461	H74	F	21.91	0.016476764
CPRCMUS-04469	95A	F	13.95	0.014800614
CPRCMUS-04470	G68	F	20.15	0.011711748
CPRCMUS-04480	X87	F	15.90	0.013843321
CPRCMUS-04485	97S	M	3.26	0.013204142
CPRCMUS-04486	R75	F	17.98	0.015446746
CPRCMUS-04487	Z99	F	14.75	0.014804412
CPRCMUS-04493	39S	F	4.19	0.01002932
CPRCMUS-04497	79O	M	5.25	0.01391728
CPRCMUS-04507	H79	M	16.38	0.013209255
CPRCMUS-04508	27C	F	9.30	0.011354414
CPRCMUS-04509	25I	M	10.66	0.011844786
CPRCMUS-04514	90S	F	5.83	0.012082242
CPRCMUS-04532	6C8	F	2.95	0.014787796
CPRCMUS-04538	23V	F	4.03	0.023993184
CPRCMUS-04540	94V	F	4.84	0.011720183

Table 2.S1 (Cont.)

Museum Number	Tattoo	Sex	Age at Death	Procrustes FA Score
CPRCMUS-04543	X53	F	3.46	0.019084778
CPRCMUS-04548	99I	F	11.29	0.012028326
CPRCMUS-04571	80J	F	1.38	0.024895019
CPRCMUS-04622	O55	F	19.68	0.019675947
CPRCMUS-04631	01P	F	7.90	0.011312285
CPRCMUS-04634	35J	F	10.72	0.012987542
CPRCMUS-04640	37T	F	4.86	0.013660908
CPRCMUS-04652	43K	F	4.24	0.017970222
CPRCMUS-04654	985	F	24.11	0.014469128
CPRCMUS-04656	F25	F	24.06	0.014878948
CPRCMUS-04669	874	F	19.74	0.012220138
CPRCMUS-04675	V24	F	11.05	0.01259001
CPRCMUS-04778	89C	F	13.63	0.015737357
CPRCMUS-04780	V44	F	17.57	0.010885244
CPRCMUS-04781	91K	F	8.70	0.015105153
CPRCMUS-04782	29I	F	10.00	0.013420656
CPRCMUS-04784	04O	F	9.23	0.022943777
CPRCMUS-04786	K86	M	7.39	0.0142275
CPRCMUS-04788	33C	F	15.14	0.012950718
CPRCMUS-04795	47L	F	8.89	0.015967086
CPRCMUS-04810	35O	F	11.33	0.011238466
CPRCMUS-04811	26P	M	11.37	0.017471067
CPRCMUS-04812	28N	F	12.44	0.012690708
CPRCMUS-04813	9A9	M	7.64	0.018685941
CPRCMUS-04815	17T	M	9.42	0.017498986
CPRCMUS-04817	45F	F	16.12	0.015399439

Chapter 3: Pathology- and secular-related changes in fluctuating asymmetry detected in olive baboons

Ashly N. Romero[1], Katherine E. Willmore[2], Claire E. Terhune[1]

[1]Department of Anthropology, University of Arkansas, [2]Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario

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Abstract:

Fluctuating asymmetry (FA), or random deviations from bilateral symmetry, reflects developmental instability in that disruptions during development cause increased dysregulation of the mechanisms maintaining symmetrical growth. These disruptions are known to occur from various environmental and genetic stressors. How FA changes across the adult period and over decades of time is poorly understand, as is the relationship of FA to pathology. This study investigates FA and its relationship to sex, age, decade of birth, and antemortem tooth loss in an adult sample from a captive colony of olive baboons (*Papio hamadryas anubis*; *N*=154). Using geometric morphometrics to quantify FA, t-test, regressions, Fisher's exact tests, and two-way ANOVAs to assess the relationship of FA to sex (t-test), age (regression), decade of birth (ANOVA), and antemortem tooth loss (Fisher's exact test, ANOVA), we found that levels of FA differed by sex, decreased across decades in males, and increased with antemortem loss of the premolar teeth. Further, males exhibit antemortem tooth loss more frequently than females in all teeth except molars. These results indicate that there could be evolutionary mechanisms affecting levels of FA in this population and that biomechanical influences may confuse estimation of developmental instability from FA. While suggesting caution, these results also provide insight into the influences on FA and warrant further study into secular change in populations using museum collections that span decades.

Introduction

Bilateral symmetry is a ubiquitous trait within its namesake clade Bilateria. This trait is an incredibly conserved phenotype, and ideally organisms should grow and develop symmetrically. When they do not, there has typically been some issue preventing this more optimal phenotype. Because both the left and right side of an organism develop from the same genetic template and in the same environment, differences are thought to reflect instability during the developmental period (Klingenberg, 2003; Palmer & Strobeck, 1986). Random deviations from symmetry are termed fluctuating asymmetry (FA), and these deviations are thought to reflect developmental instability (Klingenberg, 2015); more FA, therefore, reflects less developmental stability. Specific gaps in our knowledge of FA include how FA changes with demographic factors such as age, sex, and year of birth and the relationship between FA and pathology. Investigation of these relationships helps tease apart the factors influencing FA and its change over time. This study aims to address each of these gaps by examining FA in adult olive baboon crania (*Papio hamadryas anubis*) from the Southwest National Primate Research Center (SNPRC).

Most work on FA focuses on non-primate mammals (e.g. Badyaev et al., 2000; Hopton et al., 2009) or birds (e.g., Kellner & Alford, 2003), lizards (e.g., Simbula et al., 2021), and insects (Fuciarelli & Rollo, 2021), but previous work in primates has found that FA increased over ontogeny in long-tailed macaques (*Macaca fascicularis*), rhesus macaques (*Macaca mulatta*), and humans (Hallgrímsson, 1993; Hallgrímsson, 1999). This author suggests that the ratio of bone turnover to bone growth in part determines the amount of FA that accumulates, so faster growth with less background bone turnover and more directed bone growth (as in juvenility) likely results in higher levels of FA (Hallgrímsson, 1993, 1998). He further suggests that FA is a result of accumulation of asymmetric mechanical factors, variation in growth regulation, and the tendency for morphological drift during bone remodeling (Hallgrímsson, 1999).

The timing at which fluctuations and perturbations occur during an organism's lifetime is likely important. While vulnerability during development has been a point of interest (Alados et al., 1998; Moes et al., 2022; Hollebone & Hough, 1991; Vrijenhoek, 1985), development is not the only vulnerable window in an individual's life. Senescence is a period in which individuals are particularly vulnerable as well. During human senescence, resorption of bone occurs at higher rates than formation of bone due to a major decrease in osteoblastogenesis (Jilka et al., 1996). Additionally, trabecular thickness and number decrease, cortical bone is lost, and cortical bone porosity increases (Pignolo et al., 2021). This dysregulation of bone modeling and remodeling could cause FA to increase during the senescent period. However, no such relationship was found in sample of adult gorillas, indicating that FA may develop solely during ontogeny (McGrath et al., 2022).

Studies on FA differences between sexes in primates yields a more ambiguous result, though it appears that sex is most often not a significant influence on FA. Males have shown greater dental FA than females in olive baboons (Hoover et al., 2021), and males in sexually dimorphic species tend to exhibit higher levels of dental FA than females (Martin, 2013). Additionally, male gorillas exhibited higher levels of craniofacial FA than females (Romero et al., 2022). However, other studies of craniofacial FA have found no differences between sexes in macaques or humans (Hallgrímsson, 1993; Hallgrímsson, 1999). The degree of masculinity or femininity of male and female primates seems to have no effect on the level of FA either (Van Dongen, 2015).

Secular change in FA is a relatively unexplored avenue due to the difficulty of obtaining samples that cross large enough time spans to assess change across generations. One study in bumble bees found that FA increased over time in association with climatic trends toward warmer and wetter weather (Arce et al., in press). This finding suggests that changes over time can be detected, though may be related to climatic shifts rather than an evolutionary mechanism. Further, Lens et al. (1999) found that FA in bird tarsus length increased over time

in individuals living in a degraded environments when compared to other individuals that lived in a more suitable habitat, supporting the use of FA as a measure of environmental stress.

While no study to date has examined levels of dental pathology and FA in primates, baboons typically exhibit dental pathologies, especially males (Bramblett, 1965; Bramblett, 1967). For example, male olive baboons have higher rates of antemortem tooth loss and pulp cavity exposure than females (Kirchhoff et al., in review). The relationship between dental pathology and FA is worth exploring due to the potential mechanical disadvantage of pathology for mastication and the association of pathology with an increase in bone remodeling.

Research Goals and Predictions

To further our understanding of factors associated with FA in primate species, this study examines the relationship between craniofacial FA and (1) demographic factors such as age, sex, and decade of birth and (2) dental pathology presence in olive baboons (*Papio hamadryas anubis*). The captive baboons in the SNPRC colony provide an ideal sample for this study as they have limited exposure to climatic events, which reduces confounding factors that could influence FA. We hypothesize that age and pathology influence levels of FA, while sex and decade of birth do not.

Previous literature indicates that FA accumulates over the lifetime, especially due to morphological drift during bone remodeling (Hallgrímsson, 1999), which suggests that FA across the adult developmental period should increase with age. We predict that bone modeling and especially remodeling during adulthood will cause morphological drift and increase FA throughout the adult period, resulting in the highest levels of FA in the oldest individuals. Further, we do not expect to find sex-specific differences in FA because previous studies in cercopithecines have not found this to be an influential factor for craniofacial FA (Hallgrímsson, 1999; Romero et al., 2022; Van Dongen, 2015). The studies to date examining changes in FA in a population over time have found increases in FA across decades of time (i.e., secular

changes) associated with climatic shifts (Arce et al., in press; Lens et al., 1999). Because our sample consists of captive baboons in stable climatic conditions, we do not expect to see any secular change in FA in our sample. Additionally, while no literature exists to frame the relationship between FA and pathology in baboons, we expect that pathology presence will increase FA due to increased bone remodeling and asymmetric mechanical strains.

Materials and Methods

Sample Composition

The skeletal samples used in this study are exclusively olive baboons (*Papio hamadryas anubis*; Jolly, 1993) born at the Southwest National Primate Research Center (SNPRC), which is hosted by the Texas Biomedical Research Institute and is located in San Antonio, Texas. The founders of this population are mostly of the subspecies *Papio hamadryas anubis* (olive baboon) from Darajani, Kenya in 1960 (VandeBerg, 2009). It is important to note that as of 2009, common baboons were a polytypic species assigned to *Papio hamadryas* with five subspecies, though these subspecies are recognized as separate species in Mittermeier et al. (2013). Today, there are over 1,000 baboons at SNPRC (Southwest National Primate Research Center, 2022).

The baboons at SNPRC are housed in groups except when specific protocols require paired or single housing. Feeding has occurred in a number of ways over the years, but the baboons are currently fed 5LEO brand monkey chow that is supplemented with foods for foraging on weekdays (fruits, vegetables, nuts, seeds, etc.; Kenneth A Sayers, personal communication, January 24, 2022). Breeding management has occurred in two controlled forms: targeted, single-male, multi-female groups and multi-male, multi-female groups. Corral breeding was discontinued in 2005. The female to male sex ratio at SNPRC is 2:1 (Hlusko, 2006). Captive olive baboons exhibit more hierarchical groups than wild baboons (Brent, 2009). Additionally, captive conditions lead to more tension, aggression, and behavioral disturbance in

olive baboons when compared with their wild counterparts. Research has shown that enrichment (such as manipulable objects, swings, and wooden logs) significantly reduces abnormal behaviors in baboons, so this tactic is employed by research colony managers. From the 1980s-1990s, canine teeth were blunted in male baboons, and after that period, male canines have been filed on the tips and lingual side of the tooth (Kenneth A Sayers, personal communication, May 3, 2022; Sharon Price, personal communication, May 3, 2022). Further, canines can be broken during conflict, and veterinarians may shorten or remove the canines if deemed necessary (Sharon Price, personal communication, May 3, 2022).

This study includes 154 olive baboon crania (*Papio hamadryas anubis*) from SNPRC including male and female individuals (F=77, M=77; Table 3.S1). All individuals in this sample were between 6 and 29 years of age at the time of death and all were born in the SNPRC colony and sacrificed for humane or management reasons. By 6 years of age, all three molars have erupted in baboons, with little craniofacial growth occurring after this time (Hlusko & Mahaney, 2009; Kahumbu & Eley, 1991; Leigh, 2009).

Data Collection

Surface models of the baboon crania used in this study were downloaded as .ply files from the Texas Biomedical Research Institute Southwest National Primate Research Center organization on Morphosource.org (Table 3.S1), which houses CT scans and surface models generated from these scans of over 930 baboons (Roseman et al., 2010; Willmore et al., 2009).

The cranial models in this study were imported into 3D Slicer (Version 4.11.20210226; Fedorov et al., 2012) for landmarking. A total of 34 fixed landmarks were placed on the cranium using the “markups module” (Table 3.1; Figure 3.1). Midline (8) and bilateral (13 pairs) landmarks were used. Semi-landmarks were not collected for this study due to their non-homologous nature. Landmarks were exported as .fcsv files, imported into R (R Core Team,

2020) for collation, and saved as a .tps file for import into MorphoJ (Klingenberg, 2011). Fixed landmarks were placed twice on each of the 154 individuals to assess measurement error.

Antemortem tooth loss (AMTL), was scored as present or absent for all individuals in the sample by visually assessing the cranial 3D surface models. AMTL was scored as present if a tooth was missing and the surrounding bone had started to remodel accordingly. All tooth types were scored (incisors, canines, premolars, and molars), and presence/absence data was also grouped into anterior (incisors, canines) and posterior (premolars, molars) teeth. Additionally, canine alteration (filing or clipping) was scored as present or absent, and supernumerary molars (unilateral and bilateral) were scored as present or absent as well.

Data Analysis

Quantification of Fluctuating Asymmetry

After digitization of all the individuals in the sample, a Procrustes superimposition or Procrustes fit was performed on all landmark configurations in MorphoJ. This generalized Procrustes analysis (GPA) moves all landmark configurations to the same position (translation), rotates all landmark configurations to the same orientation (rotation), and makes each landmark configuration the same size (scaling) to “fit” the configurations using a least squares approach (Dryden & Mardia, 1998; Goodall, 1991; Gower, 1975; Klingenberg, 2015). These translation, rotation, and scaling steps are important for eliminating location, orientation, and size variables and leaving only shape (i.e., everything that remains after superimposition) for analysis.

