

**Facial fluctuating asymmetry in three species of colobus monkeys**

**by**

**Jimmy Erkens**

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## Abstract

Fluctuating asymmetry (FA) in biological structures has historically been used as a proxy for developmental instability (DI), and therefore stress in development. Facial bones, specifically cranial bones, are of particular interest for FA analysis. To better understand differences in environmental stressors, particularly in the context of anthropogenic change, facial FA has been quantified in three species of colobus monkeys; two of these species (*Piliocolobus badius* and *Colobus polykomos*) are sympatric and inhabit the Taï region of Côte d'Ivoire. An additional population of southern Kenyan *C. angolensis palliatus* was included for comparative analysis; these populations represent a gradient of stress. Landmarks were imposed on primate crania using Viewbox 4 software; using *geomorph* and *morphoJ*, relative differences in FA were quantified through PCAs and FA component variance tests. The results from this project offer weak support in favor of this gradient, with significant evidence ( $p=0.035$ ) suggesting this population of Taï *P. badius* demonstrates a greater response to stress than the population of *C. angolensis palliatus*. Given recent alarming trends in *P. badius* conservation, future work comparing responses to stress among various populations and species of *Piliocolobus* is desired.

## Introduction

While most primate populations have sharply declined over the course of the colloquially defined Anthropocene, taxa vary in their vulnerability to environmental disruption. West African red colobus monkeys have been demonstrated to be uniquely sensitive to anthropogenic change (Isaac and Cowlshaw, 2004; Linder et al., 2021; Ruiz-Lopez et al., 2015). For example, the probable extinction of Miss Waldron's red colobus (*Piliocolobus badius waldronae*) has been reported on extensively (McGraw, 1998b; McGraw, 2005; Oates et al., 2000). It is clear the future of red colobus monkeys is precarious; without extensive efforts to ameliorate and rectify population declines in West Africa, one can expect red colobus conservation trends to worsen. The loss of primate species carries disastrous 'ripple effect' consequences for the broader ecosystems in which they inhabit (Estrada et al., 2017). Moreover, outside of commercialized ventures, the loss of red colobus monkeys has the potential to eliminate historical lifeways regarding bushmeat hunting and consumption for West African peoples inhabiting regions near red colobus habitats (van Vliet & Mbazza, 2011; Wilkie et al., 2016). Consequently, in reconciling with the inevitabilities of the Anthropocene, given their sensitivity to environmental change, role in forest ecosystems, and sociocultural relevance, red colobus monkeys are pertinent taxa for analysis.

A comparative comprehensive analysis of species-level stress is desired to assess red colobus monkeys' relative robusticity to anthropogenic stress. On an individual level, stress embodies a state of threatened homeostasis brought on by external and internal forces (Tsigos et al., 2020). Quantifying stress can bring relevant insights to conservation efforts as heightened stress has been demonstrated to decrease an organism's fitness as opportunities for reproduction become disrupted due to normative and novel anthropogenic stressors (Hofer & East, 1998;

Wingfield et al., 1997). Stress therefore serves as a relevant proxy for a species' overall wellbeing and robusticity to anthropogenic change.

A goal of this research is to guide current conservation efforts in sub-Saharan Africa within the greater context of increased anthropogenic change. Here we have compared developmental responses to stress between three colobus monkey species: Bay red colobus monkeys from Taï Côte d'Ivoire (*Piliocolobus badius*), King black-and-white colobus monkeys also from Taï (*Colobus polykomos*), and Angolan black-and-white colobus monkeys from southern Kenya (*C. angolensis palliatus*). Given environmental differences, in addition to historic conservation trends, these three species are hypothesized to demonstrate a gradient of responses to stress. *P. badius* is hypothesized to demonstrate a heightened response to stress relative to sympatric *C. polykomos*. *C. polykomos* is in turn hypothesized to demonstrate a greater response to stress compared to *C. angolensis palliatus*.

## Literature Review

An organism's incapacity to produce an 'ideal' structure under a particular set of conditions is reflective of its developmental instability (DI) (Zakharov, 1992). The lower an organism's stability, the more likely it is that deviations from an 'ideal' structure are produced. DI has been shown to be positively associated with the level of stress an organism experiences during development (Lens et al., 2002; Palmer, 1996; Pertoldi et al., 2005). Stress can be expected to disturb an organism's check mechanisms and consequently augment perturbations to an organism's development (Emlen et al., 1993). Moreover, population-level DI is positively associated with the degree of inbreeding within a given population, indicating that some aspect of DI is related to genetic stress (Lens et al., 2000; Loy et al., 2021).