To measure object symmetry, the entire configuration of landmarks was reflected across the midline, and the bilateral landmarks of the reflected copy are relabeled to match the original shape (Kent & Mardia, 2001; Klingenberg et al., 2002; Mardia et al., 2000). A consensus of the original and reflected/relabeled copy that is perfectly symmetric was created via a Procrustes fit of the original and reflected/relabeled configurations (see Klingenberg, 2015 for visual). Then, the original, reflected/relabeled, and consensus configuration undergo a Procrustes fit where the

sum of squared deviations of the original and reflected/relabeled copy were minimized from the consensus shape. Both the individual consensus shapes and the overall consensus shape are perfectly symmetric, which allows the midline landmarks to lie in a plane that represents an informed anatomical midline, and the bilateral landmarks are connected by lines perpendicular to this plane. The differences between corresponding landmarks in an original and reflected/relabeled configuration represent the asymmetry present. This is the same as the difference between the original configuration and the symmetric consensus or the difference between the reflected/relabeled configuration and the symmetric consensus.

After GPA, a Procrustes ANOVA (analysis of variance) was performed in MorphoJ (Klingenberg et al., 2002; Klingenberg & McIntyre, 1998; Palmer & Strobeck, 1986). This ANOVA is a two-factor, mixed-effect model using individuals (right/left of each specimen) and sides (right/left) as main effects, expanded to include replicate measurements to estimate error. In this study, statistical significance for all analyses is achieved at the $\alpha = 0.05$ level. The individual effect is the variation in the right and left trait values for each individual, while the sides effect is the average difference between the right and left sides (directional asymmetry) of the whole sample. The interaction effect of individual by side is the difference in individuals due to their differences in left and right sides, which represents fluctuating asymmetry and/or antisymmetry. The error term in this analysis calculates measurement error as the residual variation in the model (McGrath et al., 2022) and helps assess the ratio of FA signal to error noise in the sample. Ideally, the measurement error is less than the FA signal. The percent of variation that each term contributes to the sample was calculated by summing the sum of squares for each term in the model and then calculating each term's contribution to the total (Gómez-Robles et al., 2013).

The Procrustes ANOVA results in MorphoJ include a centroid size for each specimen, coordinates for the symmetric and asymmetric component of shape, and both Procrustes and Mahalanobis FA scores (magnitude of fluctuating asymmetry; Klingenberg & Monteiro, 2005).

Procrustes, rather than Mahalanobis, FA scores were used in this study. The calculation of Mahalanobis distance is the same as the Procrustes distance, using the right-left differences between sides and subtracting the mean asymmetry, except there is additional scaling in each direction so there is an equal amount of asymmetry in each direction. This method requires larger sample sizes to reliably estimate the covariance matrix. Additionally, Mahalanobis distances are difficult to interpret because they are not comparable to other measures of shape variation (Klingenberg, 2015). The centroid size and Procrustes FA scores from MorphoJ were used for further analysis in R. Descriptive statistics were performed on the Procrustes FA scores to examine the general parameters of the data in this study (Figure 3.2).

Demographic Associations with Fluctuating Asymmetry

To investigate differences between FA level in males and females, an f-test was performed to assess data normality using the "var.test" function in the *stats* package (R Core Team, 2020), and then a t-test was performed to assess the difference in mean FA between sexes using the "t.test" function in the *stats* package. To control for changes in cranial size associated with age, we used a linear regression of centroid size on sex + age at death and planned to use the residuals from this analysis for cranial size if statistically significant. We then regressed FA magnitude on sex + centroid size (original values, not needing adjustment) to assess allometry of FA in our sample. Lastly, to investigate changes in FA associated with age, we performed a linear regression of FA magnitude on sex + age at death. All linear regressions were performed using the "lm" function in the *stats* package (R Core Team, 2020).

To investigate changes in FA magnitude over time in the SNPRC baboon population, we performed a two-way ANOVA using sex and decade of birth as main effects and a sex*decade of birth interaction term using the "aov" function in the *stats* package (R Core Team, 2020). This allows for investigation of sex-related changes in FA over time in addition to the differences in

FA by sex and by decade. If results were significant, differences in FA mean were tested with a one-way ANOVA within each sex.

Pathology and Fluctuating Asymmetry

Before assessing the relationship between FA and pathology, we first quantified AMTL (using presence/absence) and tested the difference in pathology frequency between male and female baboons using Fisher's exact tests for each pathology variable with the "fisher.test" function in the *stats* package (R Core Team, 2020). This allowed us to assess if pathology occurs more frequently in either sex more than would be expected by chance. Then, changes in FA related to pathology were assessed using two-way ANOVAs including sex and various tooth-related pathologies as main effects and an interaction term between sex and the pathology using the "aov" function in the *stats* package (R Core Team, 2020). The Fisher's exact tests and ANOVAs were performed using presence/absence data for canine alteration, anterior AMTL, posterior AMTL, and supernumerary molars as a main effect. Anterior and posterior AMTL were further investigated if they were statistically significant using data on incisor AMTL, canine AMTL, premolar AMTL, molar AMTL more specifically.

Results

Fluctuating Asymmetry

Both craniofacial directional asymmetry and FA are present in the sample ($p<0.001$ for both in the Procrustes ANOVA), where FA is distributed relatively normally with some outliers on the positive end (Figure 3.2A). Most shape variation comes from differences between the left and right sides of each individual (90.17%; Table 3.2). The FA signal (7.6% of total variation) in this sample is more than twice the error (2.01% of total variation), indicating that we are adequately able to investigate our questions in this study. The Procrustes FA scores extracted

from MorphoJ had a total mean of 0.01848, median of 0.01761, variance of 3.30626e-05 and standard error of 0.00575 (Table 3.3).

Demographic Associations

The FA variance in male and female baboons are significantly different ($p=0.02$), and FA levels are significantly different between sexes per the t-test with unequal variances ($p<0.001$), where males exhibit higher levels of FA than females (Figure 3.2B).

Cranial size is not significantly related to age in these adult baboons as tested with a regression of centroid size on sex and age at death, though there is a general trend of increased size with age as one would expect (Table 3.4; Figure 3.S1). Though sex has a significant influence on FA in this sample, no relationship exists between cranial size and FA when sex is accounted for, which indicates allometry is not detected in this sample (Table 3.4). Additionally, no relationship between FA and age at death was detected ($p=0.64$) in a regression of FA on age at death, though there is a trend toward FA increasing with age in male baboons (Figure 3.3A).

Secular change in FA levels was detected in this sample using a two-way ANOVA with sex and decade of birth (Table 3.4). Differences in FA by sex were present ($p<0.001$) and in the interaction between sex and decade of birth ($p=0.008$). In males specifically, FA significantly decreases over time through the 1970s, 1980s, and 1990s at SNPRC ($p<0.001$; Figure 3.3B). No difference in females was detected over these decades ($p=0.9$).

Pathology and FA

Overall, male baboons exhibited significantly higher frequency of pathologies than females except for missing molars and supernumerary molars (Table 3.5). Fisher's exact tests demonstrated that male baboons had higher frequency of canine alteration, missing incisors, missing canines, general anterior AMTL, missing premolars, and general posterior AMTL than

females (all $p<0.05$; Table 3.6). Two-way ANOVAs testing differences in FA between sexes and presence/absence of all pathologies only showed differences in FA for individuals with posterior AMTL and those without, where individuals with posterior AMTL had higher levels of FA ($p=0.003$; Table 3.6). This result is driven by individuals with missing premolars exhibiting higher levels of FA ($p=0.001$).

Discussion

This study assesses craniofacial FA in relation to demographic factors (age, sex), secular change, and dental pathology presence in the captive colony of olive baboons (*Papio hamadryas anubis*) at SNPRC. Our major findings are that male baboons exhibit higher levels of FA than females, FA does not increase ontogenetically in adult baboons (though there is a slight trend toward increasing in males), male baboons exhibit secular change in FA with FA decreasing over time, males exhibit higher levels of AMTL than females in this sample, and only premolar AMTL is related to higher levels of FA while other AMTL has seemingly no relationship. In all, these findings suggest that the degree of sexual dimorphism may impact levels of FA, secular change in FA may be linked to evolutionary mechanisms apart from environmental influence, and pathology cannot be discounted when considering potential influences on FA.

Sex and Time but Not Age Influence FA Levels

In this assessment of the demographic and pathological factors related to FA magnitude, results indicate that levels of craniofacial FA are higher in male baboons than females, though we predicted no difference in sex. Our results support the findings of Martin (2013), where males in sexually dimorphic species exhibit higher levels of dental FA, and these results specifically support Hoover et al. (2021) that found male olive baboons exhibit more dental FA than females. While others have found no difference in FA between sexes in macaques

(Hallgrímsson, 1993; Hallgrímsson, 1999; Romero et al., unpublished data), these results could indicate that the degree of sexual dimorphism is important to FA magnitude within a species as baboons have a higher degree of sexual dimorphism than macaques in both canine height and facial length (Plavcan, 2001). Romero et al. (2022) found that gorillas also have sex-dependent levels of FA where males exhibit more FA than females, and this taxon was the most dimorphic species in their sample. Males in sexually dimorphic primate species typically have either faster growth rates or longer growth periods (bimaturism) than females (Leigh & Shea, 1995; Plavcan, 2001; Turcotte et al., 2022). With either strategy, more bone deposition is occurring in males than females, providing increased opportunity for error when energy is diverted from typical growth and development such as in times of stress (Gluckman & Hanson, 2006; Halgrimsson, 1999).

Unlike earlier studies in macaques, we found that FA does not change significantly over the adult life stage in olive baboons, though there was a trend toward increasing FA with age in adult males older than 7 years. We predicted that FA would increase across the adult life stage, especially in advanced ages, but this was not necessarily the case for this sample. These results complicate the idea that FA accumulates with age in primates (Hallgrímsson, 1993; 1998; 1999), at least during the adult developmental period. These results indicate that other factors influence changes in FA in addition to accumulation of errors with age and suggest that neither bone remodeling and resorption during senescence nor morphological drift are the major drivers for FA in this population. The youngest individual in this study was a 7-year-old male. By 7 years old, all permanent dentition has erupted, and craniofacial growth has ceased in olive baboons (Leigh, 2009; Leigh & Cheverud, 1991), though cranial sutures never fully close in most individuals (Zuckerman, 1926). Levels of FA may not change during the adult developmental stage in olive baboons because of the early cessation of growth compared to other species (i.e., *Macaca mulatta* at 8yo for males and 15yo for females). The trend observed here toward FA increasing during adult life in male baboons could indicate that greater stresses

or pathologies due to male-male competition are influencing FA over time, seeing as craniofacial growth ceases earlier in males than females.

We predicted no change in FA over time due to the captive environment of our sample, but FA decreases over time in these male baboons. Secular change in FA levels in male baboons is a novel result for a novel question, as no studies to date have investigated this topic in primates or even mammals more broadly. Levels of FA decreasing over three decades in a captive baboon colony is not likely due to any release on climatic stress because the colony is protected from adverse climatic events, pointing to alternative and potentially evolutionary explanations. Additionally, this result was only observed in males, which further complicates any explanation. One might expect levels of FA to decrease over time in a population without any environmental stresses as the system would be expected to stay the same or increase in efficiency over time. As efficiency in the system increases, FA decreases, though this does not explain the sex-specific results of this study. Previous studies have found increases in FA with climatic disturbances (Arce et al., in press; Lens et al., 1999), though there does not appear to be evidence of decreases in FA in these studies as is seen in our sample of male baboons here. Conversely, this secular change observed in FA levels could be due to genetic drift rather than an adaptation-driven mechanism like directional selection, which would make the sex-specific results even more complicated.

Premolar AMTL Impacts FA Levels

Males in this study exhibited both increased frequency of pathology and higher levels of FA than females in the sample. Only males in the SNPRC colony have clipped or filed canines, but AMTL specifically (not just clipping/filing) is increased across every tooth type (except molars) in males in this sample when compared to females. This result is consistent with assessments of wild baboons (Kirchhoff et al., in review), where male olive baboons exhibit higher rates of AMTL and pulp cavity exposure, particularly in the anterior dentition. Kirchhoff et

al. suggest that this is related to higher male-male competition rates in this species; this is also a potential explanation for our similar findings here, since baboons at SNPRC are group housed.

Our finding that missing premolar teeth in particular influence levels of FA is a good indicator that factors apart from the developmental instability of an organism contribute to FA. The first of its kind, this finding suggests that caution is warranted when estimating developmental instability from FA because mechanical strains may affect levels of FA. Though other studies found no relationship between mastication and FA (Landi et al., 2021), these results suggest that changes in mastication due to AMTL may be an important contributor to FA levels. This, in part, could be due to additional bone remodeling associated with AMTL, causing drift in morphological symmetry of the craniofacial region (Hallgrímsson, 1999). Additional analyses are necessary to better understand these relationships and why this particular tooth class is significant to levels of FA.

Conclusion

This work provides characterization of craniofacial FA in olive baboons across the adult developmental period, the first examination of secular changes in FA in a captive colony of baboons, and a novel investigation into the influence of pathology on FA in a species of primate that exhibits high frequency of tooth-related pathologies. No relationship between adult age and FA was detected, but differences in FA were found between sexes, over time in male baboons, and with antemortem premolar loss. These results further contribute to teasing apart the influences on FA levels and suggest caution with estimating developmental instability from FA. Further, secular change in FA levels in this population suggests an evolutionary mechanism is contributing to FA levels, requiring additional investigation into museum collections to assess changes over time in various populations.

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Competing Interests

Funding for this work was provided by P.E.O. International. All authors declare no competing interests.

Data Availability

The 3D surface models used in this study are freely available for download from Morphosource.org under the organization Southwest National Primate Research Center. All demographic data must be requested from SNPRC, and associated fees are required. The Procrustes FA scores for each specimen are included in the supplementary material of this publication.

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Tables and Figures

Table 3.1. Descriptions of the 34 landmarks used in this study.

Landmark	Midline /Bilateral	Location	Description
1	Midline	Face	Nasion (point where two nasal bones and frontal bone intersect)
2	Midline	Face	Premaxillary midline suture (superior point)
3	Midline	Face	Nasospinale (midpoint on lower border of nasal aperture)
4	Midline	Face	Alveolare (inferior tip of bone between upper central incisors)
5, 6	Bilateral	Face	Frontozygomatic suture at orbital rim
7, 8	Bilateral	Face	Zygomaxillare superior
9, 10	Bilateral	Face	Infraorbital foramen (most medial and superior)
11, 12	Bilateral	Face	Zygomaxillare inferior
13, 14	Bilateral	Face	Premaxilla-maxilla junction at alveolus
15, 18	Bilateral	Face	Midpoint on alveolus between the 4th premolar and the first molar
16, 19	Bilateral	Face	Temporozygomatic suture (superior point)
17, 20	Bilateral	Face	External auditory meatus (most superior point)
21	Midline	Face	Incisive fossa (most posterior and inferior point on the incisive fossa; between incisive foramina when there are two)
22	Midline	Face	Interpalatine suture (posterior point)
23	Midline	Base	Basion (anterior margin of foramen magnum)
24	Midline	Base	Opisthion (posterior margin of foramen magnum)
25, 26	Bilateral	Face	Maxillary tuberosity (intersection of maxilla and palatine)
27, 28	Bilateral	Face	Sphenosquamosal suture along infratemporal crest
29, 30	Bilateral	Base	Lateral joining of spheno-occipital suture
31, 32	Bilateral	Base	Carotid canal (anterior point)
33, 34	Bilateral	Base	Posteriomedial junction of occipital condyle and foramen magnum

Table 3.2. Results of the Procrustes ANOVA performed on all landmark configurations after a Procrustes fit. The side effect represents the directions asymmetry (DA) in the sample, and the individual*side effect represents fluctuating asymmetry (FA). The percent variation that each effect contributes to the sample is calculated in the last column (% var). Asterisk notes statistically significant relationships below the α=0.05 level.

Effect	**df**	**Sum of Squares**	**Mean Squares**	**F**	**p**	**% var**
Individual	7803	1.36852193	0.0001753841	10.24	<0.001*	90.17%
Side (DA)	44	0.00330546	0.0000751240	4.38	<0.001*	0.22%
Individual*Side (FA)	6732	0.11535034	0.0000171346	8.22	<0.001*	7.60%
Error	14630	0.03047985	0.0000020834			2.01%

Table 3.3. Descriptive statistics of Procrustes FA scores.

	Mean	**Median**	**Variance**	**Standard Error**
All	0.018	0.018	0.0000331	0.000463
Female	0.017	0.016	0.0000219	0.000533
Male	0.020	0.019	0.0000379	0.000701

Table 3.4. Test statistics for the regressions and ANOVAs performed in this study to assess the association between various demographic factors and pathologies with FA. “x” notes the independent variable for each analysis. Asterisk notes statistically significant relationships below the α=0.05 level.

Demographic Associations			
Test	**Variables**	**P value**	**Model R^2**
Regression *Is centroid size related to age?*	Centroid size (x)	-	0.860
	Sex	<0.001*	
	Age	0.127	
	Sex x Age interaction	0.965	
	Whole Model	<0.001*	
Regression *Is FA related to centroid size?*	FA (x)	-	0.087
	Sex	0.698	
	Centroid size	0.475	
	Sex x Centroid size interaction	0.566	
	Whole Model	<0.001*	
Regression *Is FA related to age?*	FA (x)	-	0.111
	Sex	0.811	
	Age	0.640	
	Sex x Age interaction	0.207	
	Whole Model	<0.001*	
ANOVA *Does FA change with decade of birth?*	FA (x)	-	-
	Sex	<0.001*	
	Decade of birth	0.081	
	Sex x Decade of birth interaction	0.008*	
ANOVA *Does FA differ with decade of birth in males?*	FA (x)	-	-
	Decade of birth	<0.001*	
ANOVA *Does FA differ with decade of birth in females?*	FA (x)	-	-
	Decade of birth	0.9	

Table 3.5. Frequency table for tooth pathologies assessed in olive baboons in the sample for this study. Canine alteration is performed on all (or most) male baboons in this colony, explaining the high rate of canine alteration for males only. Two males in this sample have bilateral supernumerary molars, the remaining male and two female individuals have a unilateral supernumerary molar.

	Female	**Male**
Total individuals	77	77
Canines altered	0	70
Incisors missing	5	36
Canines missing	0	26
Premolars missing	0	9
Molars missing	3	2
Supernumerary molars	2	3

Table 3.6. Test statistics for the Fisher's exact tests and ANOVAs performed in this study to assess the association between various pathologies with FA. "x" notes the independent variable for each analysis. Asterisk notes statistically significant relationships below the α=0.05 level.

Pathology and FA			
Question	**Test**	**Variables**	**P value**
Do tooth pathology frequencies differ by sex?	Fisher's exact	FA (x)	-
		Canine alteration	<0.001*
	Fisher's exact	FA (x)	-
		Anterior AMTL	<0.001*
	Fisher's exact	FA (x)	-
		Incisor AMTL	<0.001*
	Fisher's exact	FA (x)	-
		Canine AMTL	<0.001*
	Fisher's exact	FA (x)	-
		Posterior AMTL	0.046*
	Fisher's exact	FA (x)	-
		Premolar AMTL	0.003*
	Fisher's exact	FA (x)	-
		Molar AMTL	1
	Fisher's exact	FA (x)	-
		Supernumerary molars	0.62
Does FA differ with canine alteration?	ANOVA	Sex	<0.001*
		Canine alteration	0.349
		Sex x Canine alteration interaction	No females with canine alteration
Does FA differ with anterior AMTL?	ANOVA	Sex	<0.001*
		Anterior AMTL	0.232
		Sex x Anterior AMTL interaction	0.572
Does FA differ with posterior AMTL?	ANOVA	Sex	<0.001*
		Posterior AMTL	0.003*
		Sex x Posterior AMTL interaction	0.751
Does FA differ with supernumerary molars?	ANOVA	Sex	<0.001*
		Supernumerary molars	0.973
		Sex x Supernumerary molars interaction	0.741
Does FA differ with premolar AMTL?	ANOVA	Sex	<0.001*
		Premolar AMTL	0.001*
		Sex x Premolar AMTL interaction	No females with premolars missing
Does FA differ with molar AMTL?	ANOVA	Sex	<0.001*
		Molar AMTL	0.171
		Sex x Molar AMTL interaction	0.894

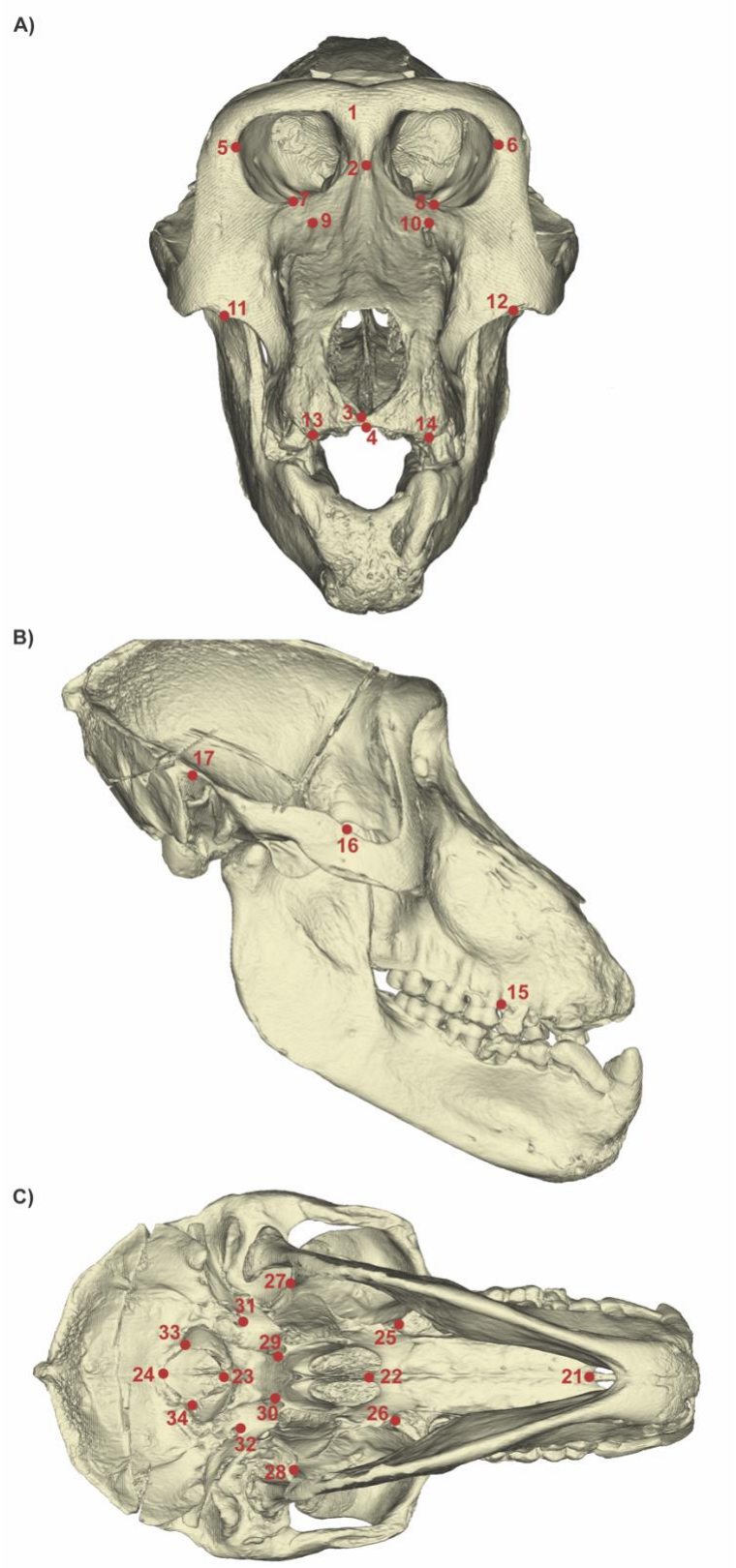

Figure 3.1. Landmarks used for calculating fluctuating asymmetry (FA) in the baboon cranium in the anterior (A), right lateral (B), and inferior (C) views (SNPRC7663M).

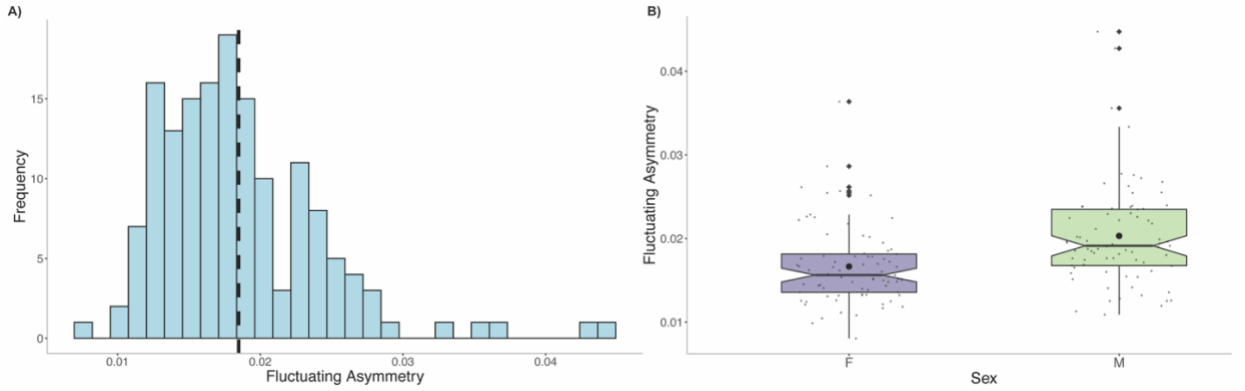

Figure 3.2. A) Distribution of Procrustes fluctuating asymmetry (FA) scores from MorphoJ. The black, dashed line represents the mean FA score. B) FA scores for female (purple) and male (green) baboons.

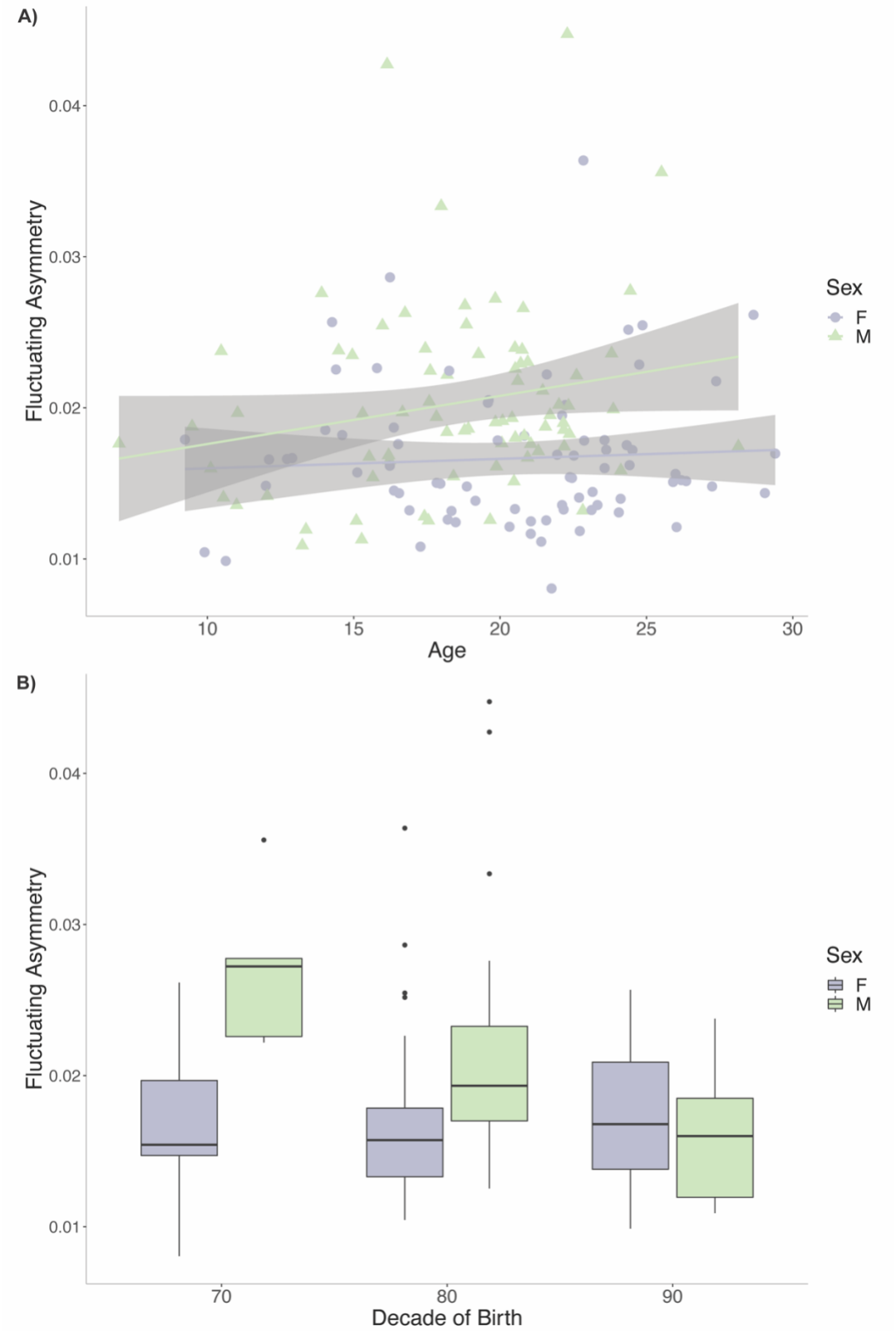

Figure 3.3. A) Scatterplot of fluctuating asymmetry (FA) scores plotted on age at death. B) Boxplot of FA scores binned by decade of birth. Female individuals are colored purple and male individuals are colored green.