The genetic component of DI is controversial and complex, as environmental stresses, and inbreeding depression result in similar deviations in development. For example, it is impossible to quantify inbreeding within a population through analysis of DI (Gilligan et al., 2000; Waldmann, 2001). However, if inbreeding in a population is known, greater DI should be expected within the population (Gomendio et al., 2000). Aside from inbreeding depression, the genetic component of DI in an organism should be conceptualized as its proneness to DI (Klingenberg & Nijhout, 1999); there is little evidence for specific genes with explicit impacts for DI, hence DI likely has a nonadditive genetic basis (Leamy & Klingenberg, 2005). Regardless, the heritability of FA itself has been demonstrated to be insignificant (Fuller & Houle, 2003). While the relation between genetic and environmental components of DI are still being understood, comparative studies of DI can reveal population level idiosyncrasies in responses to stress; asymmetry in biological structures has historically been used as a proxy for DI.

First, it must be understood that in this discussion of asymmetry, only deviations from bilateral symmetry are considered. Traditionally three types of symmetry have been identified within biological systems: fluctuating asymmetry (FA), directional asymmetry (DA), and antisymmetry (Klingenberg, 2015; Valen, 1962). Antisymmetry refers to asymmetries, common throughout an entire population, relating to an underlying genetic basis. Popular examples of this phenomenon include asymmetries in crustacean appendages (i.e., male fiddler crab signaling claws are naturally unequally proportioned) (Palmer, 2005). In this study, the structures analyzed adhere to bilateral asymmetry. Consequently, antisymmetry is not relevant to this discussion.

Directional asymmetry, in biological structures, refers to systematic deviations from perfect bilateral symmetry (Budečević, 2022). DA affects one side of a bilaterally symmetric

structure more than the other due to increased use of the preferred or dominant side (Cole et al., 2020; Franks and Cabo, 2014). Right and left-handedness in humans exemplifies DA as continued use and wear of the dominant side results in expected asymmetries in structures (Little et al., 2002; Palmer, 1996). Conversely, fluctuating asymmetry is best understood as “developmental noise,” or random deviations from symmetry in biological structures occurring during development (Adams & Niswander, 1967; Valen, 1962). For example, in humans, FA has been demonstrated to steadily increase before beginning to plateau or decrease at thirteen years of age, likely as a reflection of developmental regulation post-puberty (Palestis & Trivers, 2016). On a population level, measures of FA therefore demonstrate an individual’s cumulative response to stress in development.

FA remains one of the most popular and controversial proxies for developmental instability. FA has been illustrated to be a sensitive biomarker for environmental stress, and therefore a proxy for DI (Badyaev et al., 2000; Beasley et al., 2013; Emlen et al., 1993; Gomendio et al., 2000; Graham et al., 2010; Hoover et al., 2021; Hoover & Hudson, 2016; Jones, 1987; King et al., 2009; Manning, 1994; Palmer & Strobeck, 1986; Willmore et al., 2005). Stressors such as obesity, exposure to heavy metals and pollutants, and severe weather have all been demonstrated to significantly impact FA (King et al., 2009; King et al., 2012; Sánchez-Chardi et al., 2013; Savriama et al., 2015; Wells et al., 2006). Conversely, the presence of similar environmental stressors has also been demonstrated to have an insignificant impact on population FA (Graham & Özener, 2016; Leamy et al., 1999; Wuytack et al., 2011). Moreover, secondary sexual characteristics, particularly among males, appear to be more prone to FA (Gomendio et al., 2000; Manning, 1993; Manning, 1994). Ultimately, the conflicting and inconsistent results of FA analysis appear to be derived from a lack of understanding of the



mechanics behind DI and the impacts of individual stressors (Dongen, 2006). However, other biomarkers of DI evident in morphology may not yield insight for these taxa; for example, enamel defects stemming from linear enamel hypoplasias, remain relatively inconspicuous in monkeys and are not necessarily robust to enamel wear (Guatelli-Steinberg & Skinner, 2000). This study acknowledges the limitations and inconsistencies of FA as a proxy for DI. Regardless, FA's historical precedence, utility among the taxa highlighted in this study, and significance as a proxy for DI justify its use as a proxy for responses to stress.