Supplementary Information

Table 3.S1. Individuals used in this study that are available from the Southwest National Primate Research Center on Morphosource.org.

SNPRC/Morphosource Collection Number	Sex	Age at Death (years)	Procrustes FA Score
SNPRC10046	F	14.39	0.0225
SNPRC10544	M	13.37	0.0119
SNPRC11769	F	14.03	0.0185
SNPRC12457	F	14.26	0.0257
SNPRC14266	M	6.98	0.0176
SNPRC14796	M	10.12	0.0160
SNPRC14909	F	10.63	0.0099
SNPRC14925	M	10.47	0.0238
SNPRC15150	M	9.48	0.0188
SNPRC1X2001	F	27.38	0.0218
SNPRC1X2124	F	27.24	0.0148
SNPRC1X2315	F	21.76	0.0080
SNPRC1X2361	F	29.40	0.0170
SNPRC1X2365	F	23.16	0.0144
SNPRC1X2572	F	25.99	0.0156
SNPRC1X2574	F	24.75	0.0229
SNPRC1X2576	M	20.52	0.0226
SNPRC1X2589	F	26.03	0.0121
SNPRC1X2594	F	25.91	0.0151
SNPRC1X2716	F	26.19	0.0152
SNPRC1X2816	M	19.84	0.0272
SNPRC1X2825	F	26.36	0.0152
SNPRC1X2996	F	28.66	0.0262
SNPRC1X3200	F	21.05	0.0125
SNPRC1X3291	F	22.12	0.0195
SNPRC1X3310	M	18.19	0.0222
SNPRC1X3347	M	25.51	0.0356
SNPRC1X3420	M	24.45	0.0277
SNPRC1X3432	F	22.23	0.0202
SNPRC1X3445	F	24.33	0.0175
SNPRC1X3581	M	22.83	0.0132
SNPRC1X3582	F	24.06	0.0131
SNPRC1X3649	F	29.04	0.0144
SNPRC1X3655	M	19.84	0.0190
SNPRC1X3697	M	22.16	0.0190
SNPRC1X3739	F	24.86	0.0255
SNPRC1X3757	M	22.61	0.0221
SNPRC1X3818	M	18.41	0.0155
SNPRC1X3822	M	20.94	0.0167
SNPRC1X3834	M	28.14	0.0174
SNPRC1X3887	M	20.41	0.0193
SNPRC1X3938	M	24.13	0.0159

Table 3.S1 (Cont.)

SNPRC/Morphosource Collection Number	Sex	Age at Death (years)	Procrustes FA Score
SNPRC1X4013	M	22.01	0.0202
SNPRC1X4022	M	22.37	0.0183
SNPRC1X4041	F	18.21	0.0126
SNPRC1X4156	F	17.96	0.0150
SNPRC1X4179	M	20.79	0.0266
SNPRC1X4184	F	15.79	0.0226
SNPRC1X4284	M	21.71	0.0195
SNPRC1X4637	M	18.85	0.0255
SNPRC1X4645	M	20.70	0.0229
SNPRC1X4647	M	20.51	0.0240
SNPRC1X4703	F	12.90	0.0167
SNPRC1X4714	F	18.26	0.0225
SNPRC1X4736	M	23.87	0.0199
SNPRC1X4746	F	18.34	0.0132
SNPRC1X4752	F	20.83	0.0181
SNPRC1X4782	M	15.65	0.0154
SNPRC1X4802	M	13.91	0.0276
SNPRC1X4810	M	16.67	0.0197
SNPRC6218	M	16.75	0.0263
SNPRC6265	M	20.94	0.0230
SNPRC6290	F	22.84	0.0364
SNPRC6301	M	17.58	0.0204
SNPRC6335	F	24.52	0.0172
SNPRC6342	F	22.70	0.0141
SNPRC6450	M	14.95	0.0235
SNPRC6451	M	16.22	0.0165
SNPRC6548	F	24.38	0.0252
SNPRC6585	M	21.05	0.0176
SNPRC6609	M	21.46	0.0211
SNPRC6622	F	24.12	0.0140
SNPRC6732	F	20.32	0.0121
SNPRC6738	F	22.52	0.0168
SNPRC6812	F	23.63	0.0172
SNPRC6819	M	18.92	0.0186
SNPRC6860	F	16.23	0.0162
SNPRC6937	F	22.17	0.0133
SNPRC6955	M	23.81	0.0236
SNPRC6965	M	15.31	0.0196
SNPRC6971	F	23.57	0.0179
SNPRC6977	M	15.53	0.0168
SNPRC7002	F	21.05	0.0117
SNPRC7017	F	24.42	0.0162
SNPRC7113	F	23.13	0.0132

Table 3.S1 (Cont.)

SNPRC/Morphosource Collection Number	Sex	Age at Death (years)	Procrustes FA Score
SNPRC7122	F	23.57	0.0160
SNPRC7210	F	20.51	0.0133
SNPRC7307	M	16.19	0.0169
SNPRC7311	F	23.33	0.0136
SNPRC7368	F	22.87	0.0178
SNPRC7538	F	21.59	0.0222
SNPRC7606	F	22.38	0.0154
SNPRC7645	F	12.00	0.0148
SNPRC7646	M	20.52	0.0180
SNPRC7663	M	22.29	0.0447
SNPRC7727	F	22.46	0.0154
SNPRC7735	M	22.19	0.0174
SNPRC7764	F	22.72	0.0118
SNPRC7784	F	22.12	0.0136
SNPRC7823	F	16.38	0.0145
SNPRC7844	M	22.18	0.0185
SNPRC7850	M	20.83	0.0181
SNPRC7866	F	16.37	0.0187
SNPRC7895	F	16.52	0.0176
SNPRC7937	M	14.49	0.0238
SNPRC7944	M	16.14	0.0427
SNPRC8000	F	14.61	0.0182
SNPRC8001	M	15.98	0.0255
SNPRC8010	M	12.05	0.0142
SNPRC8062	F	16.24	0.0286
SNPRC8070	M	17.55	0.0125
SNPRC8091	M	22.34	0.0201
SNPRC8129	F	16.55	0.0144
SNPRC8212	M	20.75	0.0238
SNPRC8229	M	11.03	0.0197
SNPRC8250	F	9.24	0.0179
SNPRC8288	M	21.55	0.0188
SNPRC8291	F	18.48	0.0124
SNPRC8292	M	17.83	0.0194
SNPRC8307	F	21.58	0.0126
SNPRC8477	M	21.31	0.0171
SNPRC8499	F	21.95	0.0169
SNPRC8510	F	21.41	0.0111
SNPRC8517	M	18.18	0.0184
SNPRC8518	M	20.60	0.0218
SNPRC8576	M	10.55	0.0141
SNPRC8589	F	19.16	0.0139
SNPRC8597	F	15.12	0.0157

Table 3.S1 (Cont.)

SNPRC/Morphosource Collection Number	Sex	Age at Death (years)	Procrustes FA Score
SNPRC8615	M	18.80	0.0268
SNPRC8623	M	20.06	0.0177
SNPRC8679	M	15.09	0.0125
SNPRC8681	F	12.11	0.0166
SNPRC8698	M	20.08	0.0191
SNPRC8778	F	16.90	0.0132
SNPRC8780	M	17.45	0.0239
SNPRC8807	M	19.66	0.0126
SNPRC8860	M	20.48	0.0151
SNPRC8979	M	11.00	0.0136
SNPRC8980	F	19.92	0.0178
SNPRC9045	F	12.73	0.0166
SNPRC9097	M	17.99	0.0334
SNPRC9282	M	19.26	0.0236
SNPRC9285	M	19.88	0.0161
SNPRC9326	M	17.61	0.0225
SNPRC9344	F	9.91	0.0104
SNPRC9361	F	19.61	0.0205
SNPRC9494	F	17.28	0.0108
SNPRC9515	F	17.83	0.0150
SNPRC9562	M	17.42	0.0128
SNPRC9576	F	18.86	0.0148
SNPRC9625	M	18.79	0.0185
SNPRC9841	F	19.58	0.0203
SNPRC9892	M	15.27	0.0113
SNPRC9906	M	13.24	0.0109

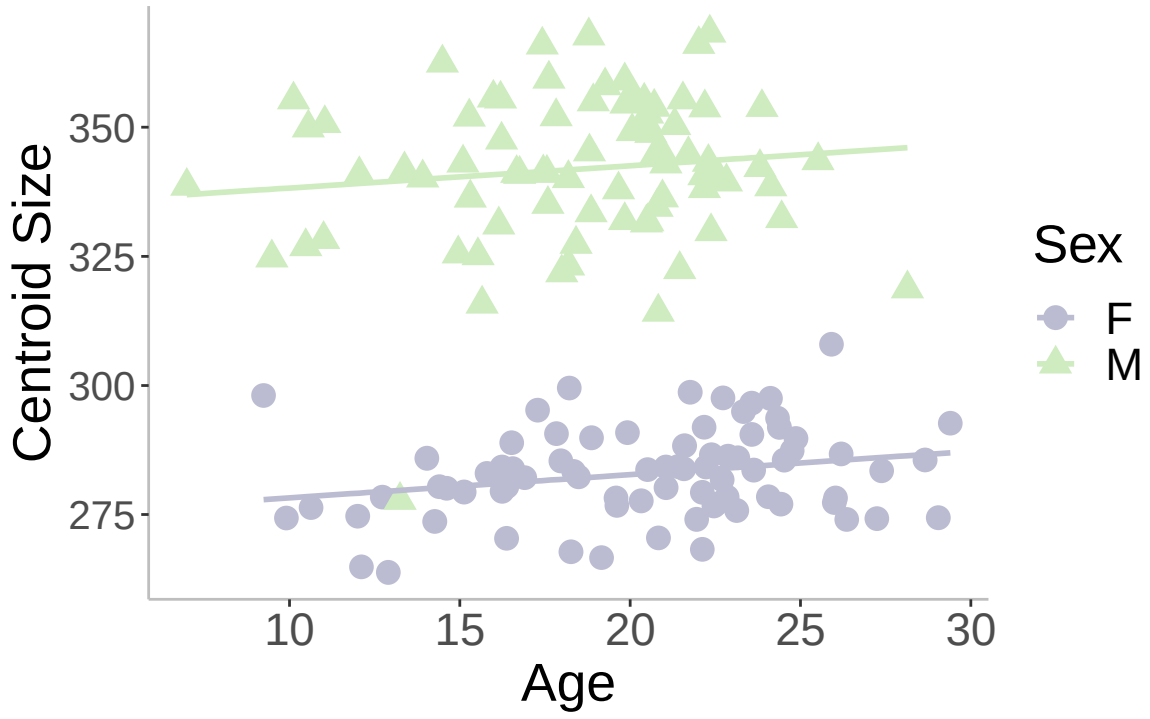

Figure 3.S1. Scatterplot of the relationship between centroid size (y) and age (x). Centroid size is measured in millimeters, while age is in years.

Chapter 4: Heritability and evolvability of fluctuating asymmetry in rhesus macaques and olive baboons

Ashly N. Romero[1], An-Di Yim[2], Claire E. Terhune[1]

[1]Department of Anthropology, University of Arkansas, [2]Department of Health and Exercise Sciences, Truman State University

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Abstract:

The possibility of evolutionary influence on craniofacial fluctuating asymmetry (FA), or random deviations from symmetry that reflect developmental instability, is unknown in primate models. Estimating the narrow sense heritability and evolvability of FA allows for understanding of how well the genetic structure of a population explains the phenotypic variance of FA and how accessible it is to natural selection. This study is the first to estimate heritability and evolvability of craniofacial FA in a free-ranging (*Macaca mulatta*) and captive (*Papio hamadryas anubis*) primate samples. Using geometric morphometrics to quantify craniofacial FA and a Bayesian approach to the animal model to estimate narrow sense heritability, we found that the estimates for heritability and evolvability were significantly non-zero but lower than expected based on previous studies. This suggests that FA is influenced to some small degree by the genetic structure of the population and somewhat accessible to selection. The evolvability estimates in these populations were even lower than the heritability estimates. This suggests that either the additive genetic variance is very low or the trait mean is unusually high in these samples. Comparing the estimates between the primate models (higher in baboons than macaques for both estimates) indicates that captive colony is in a more favorable environment than the free-ranging colony and that the additive genetic variance in baboons is higher than that in macaques. While providing insight into the evolutionary potential of FA, further investigation is warranted with larger sample sizes to achieve more accurate estimates.

Introduction

The right and left sides of all bilaterally symmetric organisms should be exact reflections of one another and develop under the same genome and the same environment. An organism's genome directs the growth and development of both reflected sides of a bilaterally symmetric organism and the whole organism exists, grows, and develops in one single environment. Therefore, random deviations from bilateral symmetry – fluctuating asymmetry (FA) – reflect developmental instability or the inability of a developmental system to accommodate fluctuations and buffer perturbations (Ludwig, 1932; Mather, 1953; Van Valen, 1962). While thought to be non-zero, the degree to which the genome influences FA and its potential for change over time remains uncertain. Here, we estimate the heritability and evolvability of craniofacial FA in two extant primate species, *Papio hamadryas anubis* and *Macaca mulatta*, to examine how well the genetic structure of these two primate populations explains the phenotypic variance of FA observed and how accessible it is to selection. Further, we estimate the heritability of developmental instability to understand the true nature of this trait, rather than just FA as its proxy.