When examining developmental instability, determining what qualifies as an 'ideal' biological structure is often difficult. To mitigate this uncertainty, bilaterally symmetric structures are frequently used to quantify DI, as symmetry in these structures is 'ideal' (Palmer, 1996). Both FA and DA result in bilateral asymmetry; consequently, it is necessary to analyze structures in which these two components of asymmetry can be isolated. Previous analyses of bilateral asymmetry in post-cranial structures in human and non-human primates have failed in differentiating FA and DA. For example, among hominins, normative and preferential (i.e., right or left-handedness) mechanical wear in appendages have resulted in expected asymmetries in structures (Hallgrímson et al., 2002; Latimer & Lawrence, 1965; Little et al., 2002; Sarringhaus et al., 2005). Consequently, analyses of FA should be conducted on structures that are not prone to mechanical wear (Palmer & Strobeck, 2003). In primates, craniofacial structures appear to be good candidates for FA analysis (McGrath et al., 2022; Romero et al., 2022).

Craniofacial asymmetry also has relevant consequences for mate selection. Given that secondary sexual structures are already more prone to FA, it has been hypothesized that FA has an important role in sexual selection (Møller & Pomiankowski, 1993). In humans, craniofacial symmetry is significantly correlated with averageness, parasite resistance, and immune response

(Enquist & Arak, 1994; Grammer & Thornhill, 1994; Thornhill & Gangestad, 1993). In contrast, it has been demonstrated that certain, asymmetric, facial features that deviate from averageness are perceived as attractive by large portions of the human population (DeBruine et al., 2007). Concerning non-human primates, preferences for symmetry have been illustrated in macaques (Waitt & Little, 2006). On the other hand, among *Sapajus apella*, facial asymmetry has little impact on sexed mate preference, but rather plays an important role in male-male aggression (Paukner et al., 2017). While facial asymmetry and symmetry influence mate selection and are therefore relevant for discussions of fitness, these relations are highly complex and cannot be reduced to a single causal link. Ultimately, while behavior, during and beyond organismal development, impacts bilateral symmetry, the inverse relation is considerably less consistent in the literature.

Habitat loss and forest fragmentation are likely the most conspicuous anthropogenic stressors in Taï, Côte d'Ivoire. One of the largest motivators for these phenomena in Côte d'Ivoire is the cocoa industry. As Côte d'Ivoire is the world's largest cocoa producer, the growth of this industry has consequently resulted in mass deforestation across the region (Bitty et al., 2015). Not only are forest fragments unable to sustain populations of colobus monkeys, specifically *P. badius*, but may also heighten the severity of present stressors such as food insecurity and parasitism (Chapman et al., 2006; Chapman et al., 2007a; Chapman et al., 2007b; Pride, 2005; Schoof & Jack, 2013). The social groups of primates in forests experiencing fragmentation may also be disrupted (Aronsen et al., 2015; Jaimez et al., 2011; Snaith et al., 2008). In this study, forest fragmentation, as a consequence of the expanding cocoa agricultural industry, is a relevant stressor for *P. badius* and *C. polykomos*.

*P. badius* has historically fared worse than *C. polykomos* in coping with forest fragmentation. Moreover, deforestation has the potential to change existing environmental stressors in Taï, such as chimpanzee predation (McGraw et al., 2007). Prevalence of retroviruses, like simian immunodeficiency virus (SIV), simian T-cell lymphotropic virus type-1 (STLV-1), or simian foamy virus (SFV), also has the potential to change because of habitat change. Already, *P. badius* demonstrate some of the highest rates of SIV (82%), SFV (86%), and STLV-1 (50%) compared to other primates (Gogarten et al., 2014; Goldberg et al., 2009; Leendertz et al., 2010; Locatelli et al., 2011). Forest fragmentation also has the potential to increase rates of inbreeding within a population (Mathiasen et al., 2007). Consequently, among these species, genetic stress, another component of FA, is hypothesized to be more severe because of this stressor. Ultimately, in Taï both *P. badius* and *C. polykomos* encounter intensive anthropogenic environmental change and are hypothesized to be exposed to many of the same stressors. However, *P. badius*, within the context of anthropogenic change, has encountered intense population decline relative to *C. polykomos*. We therefore hypothesize that it is less able to withstand stress and more likely to demonstrate FA.

Outside of forest fragmentation, bushmeat consumption is another relevant anthropogenic stressor for all Taï monkeys and has been identified as a primary motivator for the likely extinction of Miss Waldron's red colobus monkeys (McGraw, 1998; Oates et al., 2000; McGraw, 2005). Bushmeat has historically been consumed to mitigate food insecurity due to poverty. However, the bushmeat trade has become increasingly commercialized in the Taï region (Casparly, 2001; Lindsey et al., 2013). Consequently, all monkeys in the Taï region except for, surprisingly, *P. badius*, are hunted for bushmeat at unsustainable rates (Refisch & Koné, 2005a; Refisch & Koné, 2005b). Restaurant and market owners, as well as intermediaries, largely

exploit bushmeat hunters. In order for hunters to profit, it is necessary to kill large numbers of primates. Although *P. badius* are hunted at slightly lower rates than other primates, human predation should not be overlooked as a significant stressor for this taxon, especially given historical trends in population loss.

Compared to the populations of *P. badius* and *C. polykomos*, one population of Kenyan *C. angolensis palliatus* is hypothesized to be exposed to considerably less stressors. Relative to other primates in southern Kenya, *C. angolensis palliatus* is hunted considerably less. Additionally, although habitat loss remains a salient stressor for this population (as is for most non-human primates), the population highlighted in this study is hypothesized to be buffered from this phenomenon (Anderson et al., 2007). This species therefore serves as a control for the two Tai species.

## Predictions

*P. badius* will display greater fluctuating asymmetry than both *C. polykomos* and *C. angolensis palliatus*. Regardless of relative exposures to stressors, it is hypothesized *P. badius* is more vulnerable to stress than *C. polykomos* (particularly in the context of increased anthropogenic change). It is also hypothesized that *P. badius* and *C. polykomos* are exposed to more stressors than *C. angolensis palliatus*. Hence, *C. polykomos* will demonstrate greater FA than *C. angolensis palliatus*. Moreover, it is hypothesized across all three primate taxa that males and females will differ in FA severity. It is not hypothesized that males and females differ in stressors. Rather, it is expected that sexually selected structures will result in greater FA and differences in biology could lead to differences in FA. For example, in male *P. badius*, the circumorbital region and brow ridges are robust. Consequently, it is predicted that male *P. badius* differ in FA compared to female *P. badius* (Fannin et al., 2021; Møller & Pomiankowski,

1993). Similarly, sexual dimorphism in other portions of craniofacial structure among male and female *P. badius*, *C. polykomos* and *C. angolensis palliatus* justify this hypothesis (Hayes et al., 1996).

## Materials and Methods

In this study, 81 *P. badius* crania from Taï National Park, Côte d'Ivoire, 21 *C. polykomos* crania also from Taï, and 34 *C. angolensis palliatus* crania from southern Kenya were examined in the OSU Department of Anthropology. From these, 63 *P. badius*, 17 *C. polykomos*, and 23 *C. angolensis palliatus* cranial models were constructed using photogrammetry (Table 2.1). *P. badius* and *C. polykomos* crania in this study were opportunistically collected over a thirty-year period as part of the ongoing Taï Monkey Project in Côte d'Ivoire. Many of the *C. polykomos* and *P. badius* crania collected from Taï were contaminated with anthrax. *C. angolensis palliatus* were opportunistically collected from a resort environment in southern Kenya. It should be noted that the sample size for *C. polykomos* and *C. angolensis palliatus* in this study is insufficient for some statistical tests (Parsons, 1990). Nevertheless, results gleaned from this analysis will be presented as preliminary analysis and should be treated with caution.

Three-dimensional textured models of primate crania were created through photogrammetry in the Emerging Technology Studio space at The Ohio State University. Each cranium was positioned on a white turntable against a white background with high-contrast lighting. For each cranium, a photo was taken while adjusting the turntable by five degrees. This process was repeated for six angles to yield at least two-hundred distinct photos for each cranium. Point clouds for each specimen's set of photos were generated using RealityCapture software (<http://capturingreality.com>). Using this software, a mesh model and texture was created for each individual point cloud. Because this method is reliant on photographic data,

these models can be adjusted alongside improvements in rendering technology. Consequently, compared to traditional scanning methods, models generated through photogrammetry are extremely robust and reduces the negative effect of model accuracy in analysis (McGlone, 2004).