Interindividual differences in an observed trait result from the conditions in which they develop and the underlying genome that produces them. The degree to which genetic factors contribute to FA remains largely unknown, though the consensus is that there is no organism-wide gene for developmental stability, developmental instability, or FA largely due to a relatively fruitless search for quantitative trait loci associated with increased FA (Leamy & Klingenberg, 2005). Heritability is an estimate of the amount of phenotypic variation in a sample that can be explained by its genetic structure (Falconer & Mackay, 1996; Vitzthum, 2003). Under quantitative genetic theory, total phenotypic variation is made up of genetic variance (σ_G^2), including the additive, dominance, and epistatic effects, and environmental variance (σ_E^2) such as variation as a result of diet, behavior, and temperature differences (Falconer & Mackay, 1996; Hardin, 2019).

Heritability estimates can be divided into broad sense heritability (H^2) and narrow sense heritability (h^2). Broad sense heritability (H^2) estimates the proportion of phenotypic variance that is due to the overall genetic variance in the sample, including dominance and epistatic variance ($H^2 = \sigma_G^2 / \sigma_P^2$). Because dominance and epistatic contributions are inconsistent from generation to generation (Falconer & Mackay, 1996), narrow sense heritability (h^2) is widely used in evolutionary quantitative genetics and includes only the additive genetic variance (σ_A^2) rather than the total genetic variance ($h^2 = \sigma_A^2 / \sigma_P^2$). Additive genetic variance is the variation in a population due to the known effect of particular alleles (Falconer & Mackay, 1996). In quantitative genetic studies, additive genetic variance can be theoretically approximated from pedigree data (i.e., relatedness coefficients; Falconer & Mackay, 1996).

Heritability estimates range from 0 to 1 because the most simple definition of this estimate is the slope of a regression of phenotypic values of offspring on the phenotypic values of parents. Heritability estimates of 0 represent little additive genetic variation and 1 represent a lot of additive genetic variation and/or little phenotypic variation. In general, heritability estimates for most traits fall between 0.2 and 0.6 and represent the effects of multiple loci, seeing as continuous and many discontinuous traits are typically polygenic (Arnold, 2023). Most estimates of the narrow sense heritability of FA are low and nonsignificant (Fuller & Houle, 2003; Leamy, 1997; Leamy & Klingenberg, 2005). Across a variety of traits and a variety of animals, the average h^2 of FA was found to be only about 0.026 (Fuller & Houle, 2003). Other meta-analyses found similar results (Van Dongen, 2000). One study examined the heritability of nonmetric cranial traits (e.g., number of foramina, supraorbital notch, divided hypoglossal canal) in the rhesus macaques from Cayo Santiago (one of the populations used in this study) and found that there were low or nonsignificant estimates of the heritability for these traits as well (Fuller & Houle, 2003; McGrath et al., 1984). As a result, they suggested that dental asymmetry is not genetically influenced.

Many studies of the heritability of FA recognize that this is an imperfect measure of developmental instability and stability, so researchers have developed methods for estimating the heritability of developmental instability as well (Whitlock, 1996). To estimate the heritability of developmental instability, the h^2 of FA is divided by the repeatability ($\Re$) of the FA measure (Whitlock, 1996, 1998). The heritability of developmental instability is generally higher than the heritability of FA as the repeatability of FA is usually below 1 and typically quite low (Carter & Houle, 2011; Fuller & Houle, 2003; Whitlock, 1996).

The heritability of FA is important for the evolutionary implications of these traits. In general, natural selection is likely reducing FA in a population, which then reduces developmental instability (Carter & Houle, 2011). The ability to reduce developmental instability may still exist because the costs of developmental precision are countered by the selection for precision, which could be due to the susceptibility of epigenetic systems to external influences (Carter & Houle, 2011). High sensitivity allows for imprecision in development, while low sensitivity makes developmental regulation difficult (Carter & Houle, 2011). Additionally, epistatic interactions may be important in the evolution of FA. While a population might respond to an environmental stress with high FA initially, epistatic interactions can develop that reduce developmental instability over time (Cheverud & Routman, 1996; Leamy & Klingenberg, 2005; McKenzie, 1997). Nevertheless, some researchers suggest that heritability is not the appropriate measure for evolutionary potential in natural populations because the selection differential (i.e., natural selection in wild populations) cannot be controlled by investigators like in artificial selection experiments (Hansen et al., 2011).

Evolvability (I_A) is a measure proposed to scale additive genetic variation (σ_A^2) by the squared trait mean ($\bar{X}^2$) rather than the phenotypic variance (σ_P^2) in a population (Houle, 1992). This measure is thought to be a better estimate of a trait's potential for evolution than heritability, and it has the advantage of being comparable across populations (Hansen et al., 2011; Hardin, 2019; Houle, 1992). An example of the difference between heritability and

evolvability is found in life history traits and morphological traits (Mousseau & Roff, 1987; Roff & Mousseau, 1987). Heritability estimates for life history traits are quite low when compared with those for morphological traits, but it is hard to imagine that life history traits have less potential for evolutionary change than morphological traits. The evolvability of life history traits, however, is much higher than for morphological traits. These differences can be attributed to inclusion of environmental variance in total phenotypic variance for heritability estimates, which is not included in measures of evolvability, and increases the denominator of the ratio used to calculate heritability but not evolvability (Hansen et al., 2011; Hardin, 2019; Houle, 1992).

This study aims to estimate the (1) narrow sense heritability, (2) evolvability of craniofacial FA, and the (3) heritability of developmental instability quantified using 3D geometric morphometric techniques in two extant primate species (*Macaca mulatta* and *Papio hamadryas anubis*). Our hypothesis is that the heritability of FA will be low, in line with previous studies of the FA heritability in other taxa and other traits. Further, the comparison of the heritability and evolvability estimates for craniofacial FA provides insight into the amount of environmental variance in the two populations sampled in this study. We predict the evolvability of FA will be relatively low but with a higher estimate than the heritability of FA based on previous literature (Hansen et al., 2011; Hardin, 2019). Lastly, we predict that the heritability of developmental stability will be higher than the heritability of FA, as is demonstrated in previous literature and likely due to the low repeatability of FA (Whitlock, 1996). This study is the first to investigate the heritability and evolvability of craniofacial FA and craniofacial developmental instability using 3D geometric morphometric techniques to quantify asymmetry in primates, providing insight into the variance and evolution of these traits.

Materials and Methods

Sample Composition

The sample for this study is derived from two populations of primates. The first population consists of rhesus macaques (*Macaca mulatta*) from the island of Cayo Santiago in Puerto Rico. These macaques live free-ranging on an approximately 18.2 hectare island with no human intervention save provisioning with fresh water and monkey chow daily (Dunbar, 2012; Kessler & Rawlins, 2016). Natural deaths and periodic culls contribute to the skeletal collection housed at the University of Puerto Rico Recinto de Ciencias Medicas in San Juan. We sampled 275 macaques (F=154, M=121) from this population ranging in age from less than 9 months to 31 years, a broad ontogenetic sample.

The other population in this study are olive baboons (*Papio hamadryas anubis*) from the Southwest National Primate Research Center in San Antonio, Texas. These olive baboons live in a captive colony that are generally housed in groups and male canines are altered to reduce injury in the population (Kenneth A Sayers, personal communication, January 24, 2022; Kenneth A Sayers, personal communication, May 3, 2022; Sharon Price, personal communication, May 3, 2022). Individuals in this study were sacrificed for humane or management purposes. We sampled 154 baboons (F=77, M=77) from this population ranging in age from 6 to 29 years, limited to only adults by data availability.

Data Collection

Macaque crania were 3D scanned using an HDI 120 blue LED scanner (LMI Technologies), then 3D surface models were processed in Geomagic Studio (3D Systems). Baboon crania were downloaded from the Texas Biomed Research Institute Southwest National Primate Research Center organization on Morphosource.org (Romero et al., in prep A). All 3D models were then imported into 3D Slicer (Version 4.11.20210226; Fedorov et al. 2012) for landmarking (Romero et al., in prep B). Fixed landmarks were placed on each cranium twice (13 bilateral pairs and 8 midline landmarks; Romero et al., in prep A, B), exported as .fcsv files,

imported and collated in R (R Core Team, 2020), and imported into MorphoJ (Klingenberg, 2011) for analysis and calculation of FA.

For the rhesus macaques on Cayo Santiago, daily census and observation backed by genotyping for many of the individuals in the skeletal collection provides a reliable pedigree for this population, especially for the maternal parent. However, paternity information is lacking for most individuals. To maximize the use of the data, we followed the approach outlined by Hardin (2019) and assigned a “dummy sire” to individuals with known mothers but unknown fathers. Two approaches were used: first, individuals with the same known mother were assigned the same dummy sire (Adams, 2011; Myers et al., 2006), thus creating a full-sib design; second, individuals were assigned different dummy sire. That is, all dummy sires were related to only one individual in the pedigree (Joganic et al., 2012; Konigsberg & Cheverud, 1992), thus creating a half-sib design. All parameters were estimated twice: once with the full-sib design, and once with the half-sib design. The “true” population heritability will fall between these two estimates, as the full-sib design will likely overestimate heritability while the half-sib design will likely underestimate heritability (Hardin, 2019). All the primates at SNPRC are genotyped and thus a reliable pedigree consisting of both parents is available for this population of olive baboons.

Data Analysis

Quantification of Fluctuating Asymmetry

A Procrustes superimposition (Procrustes fit) was performed on the 3D landmark configurations separately for each species in MorphoJ. This was done to avoid overwhelming the variation within species by the variation between species. Further, the macaque sample includes increased variation due to ontogenetic differences, whereas the baboon sample does not. After the Procrustes superimposition, a Procrustes ANOVA (analysis of variance) was performed for each species in MorphoJ to calculate the magnitude of craniofacial FA

(Klingenberg, 2015; Klingenberg et al., 2002; Klingenberg & McIntyre, 1998; Palmer & Strobeck, 1986). An α of 0.05 or below was used in this study to achieve statistical significance. Procrustes FA scores for each individual are calculated as part of the Procrustes ANOVA model, and these scores were pooled after calculation and then used in further analysis of heritability and evolvability in R (R Core Team, 2021).

Estimating the Heritability and Evolvability of Fluctuating Asymmetry

The animal model is the most commonly used method to estimate the heritability of a trait in wild populations (Kruuk, 2004). The model estimates the phenotypic covariance among all relatives in a population (Lynch & Walsh, 1998) by partitioning individual phenotype into a mixture of "fixed" and "random" effects. Fixed effects represent systematic differences among groups of individuals based on their group-level status as belonging to the same sex, age, or other factors. Random effects, on the other hand, represent individual-level variation, such as the additive genetic value (breeding value) of an individual. An animal model takes the following form:

$$y_i = \mu + \beta x_i + a_i + \epsilon_i$$

where y_i is the phenotypic value of individual *i*, μ is the grand mean (mean of the population), β is the regression coefficient for a fixed effect covariate (e.g., sex), and x_i is the fixed effect status for individual *i* (e.g., male or female), a_i is the additive genetic value of individual *i*, and ϵ_i is the residuals. The random effects and the residuals are defined as values drawn from distributions with means equal to 0 and variances equal to σ_A^2 (the additive genetic variance) and σ_R^2 (the environmental/residual variance), respectively.

To estimate the narrow sense heritability, animal models were fitted using the package *MCMCglmm* (Hadfield, 2010), with sex and age as fixed effect covariates. Covariates were considered statistically significant at or below an α of 0.05. *MCMCglmm* operates under a Bayesian framework with a Markov Chain Monte Carlo Gibbs sampler. The main parameters

estimated by the model are the variance components (Kruuk, 2004), and the Bayesian framework samples variance components from the prior distributions. Since FA generally has low variance, the 'classical' weakly informative prior with an inverse-Gamma distribution (V = 1, v = 0.002) is inappropriate (Gelman, 2006). Instead, a weakly informative, parameter expanded half-Cauchy prior distribution was used for the random effects. The default Gaussian non-informative prior was used for the fixed effects, while the inverse-Gamma distribution was used for the residuals. Parameter expansion was used to ensure convergence of the models. Each model was performed with one sampling Markov chain with a 500,000-iteration burn-in, followed by a 500,000-iteration sampling, thinning at a rate of 500. A total of 1,000 posterior samples was obtained for inference for each model. The significance of the heritability estimates was assessed by determining whether the posterior distribution crosses zero. Evolvability (I_A) of FA can be estimated as the additive genetic variation divided by the trait mean squared ($I_A = \sigma_A^2 / \bar{X}^2$; Brookfield, 2008; Hansen et al., 2011).

Estimating the Heritability of Developmental Instability

Whitlock (1996) describes how the heritability of developmental instability can be estimated by dividing the heritability of FA by the repeatability of FA. The repeatability of FA can be calculated with the following formula:

$$\Re = \frac{1}{1 + \left(1 + \frac{1}{CV^2}\right)\frac{\pi - 2}{2} + \frac{V_{me}}{V_P}}$$

where CV is the coefficient of variation of FA, V_{me} is measurement error of FA calculated from the duplicate landmark configurations for each specimen (Whitlock, 1996), and V_P is phenotypic variation of FA. After calculating maximum repeatability (assuming no measurement error), we can estimate the heritability of developmental instability ($h_{DI}^2 = h_{FA}^2/\Re$).

Results

Rhesus Macaques

Analyses for the rhesus macaques with both the half-sib and full-sib designs yielded significant, non-zero estimates of the narrow sense heritability of FA, and the narrow sense heritability of developmental instability is estimated to be 0.002 for both half- and full-sib designs (Table 4.1). The credible interval for the heritability estimate of FA in the half-sib design is 0.00000000163 – 0.0502, and the credible interval for the full-sib design is 0.00000000834 – 0.0529. Neither sex nor age were significant covariates in our model ($p>0.05$ for both covariates in both the half- and full-sib models), as shown in Figures 4.1 and 4.2 where the range of estimates for the effect of sex and age cross zero indicating that they are not significantly different from zero. Fuller & Houle (2003) suggest that estimates of the heritability of developmental instability are only reliable when the coefficient of variation of FA (CV_{FA}) is sufficiently high (>0.75). Our CV_{FA} value is 0.277 for the full-sib design, indicating that the heritability estimate for developmental instability may not be reliable. The evolvability estimate for FA ($I_A = \sigma_A^2 / \bar{X}^2$) in the rhesus macaques is between 0.0000172 (half-sib) and 0.0000332 (full-sib), suggesting low additive genetic variance seeing as mean FA for this population is not abnormally low.

Olive Baboons

Analyses for the olive baboons in our sample yielded significant, non-zero estimates of the narrow sense heritability of FA, and the narrow sense heritability estimate for developmental instability is 0.0037 (Table 4.1). The credible interval for the heritability estimate of FA is 0.00000000307 – 0.0787. Sex but not age was a significant covariate in our model ($p<0.001$; Figure 4.1 & 4.2). Our CV_{FA} value for the olive baboons is 0.311, not meeting the 0.75 threshold for a reliable estimate of the heritability of developmental instability (Fuller & Houle, 2003). The evolvability estimate for FA ($I_A = \sigma_A^2 / \bar{X}^2$) in the olive baboons is 0.0000607, suggesting low additive genetic variance seeing as mean FA for this population is not abnormally low.

Discussion

This study aimed to estimate the (1) narrow sense heritability of craniofacial FA, (2) evolvability of craniofacial FA, and (3) narrow sense heritability of developmental instability in two samples of primates, rhesus macaque and olive baboons. Our results indicate that the heritability of FA is non-zero but extremely low (Aim 1). The evolvability of FA is similarly quite low (Aim 2). Lastly, the heritability of developmental instability was not able to be reliably estimated according to a threshold set in previous literature (Aim 3; Fuller & Houle, 2003) and, therefore, no further conclusions can be drawn from this estimate in these populations. These results generally match our predictions in that the heritability of FA is low, but the evolvability of FA is not higher than the heritability of FA as we predicted. Additionally, sex was the only significant covariate, and only for the baboons in this study, which matches previous literature in these two populations (Romero et al., in prep A; B).

The heritability estimates for craniofacial FA in this study were lower than predicted in these samples (0.00022 for macaques and 0.0005 for baboons), though the credible interval for the estimates are quite large. The heritability estimate for nonmetric cranial traits in the Cayo Santiago rhesus macaques by McGrath et al. (1984) averaged 0.0525 across traits with a majority of values nonsignificant (Fuller & Houle, 2003). The authors in that study suggest that these nonmetric traits are not likely influenced by a genetic component, but our significant, non-zero results here indicate that there is some component of craniofacial FA that is influenced by the genetic structure of the population, however small. These non-zero results for FA heritability also suggest that craniofacial FA is accessible to selection to some degree in these sample populations, lending support to the idea of interplay between costs and selection for developmental precision (Carter & Houle, 2011). This idea suggests that there is a cost of developmental precision in that low sensitivity to external influences makes developmental regulation difficult, and high sensitivity to external influences allows imprecision. In this scenario,

selection for developmental precision still occurs due to its importance in developmental stability, but this interplay works toward some sort of optimal medium sensitivity for the epigenetic system to external influences (Carter & Houle, 2011). Low heritability estimates do not necessarily result from low additive genetic variation in a population (Visscher et al., 2008). Low heritability estimates can also result from greater phenotypic variation overwhelming the additive genetic variation present.

Our results that sex is a significant covariate in this baboon colony but not in the macaque population supports previous findings where males in sexually dimorphic species exhibit higher levels of dental FA (Martin, 2013), and these results specifically support Hoover et al. (2021) where male olive baboons exhibit more dental FA than females. While others have found no difference in FA between sexes in macaques (Hallgrímsson, 1993; Hallgrímsson, 1999; Romero et al., in prep A), these results could indicate that the degree of sexual dimorphism is important to FA magnitude within a species, as baboons have a higher degree of sexual dimorphism than macaques in both canine height and facial length (Plavcan, 2001). Romero et al. (2022) found that gorillas also have sex-dependent levels of FA where males exhibit more FA than females, and this taxon was the most dimorphic species in their sample. Males in sexually dimorphic primate species typically have either faster growth rates or longer growth periods (bimaturism) than females (Leigh & Shea, 1995; Plavcan, 2001; Turcotte et al., 2022). With either strategy, more bone deposition is occurring in males than females, providing increased opportunity for error when energy is diverted from typical growth and development such as in times of stress (Gluckman & Hanson, 2006; Halgrimsson, 1999).

The extremely low evolvability estimates for FA in this study were unexpected (0.0000172-0.0000332 for macaques and 0.0000607 for baboons), as previous estimates have typically exhibited higher evolvability than heritability for a given trait (Hansen et al., 2011; Hardin, 2019; Mousseau & Roff, 1987; Roff & Mousseau, 1987). Evolvability is calculated by dividing the additive genetic variance by the trait mean squared ($I_A = \sigma_A^2 / \bar{X}^2$) instead of by the

phenotypic variance as in narrow sense heritability ($h^2 = \sigma_A^2 / \sigma_P^2$). Previous literature has pointed out that heritability estimates include environmental variance in total phenotypic variance whereas evolvability does not include this component in its calculation (Hansen et al., 2011; Hardin, 2019). This would suggest that either the additive genetic variance is very low in our heritability estimate or the trait mean is unusually high for these samples. The mean FA in our sample is not unusual compared with previously published data (e.g., Romero et al., 2022), indicating that the additive genetic variance in our sample is quite low for this trait.

A cautious comparison of the heritability and evolvability of FA between this macaque population and baboon population provides insight into the influence of a free-ranging environment and a captive environment (Charmantier & Garant, 2005; Hardin, 2019). The Cayo Santiago macaques included in this study live in a natural, free-ranging, provisioned environment on a small island off the coast of Puerto Rico, whereas the baboons are from a captive breeding colony located in San Antonio, Texas. The heritability estimate for FA was higher in baboons than macaques for this study (h_{FA}^2 baboons = 0.0005, h_{FA}^2 macaques = 0.00022). Charmantier & Garant (2005) found that heritability estimates are higher in more favorable environments, especially for morphometric traits. These authors suggest that this can result from a few different possibilities: (1) the additive genetic variance (V_A) in a population can be constrained by poor growth conditions, thus limiting genetic potential; (2) environmental variance increases in environments with poor conditions that are negligible in favorable environments; (3) genotype-by-environment interactions can occur when traits are controlled by a different genetic basis in different environments and the genetic correlation of traits differs between environments. In this study, the captive colony of baboons is not exposed to the same climatic pressures that the free-ranging macaques, so the V_A could be lower in the baboons. For this same reason, environmental variance could be increased in the macaques. Further, because these populations are different species, it is entirely plausible that the genetic correlations between traits differ between them. The higher estimate of the evolvability of FA in

baboons than macaques (0.0000172-0.0000332 for macaques and 0.0000607 for baboons) suggests further differences between these populations ($I_A = \sigma_A^2 / \bar{X}^2$). Because the trait mean (FA) is higher in baboons than macaques in this study, this suggests that the additive genetic variance in baboons is also higher, as the evolvability estimate for FA in baboons remains higher than macaques despite the higher denominator.

Conclusion

This study provides the first heritability and evolvability estimates of craniofacial FA in rhesus macaques and olive baboons, which align with previous studies in that they are quite low – even lower than expected – though still significant and non-zero. The estimation of the heritability of developmental instability in this study proved unreliable due to a low coefficient of variation of FA. These results indicate that craniofacial FA is influenced genetically but to a very small degree, at least in these populations, and craniofacial FA may be minimally accessible to natural selection. Additionally, higher heritability estimates in baboons suggest captive colonies are a more favorable environment, likely primarily due to their protection from climatic stresses. Captive environments can be less stressful for many additional reasons, including lack of predators, provisioning, and wound care, so these results are not surprising. Further work should be done with a larger sample to produce more accurate heritability estimates for craniofacial FA in primates, though this study provides a first look at where results may lie.

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Competing Interests

Funding for this research was provided by P.E.O. International, the University of Arkansas Fulbright College of Arts and Sciences, and the University of Arkansas Department of Anthropology. All authors declare no competing interests.

Data Availability

The 3D surface models used in this study are freely available for download from Morphosource.org under the organization Southwest National Primate Research Center for baboons and Romero Dissertation Scans – CPRC Macaques for macaques. All baboon demographic data must be requested from SNPRC, and associated fees are required. The macaque scans are free for use with the acknowledgement of the Caribbean Primate Research Center that is provided in the project description on Morphosource.

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Tables and Figures

Table 4.1. Narrow sense heritability and evolvability estimates for the macaque and baboon samples. Sample size (*N*), heritability of FA (h_{FA}^2) with credible interval, evolvability of FA (I_A), coefficient of variation for additive genetic variance (CV_A), coefficient of variation for phenotypic variance (CV_P) or coefficient of variation for fluctuating asymmetry (CV_{FA}), maximum repeatability (R_{max}), and heritability of developmental instability (h_{DI}^2) are provided.

	N	h_{FA}^2 **(credible interval)**	I_A	CV_A	CV_P **(**CV_{FA}**)**	$\mathfrak{R}_{max}$*	h_{DI}^2
Papio hamadryas anubis	154	0.00050 (0.00000000307 – 0.0787)	0.0000607	0.779	0.311	0.134	0.0037
Macaca mulatta **(full-sib)**	275	0.00022 (0.00000000834 – 0.0529)	0.0000332	0.576	0.277	0.111	0.0020
Macaca mulatta **(half-sib)**	275	0.00022 (0.00000000163 – 0.0502)	0.0000172	0.415			0.0020

*$\mathfrak{R}_{max}$ is the maximum value of repeatability, assuming no measurement error

Table 4.2. Regression coefficients and credible intervals for the fixed effect covariates of sex and age included in our model. The asterisk (*) notes an α of <0.001.

	β	**Mean effect of sex**	**95% credible interval**	**Mean effect of age**	**95% credible interval**
Papio hamadryas anubis	1.303e-02*	4.049e-03*	1.628e-03 – 5.926e-03	1.766e-04	-6.108e-05 – 3.854e-04
***Macaca mulatta* (full-sib)**	1.550e-02*	-3.214e-05	-1.097e-03 – 1.414e-03	-5.027e-05	-1.578e-04 – 5.019e-05
***Macaca mulatta* (half-sib)**	1.551e-02*	-2.814e-05	-1.176e-03 – 1.210e-03	-5.025e-05	-1.649e-04 – 5.029e-05

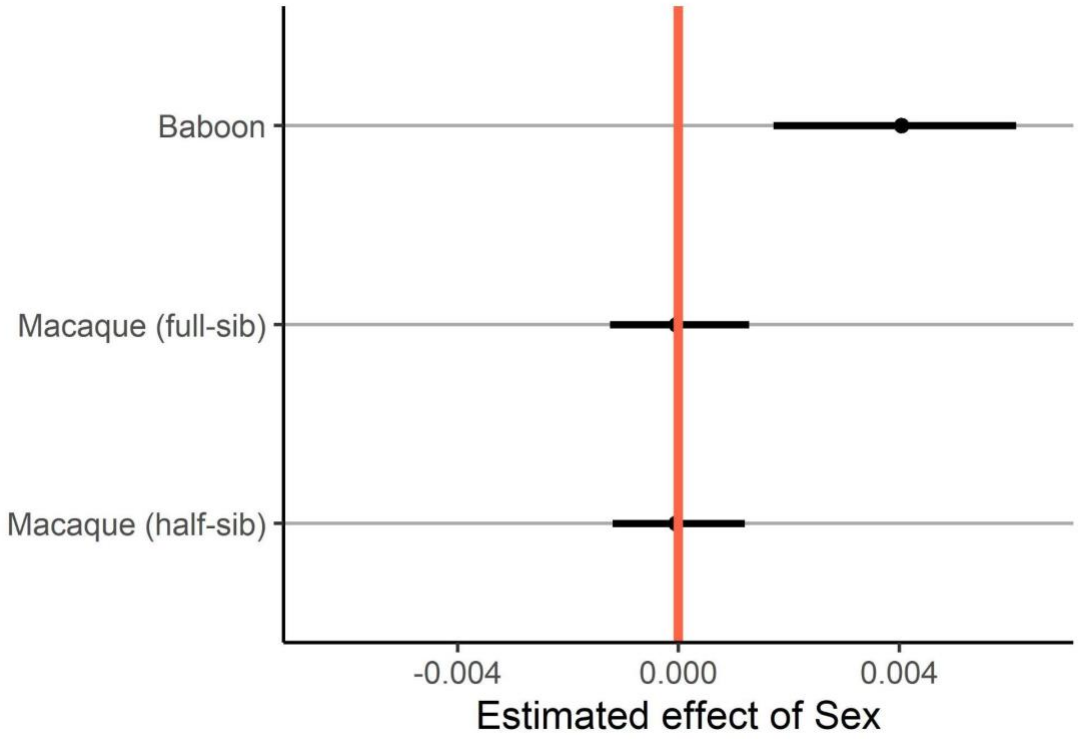

Figure 4.1. Regression coefficients for the sex covariate in the baboon, macaque (full-sib), and macaque (half-sib) models demonstrating that the only coefficient that is significantly different from zero is in the baboon model.

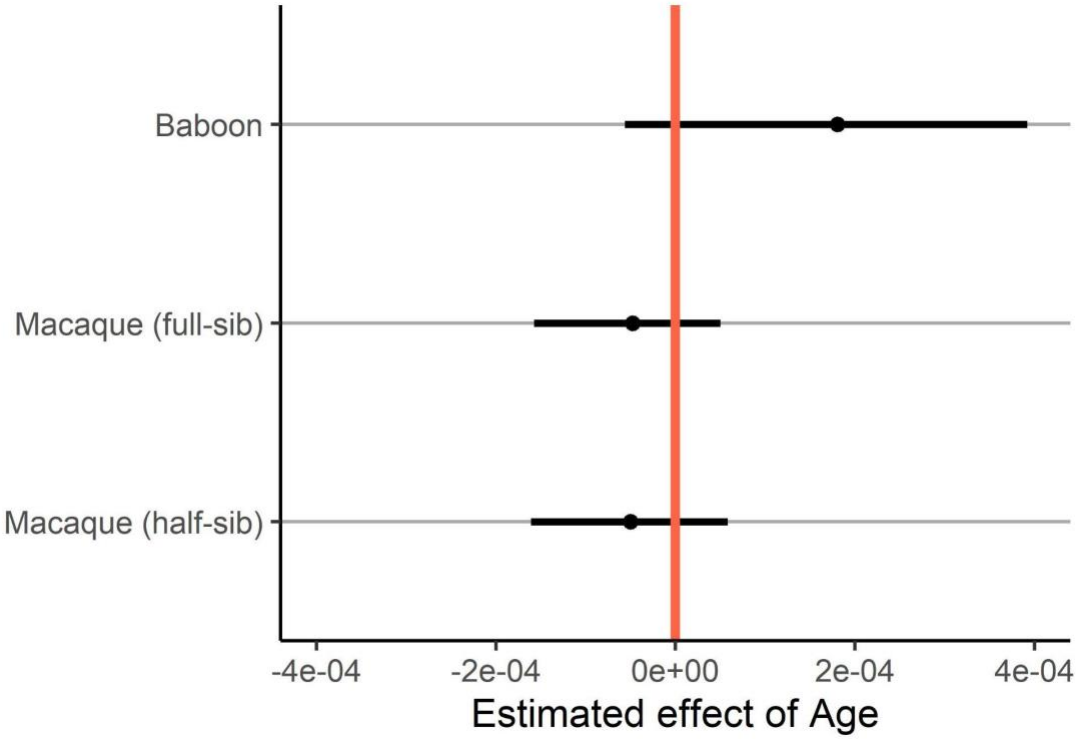

Figure 4.2. Regression coefficients for the age covariate in the baboon, macaque (full-sib), and macaque (half-sib) models.