Using Viewbox 4 software (<http://www.dhal.com/>), 22 landmarks (Table 2.2) were imposed on each model in accordance with previous studies (Romero et al., 2022). These landmarks were chosen to minimize the effect of DA in this analysis. Furthermore, landmarks derived from Romero 2022 et al. have been modified to preserve the interpretation of this analysis as facial FA. Due to time constraints, only fixed landmarks have been included in this configuration. Future work should incorporate sliding semi-landmarks in its methods to reveal greater variation in morphology. To reduce additional uncertainty, individuals missing any landmarks were systematically excluded from this study. Examples of models created via photogrammetry and a sample landmarked *P. badius* specimen are provided (Figures 3.1-3.3).

In order to quantify fluctuating asymmetry for any structure, it is first necessary to conduct a generalized Procrustes analysis (GPA). A GPA reflects, rotates, and aligns landmarks such that each object's centroid is centered at the origin and goodness-of-fit criterion is minimized (Gower, 1975). This process removes the effects of scale, orientation, and position (McGrath et al., 2022). A GPA makes it possible to describe differences in shape between individuals. It should be noted that the GPA is performed not on the  $n$ -number,  $k$ -dimensional objects but rather on the points,  $p$ , defined and landmarked by an individual. A GPA provides a least-squares correspondence of  $n$ ,  $k$ -dimensional, matrices, each consisting of  $p$  points; essentially, this process generates an approximation of an unknown optimal matrix of landmarks (Akca, 2003). In this study, the optimal matrix represents the average three-dimensional landmark coordinates for each species.

Once all specimens were landmarked, principal component analyses of the asymmetry and symmetric components of shape variation were conducted as a diagnostic procedure in *morphoJ*. Here, the symmetric component is the symmetric average of original and mirrored landmark configurations, and the asymmetry component is measured by deviations from original configurations and the symmetric component (Klingenberg, 2011). If PC1 and PC2 of the symmetric component are distinct by species, then it can be concluded that species differ in morphology. Moreover, this result indicates that landmarks capture shape variation, justifying further analysis. For an ideal PCA of the asymmetry component, PC1 and PC2 are centered at the origin (0,0) and overlap between species. Deviation from the origin is indicative of relative severity. This plot provides a visual representation of asymmetry and can key into general trends; however, it does not directly test hypotheses of interest. If this PCA appears normal, then we can proceed with formal hypothesis testing.

If PCA diagnostics are sufficient, an initial ANOVA of asymmetry component (for all individuals, and then for each species) to determine if asymmetry is significant will be conducted in R using *geomorph*. If asymmetry is present in each species, a hierarchical ANOVA model of the FA component (specimen specific side deviation adjusted for mean L/R shape among all individuals) in accordance with species, sex, and an interaction term will be performed. Tests of FA component variance will then be conducted in accordance with significant terms ( $FA \sim \text{Species}_i \times \text{Sex}_j; i \in \{1, 2, 3\}, j \in \{1, 2\}$ ). For example, if “species” is significant, then pairwise difference in FA component variance tests will be conducted to determine which species-level differences are significant. Significant differences in FA component variance by species are indicative of differences in FA severity, and therefore species-level responses to

stress (Adams et al., 2022; Baken et al., 2021; Collyer et al., 2018; Collyer et al., 2021). This procedure directly tests the hypotheses presented in this thesis.

## Results

Visual representations of average Procrustes landmarks for each species are provided (Figures 3.6 – 3.8). Visual representations of all Procrustes landmarks for each species are also presented (Figures 3.9 – 3.11). Again, these landmarks control for differences in specimen sizes and exclusively capture shape variation. Examining diagnostics, the PCA of the symmetric component (Figure 3.4) reveals morphological differences between each species. For the symmetric component, PC1 explains 32.04% of the variation in shape and PC2 explains 15.57% of the variation in shape. PC1 likely describes differences between *Piliocolobus* and *Colobus*, whereas PC2 appears to describe differences within *Colobus*. The landmarks in this study capture craniofacial shape variation. For the PCA of the asymmetry component (Figure 3.5), PC1 explains 30.40% of the variation in shape and PC2 explains 14.29% of the variation in shape. The PCs are centered at the origin and data is concentric, hence we may proceed with further analysis. There are many notable *P. badius* outliers, indicating these individuals are considerably more asymmetric than other specimens.