Chapter 5: Conclusions

Fluctuating asymmetry (FA), or random deviations from bilateral symmetry associated with environmental and genetic stressors, is reflective of developmental instability in that disruptions to typical developmental regulation cause dysregulation of symmetrical growth in an organism. This dissertation aimed to explore the impact of environmental factors on the development and perpetuation of FA and sought to understand the role evolution may play in the FA exhibited in a given population of primates. The following research questions were investigated throughout this dissertation:

1. How do demographic variables influence fluctuating asymmetry?
2. How does fluctuating asymmetry change ontogenetically?
3. Are there secular changes in fluctuating asymmetry?
4. How do external perturbations influence fluctuating asymmetry?
5. To what degree is fluctuating asymmetry influenced by genetic factors?

Specifically, this dissertation investigated FA over all ontogenetic stages, across decades, between sexes, in association with ecological catastrophes, and with tooth pathology in an effort to tease apart factors that may influence FA and developmental instability. This dissertation also estimated the heritability and evolvability of FA and used FA levels over decades to examine the role of evolutionary mechanisms on FA. These topics were studied in two primate models: rhesus macaques (*Macaca mulatta*) and olive baboons (*Papio hamadryas anubis*).

Summary of Results

Skeletal Age During Hurricane Impacts Fluctuating Asymmetry in Cayo Santiago Rhesus Macaques

As natural disasters become more frequent with climate change, understanding the biological impact of these ecological catastrophes on wild populations becomes increasingly pertinent. Fluctuating asymmetry is reflective of developmental instability and has long been

positively associated with increases in environmental stress. Chapter 2 of this dissertation investigated craniofacial FA in a population of free-ranging rhesus macaques (*Macaca mulatta*) that has experienced multiple Category 3 hurricanes since the colony's inception on Cayo Santiago. Using geometric morphometrics to quantify FA and a linear mixed-effect model for analysis, we found that sex, age, and decade of birth did not influence the amount of FA in the individuals included in the study, but the developmental stage at which individuals experienced these catastrophic events greatly impacted the amount of FA exhibited ($p=0.001$). Individuals that experienced these hurricanes during fetal life exhibited greater FA than any other post-natal developmental period. These results indicate that natural disasters may be associated with developmental disruption that results in long-term effects if occurring during the pre-natal period, possibly due to increases in maternal stress-related hormones. Maternal stress-related hormones such as cortisol and glucocorticoids that increase in response to perceived and/or actual stress can be transmitted to a gestating fetus, causing dysregulation of the fetal HPA axis and disruption of histone modifier transport that result in disruption to tissue development (Argyraki et al., 2019; Provencal & Binder 2015; Weaver et al., 2004).

Pathology- and Secular-related Changes in Fluctuating Asymmetry Detected in Olive Baboons

How FA changes across the adult period and over decades of time is poorly understood, as is its relationship to pathology. Chapter 3 of this dissertation investigated FA and its relationship to sex, age, decade of birth, and antemortem tooth loss (AMTL) in an adult sample from a captive colony of olive baboons (*Papio hamadryas anubis*). We found that levels of FA differed by sex, decreased across decades in males, and increased with AMTL of the premolar teeth. Further, males exhibit AMTL more frequently than females in all teeth except molars. These results indicate that there could be evolutionary mechanisms affecting levels of FA in this population and that biomechanical influences may confuse estimation of developmental instability from FA. No environmental change is occurring over time in this population because

they are a captive colony, which points to an evolutionary explanation for this shift. The sex-specific decrease in FA presents an additional challenge. Further, the relationship between AMTL and FA indicates that biomechanical changes to mastication caused by losing teeth may influence the bone and result in additional FA. While suggesting caution, these results also provide insight into the influences on FA and warrant further study into secular change in populations using museum collections that span decades.

Low Heritability and Evolvability of Fluctuating Asymmetry Found in Rhesus Macaques and Olive Baboons

The possibility of evolutionary influence on craniofacial FA is unknown in primate models. Estimating the narrow sense heritability and evolvability of FA allows for understanding how well the genetic structure of a population explains the phenotypic variance of FA and how accessible it is to natural selection. Chapter 4 of this dissertation estimated heritability and evolvability of craniofacial FA in two primate samples: free-ranging (*Macaca mulatta*) and captive (*Papio hamadryas anubis*). We found that the estimates for heritability and evolvability for both populations were significantly non-zero but lower than expected based on previous studies. The non-zero heritability estimates suggest that FA is influenced to some small degree by the genetic structure of the population and somewhat accessible to selection because the additive genetic variation is not completely overwhelmed by the environmental variation. The evolvability estimates in these populations were even lower than the heritability estimates, which has not been the case in previous studies (Hansen et al., 2011; Hardin, 2019; Mousseau & Roff, 1987; Roff & Mousseau, 1987). This suggests that either the additive genetic variance is very low or the trait mean is unusually high in these samples. The trait mean is not unusual compared with other published data, indicating that the additive genetic variation for this trait is quite low. Comparing the estimates between the primate models (higher in baboons than macaques for both estimates) indicates that the captive colony is in a more favorable

environment than the free-ranging colony and that the additive genetic variance in baboons is higher than that in macaques (Charmantier & Garant, 2005). Captive environments can be less stressful for many reasons such as lack of predators, climatic stability, provisioning, and medical care. While providing insight into the evolutionary potential of FA, further investigation is warranted with larger sample sizes to achieve more accurate estimates.

Discussion

The results of this dissertation contribute to existing literature on FA in primates through numerous insights into relatively unexplored questions about the influence of certain factors on FA and, therefore, developmental instability. Specifically, this work identifies stages of skeletal development that are particularly vulnerable to environmental stress (Chapter 2), finds elevated FA related to potential changes in biomechanical strain (Chapter 3), and suggest evolutionary mechanisms work on FA over time in populations (Chapter 3 & 4). This work supports some hypotheses such as the developmental origin of health and disease (DOHaD) hypothesis, which proposes that early life environment influences health and disease later in life (see Gluckman et al., 2010). The results of this dissertation also support the idea that FA has some underlying genetic influence because of the non-zero heritability estimates for craniofacial FA in both macaques and baboons (Leamy & Klingenberg, 2005). However, these findings also suggest caution with estimating developmental instability from FA without considering other influences that may occur in the population samples (e.g., unusual biomechanical strains).

How Do External Perturbations Influence Fluctuating Asymmetry?

The investigation into the rhesus macaques that experienced Category 3 hurricanes on the Caribbean island of Cayo Santiago revealed that individuals that experienced the hurricanes during the fetal developmental stage had the highest levels of craniofacial FA at age of death (Chapter 2). This supports previous work by Moes et al. (2022) that found that early stages of

postnatal development are more susceptible to stress based on higher levels of both craniofacial FA and linear enamel defects in individuals in early developmental periods. It is possible that in the rhesus macaques, increases in maternal stress-related hormones such as cortisol and glucocorticoids cause disruptions to fetal development and result in abnormal skeletal morphology later in life; this aligns well with the developmental origin of health and disease hypothesis (Gluckman & Hanson, 2006). This hypothesis suggests that stressful environments early in life have adverse health and disease outcomes in adulthood. Higher FA found in macaques that experienced hurricanes as a fetus lends support to this hypothesis, as the results here suggest that this stress caused skeletal effects seen later in life. This work provides insights into the biological vulnerabilities to natural disasters and suggests long-term consequences of experiencing such stressful events early in development.

An important discovery in this dissertation is that antemortem tooth loss impacts levels of craniofacial FA, at least in olive baboons (a species with exceptionally high levels of antemortem tooth loss as shown in Kirchhoff et al., in review, and observed here in Chapter 3). In examining levels of FA related to antemortem tooth loss of various tooth types, premolar antemortem tooth loss had a significant relationship with elevated levels of craniofacial FA. This is critical information for understanding influences on levels of FA as it suggests that factors apart from developmental instability may be important. Previous work in bats suggests that masticatory function is important for levels of FA. For example, López-Aguirre & Pérez-Torres (2015) found that anatomical regions critical to mastication exhibited lower levels of FA, meaning that changes to mastication via AMTL could result in changes to FA levels. These increases in FA could be the result of additional bone remodeling in the alveolus where the teeth are lost causing drift in morphological symmetry, or from changes to muscle attachment sites on the cranium that shift with changes in biomechanical strains related to mastication (Hallgrímsson, 1999). Researchers should exercise caution when estimating developmental instability from FA levels. At the very least, investigators need to know enough about the

population under study to know if there are abnormal biomechanical strains or changes in biomechanical strains that could influence their results.

How Do Demographic Variables Influence Fluctuating Asymmetry?

In examining craniofacial FA in the two primates included here, we found sex-related differences in FA in baboons but not macaques (all chapters). Previous work has found varying results for sex-related differences in FA across organisms (Badyaev et al., 2000; Hallgrímsson 1999; Hopton et al., 2009; Romero et al., 2022; Schlager & Rüdell, 2015), but the results of this dissertation, combined with those of Hallgrímsson (1999) and Romero et al. (2022), indicate that sex-specific levels of FA may be present in some primate species but not others as this result was found twice in the same population of rhesus macaques and is likely accurate. This could be a result of differences in stress in sexes of different species that could be related to social system or could be potentially environmental in origin. Romero et al. (2022) found sex-related differences in gorillas but not long-tailed macaques or chimpanzees, and Martin (2013) found higher levels of dental FA in males in sexually dimorphic species compared to those that are monomorphic. Macaques are a sexually dimorphic species but did not exhibit FA differences in this dissertation nor in previous studies (Hallgrímsson, 1993; 1999), indicating that the degree of sexual dimorphism in a species may be important to the amount of stress experienced. The results found here could indicate species with higher levels of sexual dimorphism also exhibit increased sex differences in levels of FA where males exhibit higher levels of FA than females. The faster growth rates or longer growth periods required to achieve larger size allow increased opportunity for error in morphological symmetry development when energy is diverted from typical growth and development like during stressful experiences (Gluckman & Hanson, 2006; Hallgrímsson, 1999).

How Does Fluctuating Asymmetry Change Ontogenetically?

In both primate species investigated in this dissertation, FA did not change ontogenetically; this result is in contradiction to what has been found in previous studies. Hallgrímsson (1999) found an increase in cranial FA over ontogeny in humans and rhesus macaques, but this dissertation found no age-related changes in FA in either rhesus macaques (Chapter 2) or olive baboons (Chapter 3). In macaques, these findings could be due to the sample. This dissertation used rhesus macaques from one population, and some individuals in this population experienced ecological catastrophes that could have impacted their level of FA more than age alone. Hallgrímsson (1999) included rhesus macaques from two populations, one of which was the same as that used in this dissertation and the other was a captive colony, the skeletons from which are housed at the National Museum of Natural History. It makes sense that FA would accumulate over life, as morphological drift, biomechanical strains, and various stressors increase the amount of FA over time (Hallgrímsson, 1993), but it is possible that major stressors outweigh this signal. This explanation, however, does not explain the lack of age-related change in FA in the olive baboons examined in this dissertation.

Are There Secular Changes in Fluctuating Asymmetry?

One goal of this dissertation was to examine how FA might change over time and to understand its potential for evolution. While no changes in FA levels were observed over decades in macaques (Chapter 2), FA decreased over three decades in male baboons but not females (Chapter 3). The suggests some broader population-level changes that have yet to be explored in primates, or really most organisms. Only one study on bees has found changes over time in museum collections, though these were shown to reflect climatic changes (Arce et al., in press). This is unlikely to be a factor in the captive colony of baboons used in this sample because they are protected from climatic events in the facility in which they are housed. This protection offers stability in their environment, and therefore, the baboons are not forced to respond biologically to climatic shifts. One might expect FA to stay the same or decrease over

time in the absence of environmental changes because the system generally gets more efficient with time (Polak, 2003), but the sex-specific decrease in FA over time complicates this explanation.

To What Degree is Fluctuating Asymmetry Influenced by Genetic Factors?

To further understand the potential for FA to evolve, the heritability and evolvability of FA were estimated for both the macaque and baboon samples using known pedigrees. The heritability of FA was significantly non-zero for both populations, which demonstrates some additive genetic variation present influencing levels of FA. This indicates that FA is susceptible to selection to at least some degree, though very little considering the extremely low estimates for each of our sample populations. By comparing heritability ($h^2 = \sigma_A^2 \,/\, \sigma_P^2$) and evolvability ($I_A = \sigma_A^2 \,/\, \bar{X}^2$) estimates in these two samples as in Hardin (2019), we find that the additive genetic variation in baboons is higher than macaques. We know this because the mean FA in baboons was higher than in macaques yet the evolvability estimate was still higher in baboons despite a higher denominator. This difference suggests more potential for evolutionary changes in FA, though requires caution as these samples are from different environments and different species. Further, comparison of heritability estimates as in Hardin (2019) suggest that the captive colony of baboons lives in a more favorable environment for this species than the free-ranging environment of Cayo Santiago is for macaques (Charmantier & Garant, 2005).

Future Work

While this dissertation contributes to the existing FA literature on a number of topics, there is still much work to be done in teasing apart the contributing factors for FA and developmental instability. For example, we do not understand the exact nature of the relationship between stress and FA. Does FA increase linearly with the amount or degree of stress? Further, which types of environmental change cause the most stress, and therefore, the

most FA? Is there a rank in importance for types of stress and environmental change that affect levels of FA? In terms of natural disasters, is it the stressful event itself that causes elevated levels of FA or the stress from the ecological fallout after the event? Future work will include scanning additional specimens from the Cayo Santiago collection of rhesus macaques to get a broader sample of individuals that experienced a hurricane and further investigate the vulnerable periods of development and how FA manifests in individuals who experience natural disasters. Additionally, a sample of captive rhesus macaques from the same genetic origin as the free-ranging individuals on Cayo Santiago are available and would be a unique opportunity to investigate the role captivity and free-ranging environments play in development and perpetuation of FA. There are also samples of wild olive baboons from Kenya in museums that could serve this purpose equally well. Lastly, the relationship between FA and stress is theoretically and experimentally supported in many cases (e.g., Badyaev et al., 2000; Harrington et al., 2019; Hosken et al., 2000; Nascimento et al., 2021). These studies show that increases in temperature both on land and in the ocean, stressful seasons, and vegetation removal are all associated with elevated FA in a broad range of organisms. However, no smoking gun has illustrated the relationship between these two variables. It is possible that by using allostatic load indices, a measure of lifetime physiological stress (Edes et al., 2018), we can understand the exact nature of the relationship between FA and stress. The three projects mentioned above have been incorporated into my plan for my research program and I look forward to furthering our knowledge of the environmental impact on our physiology and morphology.

Conclusion

This dissertation explored the impact of environmental factors on the development and perpetuation of FA and sought to understand the role evolution may play in the FA exhibited in two populations of primates: the free-ranging Cayo Santiago rhesus macaques and the

Southwest National Primate Research Center olive baboons. Results suggest that FA levels may be sex-specific in species with extreme sexual dimorphism, and FA generally seems not to change over ontogeny in these populations. Secular changes in FA appear possible in primates, although the pattern remains ambiguous. Results also show that ecological catastrophes such as hurricanes are critical for increasing FA later in life if experienced as a fetus. Lastly, FA seems to have some additive genetic variation that is subject to selection, though minimal. Overall, this work offers additional resolution in teasing apart factors contributing to FA and points to minimal genetic influence on FA levels.

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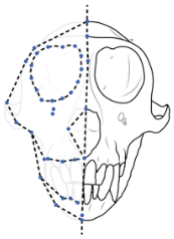

Ashly N. Romero
CV

CONTACT:
anromero@uark.edu
https://ashlyromero.wixsite.com/anromero

EDUCATION

2023	**Doctor of Philosophy**, Anthropology University of Arkansas Dissertation title: *Understanding the environmental and genetic influence on fluctuating asymmetry and developmental instability in primates* Language competency: Spanish | Microcertificate: Preparing for the Professoriate
2018	**Master of Arts**, Anthropology University of Arkansas Thesis title: *A comparison of craniofacial asymmetry in Gorilla gorilla gorilla and Pan troglodytes troglodytes*
2016	**Bachelor of Arts**, Anthropology (*Magna cum laude*) California State University – Long Beach (CSULB)

RESEARCH INTERESTS

Primate cranial morphology and evolution; comparative anatomy and morphology; fluctuating asymmetry and developmental instability; stress; ontogeny; geometric morphometrics

PUBLICATIONS

In review	**Romero AN**, Dickinson E, Turcotte CM, Terhune CE. *Skeletal age during hurricane impacts fluctuating asymmetry in Cayo Santiago rhesus macaques*. Ecology and Evolution.
In prep	**Romero AN**, Terhune CE. *Pathology and secular-related changes in fluctuating asymmetry detected in olive baboons*. International Journal of Primatology.
In prep	**Romero AN**, Yim A, Terhune CE. *Heritability and evolvability of fluctuating asymmetry in rhesus macaques and olive baboons*. American Journal of Biological Anthropology.
2022	**Romero AN**, Mitchell DR, Cooke SB, Kirchhoff CA, Terhune CE. *Craniofacial fluctuating asymmetry in* gorillas, chimpanzees, and macaques. American Journal of Physical Anthropology 177(2):286-299.
2019	Yoakum CB, **Romero AN**, Moore C, Douglas E, Gallagher K, Terhune C. *Sex and height influence neck posture when using electronic handheld devices*. Clinical Anatomy 32(8):1061-1071.
2017	**Romero AN**, Herlin M, Finnilä M, Korkalainen M, Håkansson H, Viluksela M, Sholts SB. *Skeletal and dental effects on rats following in utero/lactational exposure to the non-dioxin-like polychlorinated biphenyl PCB 180*. PloS One 12(9):e0185241

2017 Stankowich T, **Romero AN**. *The correlated evolution of antipredator defences and brain size in mammals*. Proceedings of the Royal Society London, Biology 284(1846):20161857

GRANTS AND AWARDS

2022 SEC Emerging Scholars Program ($17,000)
2022 Summer Institute in Statistical Genetics (SISG) Scholarship ($900)
2022 American Association for Anatomy Graduate Poster Award Finalist ($400)
2021 P.E.O. Scholar Award ($20,000)
2021 ARSC Graduate Dissertation Research Award ($5,000)
2021 Graduate and Professional Student Congress Research Grant ($1,500)
2020 Professional and Internship Grant Sponsorship (PIGS) Award ($500)
2019 Arkansas Graduate Fellowship in Anthropology ($8,500)
2018-2022 Distinguished Doctoral Fellowship, University of Arkansas ($22,000/year)
2018 Best Overall Presentation, Lambda Alpha Student Symposium, Wichita State University ($100)
2018 Professional Awareness, Advancement, and Development (PAAD) fellow, National Science Foundation (NSF)
2017 Honorable Mention, Graduate Research Fellowship Program, NSF
2016 College of Liberal Arts Outstanding Graduate, California State University – Long Beach (CSULB)
2016 Outstanding Baccalaureate Student in Anthropology, CSULB
2016 Award for Excellence in Anthropology, CSULB
2015 NSF REU, National Museum of Natural History
2012-2016 President's Scholars' Program, CSULB ($61,000)

PUBLISHED ABSTRACTS (*Notes mentees)

Accepted **Romero AN**, Dickinson E, Turcotte CM, Terhune CE. *Skeletally mature individuals that experienced hurricane Hugo exhibit decreased craniofacial fluctuating asymmetry*. Invited symposium: Coming to the Caribbean - Celebrating 85 Years of Rhesus macaques at Cayo Santiago; New Endeavors of Non-Human Primate Research. American Association of Biological Anthropologists.
Accepted **Romero AN**, Yoakum CB. *Examining the relationship between inferior alveolar nerve volume and dietary properties in primates*. American Association for Anatomy.
2022 **Romero AN**, Terhune CE. *The role of social group and matriline on fluctuating asymmetry in female rhesus macaques*. American Association for Anatomy. FASEB J 36(S1).
2022 **Romero AN**, Mitchell DR, Terhune CE. *Cranial fluctuating asymmetry and reproductive fitness in the Cayo Santiago rhesus macaques*. American Association of Biological Anthropologists. AJBA 177(S73):154.
2022 Bowland LA, **Romero AN**, Kelly CD. Old questions, new methods: testing hypotheses for canine dimorphism in the evolution of anthropoid primates using phylogenetic analyses. American Association of Biological Anthropologists. AJBA 177(S73):20.
2021 Barton AB*, Knox O*, **Romero AN**, Terhune CE. *Analysis of inter- and intraobserver error when scoring tooth wear in the Cayo Santiago macaques*. Undergraduate symposium. American Association of Physical Anthropologists.
2020 **Romero AN,** Mitchell DR, Terhune CE. *Analysis of landmark variation in the study of cranial fluctuating asymmetry*. American Association of Physical Anthropologists. AJPA 171(S69):238.

2019 **Romero AN**, Kirchhoff CA, Cooke SB, Terhune CE. *Examining fluctuating asymmetry in Macaca fascicularis*. American Association of Physical Anthropologists. AJPA 168(S68):207.

2019 Kirchhoff CA, Cooke SB, **Romero AN**, Terhune CE. *Covariation among dental wear, craniofacial morphology, and pathologies in Macaca fascicularis*. American Association of Physical Anthropologists. AJPA 168(S68):126.

2018 Moore CL, Yoakum CB, **Romero AN**, Douglas E, Gallagher K, Terhune C. *A Biomechanical Analysis of Cervical Spine Posture using Geometric Morphometrics*. American Association for Anatomy. FASEB J 32(S1).

2018 **Romero AN**, Terhune CE. *A comparison of fluctuating asymmetry models in non-human primate crania*. American Association of Physical Anthropologists. AJPA 165(S66):230.

2016 **Romero A**, Sholts S, Håkansson H, Viluksela M. *Craniofacial and dental effects shown in rats following in utero/lactational exposure to 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB-180)*. American Association of Physical Anthropologists. AJPA 159(S62):272.

INVITED PRESENTATIONS

2023 *Introduction to Primates*. University of Minnesota, Delivered virtually to undergraduate students; February 15, 2023. Seminar.

2020 *Cranial asymmetry in Primates: What is it and how does it get there?* Paradigm in Biological Anthropology Research Series, Delivered remotely to graduate students in India; November 1, 2020. Seminar.

2018 *Fluctuating asymmetry in gorillas and chimpanzees*. Arkansas Tech University, Russellville, AR; October 10, 2018. Seminar.

PROFESSIONAL TRAINING

2022 Evolutionary Quantitative Genetics Workshop
University of Washington

2022 Summer Institute of Statistical Genetics (SISG)
University of Washington

- Fundamentals of Population Genetics
- Quantitative Genetics
- Association Mapping: GWAS and Sequencing Data

2020 Analysis of Organismal Form short course with Dr. Chris Klingenberg
University of Manchester

2019 CITI Program

- Conflicts of interest
- Humanities Responsible Conduct of Research
- Research with data or laboratory specimens
- Working with the IACUC

2018 Taphonomic Analysis Workshop
Terhune Lab, Dr. Sabrina Curran, University of Arkansas

RELEVANT EMPLOYMENT

2022-Present **Graduate Research Assistant**
MicroCT scanning technician
Department of Anthropology, University of Arkansas

2016-2022 **Graduate Teaching Assistant**

	Introduction to Biological Anthropology Lab Department of Anthropology, University of Arkansas
Fall 2021	**Online Instructor and Course Developer** Introduction to Biological Anthropology Department of Anthropology, University of Arkansas
Summer 2021	**Graduate Teaching Fellow** Human Gross Anatomy Congdon School of Health Sciences, High Point University, NC
2018-2019	**Graduate Assistant Coordinator** Introduction to Biological Anthropology Lab Department of Anthropology, University of Arkansas
2017-2018	**Departmental Intern** Environmental Health and Safety, University of Arkansas

RESEARCH EXPERIENCE

2022-2023	Segmenting pulp, dentin, and enamel of microCT-scanned macaque teeth in Avizo to create surface models for shape analysis MICRO, Dr. Kathleen Paul, University of Arkansas
2021, 2023	Morphological measurement, external microbiome swabbing, and face photography of live macaques, occasional necropsy assistant, bone categorization and packaging, and 3D surface scanning Cayo Biobank Research Unit, Dr. Michael Montague, Puerto Rico
2019	3D surface scanning, specimen photography, and macaque colony visit Laboratory of Primate Morphology (CPRC), Terry Kensler, Puerto Rico
2017	3D surface scanning and specimen photography Cleveland Museum of Natural History, Lyman Jellema, Cleveland, OH
2017, 2019	Pleistocene survey and collections identification/organization team member Co-directed by Dr. Claire Terhune, Dr. Sabrina Curran, and Dr. Alexandru Petculescu Oltet River Valley, Romania
2017	3D surface scanning and scan editing (Flexscan & Geomagic) Terhune Lab, Dr. Claire Terhune, University of Arkansas
2017-2020	Zoological collections management University Collections Facility, Dr. Mary Suter and Dr. Nancy McCartney (since passed), University of Arkansas
2016	Collection organization and database creation NAGPRA Lab, Cindi Alvitre, California State University – Long Beach (CSULB)
2014-2016	Undergraduate Researcher Mammal Lab, Dr. Ted Stankowich, CSULB
2015	Natural History Research Experience REU Internship, Dr. Sabrina Sholts, NMNH, Smithsonian Institution
2015	Mammal specimen preparation Vertebrate Zoology Lab, Suellen Jacob, CSULB
2014	Laboratory Intern Forensic investigation, Dr. John Wang, Glasgow Caledonian University

SERVICE AND OUTREACH

Journal Reviewer

Reviewer for articles in *American Journal of Biological Anthropology*, *Evolutionary Biology*, *Journal of Anatomy*, and *Emerging Topics in Life Sciences*. 2020-Present

Graduate and Professional Student Congress

Served as an at-large representative for the Fulbright College of Arts and Sciences and co-chair/grant reviewer for the Research Council. 2019-2020, 2021-2022

Diversity and Inclusion Committee

Serve as graduate student representative on the Department of Anthropology Diversity and Inclusion Committee to help draft and implement a diversity, equity, and inclusion plan for the department. 2019-2021

American Association of Physical Anthropology Meetings

Student volunteer at the annual American Association of Physical Anthropology meetings. 2018-2021

K-12 Workshops

Hold workshops at local elementary schools in which students complete a laboratory exercise that demonstrates how the concepts they are currently learning in math or science translate into biological anthropology. 2016-Present

Primatweeps Curator

Collective twitter account that uses games and polls to engage the wider public in primatology (#PrimatePlaytime, #PrimateTrivia, #GuessThePrimate, #MonkeyOrNot, etc.). 2019-2022

Peculiar Primates

Blog series that features a different primate in each post with the aim to increase scientific literacy and engage the public in biological anthropology. 2018-2019

Open House Events

Open lab or open collection events with the University of Arkansas Department of Anthropology and Arkansas Archaeological Survey that engage the wider community in scientific research. 2016-2019

Girls in STEM

Introduce concepts in biological anthropology to girls in the Northwest Arkansas community and share scientific research via booths at various girls in STEM/STEAM events. 2016-2019

PROFESSIONAL MEMBERSHIPS

American Association for Anatomy
American Association of Biological Anthropologists
Dental Anthropology Association
Society for the Study of Evolution
Sigma Xi
Lambda Alpha

Last updated: 07/14/23