Procrustes ANOVA output and Goodall F tests show that when including all three primate taxa, side variation is significant (Table 3.1). Similarly, for *P. badius* (Table 3.2), *C. polykomos* (Table 3.3), and *C. angolensis palliatus* (Table 3.4), side variation is significant. Each species is significantly symmetric. Results from the hierarchical ANOVA model,  $FA \sim \text{Species}; \times \text{Sex}_j; i \in \{1, 2, 3\}, j \in \{1, 2\}$ , are provided (Table 3.5). Here, variation in the FA component is only significantly different between species. Although sex was found to be insignificant, analysis of variation in the FA component has been conducted to test for both effects (unsexed



individuals were systematically removed). The interaction effect was highly insignificant and subsequently excluded from individual hypothesis testing.

The resulting Procrustes variances of the FA component are provided for each species alongside pairwise absolute differences between these values are also listed (Table 3.6, 3.7; Figure 3.12). Absolute differences may not capture the true relations between species, hence pairwise ratios in FA component variation are also shown (Table 3.8). Here, it is seen that variation in the FA component for *P. badius* is 2.052 times greater than for *C. angolensis palliatus*, and 1.534 times larger than for *C. polykomos*. Additionally, variation in the FA component of *C. polykomos* is 1.338 times greater than *C. angolensis palliatus*; corresponding P-values demonstrate the strengths of these differences (Table 3.9). The only significant P-value is between the variance in FA component in *P. badius* and *C. angolensis palliatus*.

In this study, there is significant evidence to suggest that Taï *P. badius* demonstrate greater facial fluctuating asymmetry than Kenyan *C. angolensis palliatus*. Analysis pertaining to sex was insignificant at every level of analysis. While this result was reflected in the ANOVA output, analysis on sex was performed to understand the sexed component of FA in these populations. The corresponding raw Procrustes variances (Table 3.10; Figure 3.13), as well as pairwise absolute differences (Table 3.11), pairwise ratios (Table 3.12), and pairwise P-values (Table 3.13) in variance of FA component are provided.

## Discussion

This is the first comparative analysis of facial fluctuating asymmetry among species of colobus monkeys. Quantifying relative responses to stress is a necessary step in understanding these species' robusticity to anthropogenic change in the Taï region (Bitty et al., 2015; Hofer &

East, 1998; Tsigos et al., 2020). These findings therefore have the potential to confirm and influence current conservation efforts.

This thesis finds that Taï *P. badius* demonstrate significantly more variation in FA component, and therefore exhibit more severe FA, than the Kenyan population of *C. angolensis palliatus*. Although not significant, the sample of *P. badius* demonstrates greater variation in the FA component than that of *C. polykomos*, which is in turn larger than variation in the FA component for *C. angolensis palliatus*. There is therefore weak support in favor of the hypothesized gradient of stressors imposed on these three taxa. This study does not support the hypothesis that male and female colobus monkeys, across all three taxa, differ in responses to FA. This result was surprising as FA has some role in sexual selection and is more severe in secondary sexual structures, such as the *P. badius* circumorbital region (Fannin et al., 2021; Gomendio et al., 2000; Manning, 1993; Manning & Chamberlain, 1994; Møller & Pomiankowski, 1993). However, this analysis is limited by the utility of FA as a proxy for DI (and therefore responses to stress) among these populations, especially when genetic and environmental components of FA are not decoupled. Consequently, future work may be interested in quantifying responses to stress through other means.

The primary limitation in this study is sample size. In general, and especially relevant for studies of sexually dimorphic taxa, for each taxon, a sample size of at least 20 has historically been illustrated to be the minimum number of samples required to conduct relevant analyses of FA (Parsons, 1990). Because the sample size of Taï *C. polykomos* is 17 individuals, the scope of analysis is limited. This constraint does warrant some concern; however, the procurement of additional colobus crania fell outside the scope of this study. Consequently, this work should be considered preliminary and exploratory analysis of FA across these three taxa.

Analyses of *P. badius* and *Colobus* genomes are necessary to isolate the environmental component of FA and quantify relative differences in exposure to environmental stressors, in particular anthropogenic stressors. The literature is divided on the degree to which FA is a reliable indicator of environmental stress (Badyaev et al., 2000; Beasley et al., 2013; Emlen et al., 1993; Graham et al., 2010; Graham & Özener, 2016; Gomendio et al., 2000; Hoover et al., 2021; Hoover and Hudson, 2016; King et al., 2009; Jones, 1987; Leamy et al., 1999; Manning, 1994; Palmer & Strobeck, 1986; Willmore et al., 2005; Wuytack et al., 2011). However, regardless of the utility of FA as a proxy for DI, it is evident that FA is likely perpetuated by numerous genes. The complexity of this process is compounded since it has been demonstrated that an organism's propensity for FA is heritable (Leamy & Klingenberg, 2005; Klingenberg & Nijhout, 1999). Consequently, while the results from this study suggest a gradient in responses to stress among *P. badius*, *C. polykomos*, and *C. angolensis palliatus*, interpretation is limited since this study does not address the genetic component of FA.

One of the most notable genetic influences on FA is the presence and frequency of inbreeding within a population. Deleterious mutations occurring because of inbreeding, have the potential to increase an organism's proneness to FA (Charlesworth & Willis, 2009; Lens et al., 2001; Loy et al., 2021; McGrath et al. 2022). While individual intraspecific genetic variation in expression of FA is likely to have little impact on population-level comparisons, if not accounted for in analysis, inbreeding has the potential to obscure the effects of environmental stressors on FA. Data concerning inbreeding among these three populations was not accessible to the researcher at time of analysis. Future work comparing species-wide trends in FA among colobus monkeys must incorporate the relative frequencies of inbreeding within each population. Moreover, inbreeding is of interest to this study since increased forest fragmentation, such as that

which occurs at Taï, has been demonstrated to increase the frequency of inbreeding in a population (Mathiasen et al., 2007). Consequently, *P. badius* and *C. polykomos* are hypothesized to have relatively higher rates of inbreeding than *C. angolensis palliatus*. To reiterate, the data from this research do not support or refute this hypothesis as it is impossible to glean genetic information from FA analysis (Gilligan et al., 2000; Waldmann, 2001).

Taï, relative to other environments that support *Piliocolobus*, is defined by numerous unique stressors. Intense bushmeat consumption and deforestation in the Taï region have aggressively reduced populations of *P. badius* and *C. polykomos* (McGraw, 1998a; McGraw, 1998b; Oates et al., 2000; Caspary, 2001; McGraw, 2005; Lindsey et al., 2013; Bitty et al., 2015). The presence of anthrax contamination among both Taï samples also remains a potential outlet for future analysis. However, *P. badius* and *C. polykomos* are exposed to unique stressors, such as intense retrovirus prevalence as well as chimpanzee and crowned eagle predation, which are likely augmented by increased anthropogenic change in the region (Gogarten et al., 2014; Goldberg et al., 2009; Liégois et al., 2008; McGraw et al., 2006; McGraw et al., 2007; Struhsaker & Leakey, 1990). This work supports the notion that Taï colobines, in general, experience a heightened response to stress relative to *C. angolensis palliatus*. Given the conservation plight of *P. badius*, and their extreme vulnerability to anthropogenic change, this study argues for a larger comparative analysis of *P. badius* across sub-Saharan Africa.

Lastly, researchers pursuing morphological work within the context of geometric morphometrics, particularly within the context of three-dimensional geometric morphometrics, may be interested in procuring three-dimensional models through the means of photogrammetry. The textured models procured in this research exceeded expectations and will be used in future research. Photogrammetry as a method, also has great potential for student engagement and for

science communication, which may be of interest (Summers et al., 2022). Traditional scanning methods are not robust to improvements in technology. In contrast, through photogrammetry, once photographs of skeletal structures have been procured, three-dimensional models can easily be updated as processing software improves. Photogrammetry may be a cheap and practical option for generating three-dimensional models.

## Conclusions

Fluctuating asymmetry has historically been used as a proxy for species-level responses to stress, both environmental and genetic. This work finds that *P. badius* at Tai, exhibit more severe FA than Tai *C. polykomos*, which in turn exhibit more severe FA than Kenyan *C. angolensis palliatus*. However, only differences in FA severity between *P. badius* and *C. angolensis palliatus* are significant (increasing sample sizes of *C. polykomos* and *C. angolensis palliatus* should clarify this hypothesized relation). *P. badius* demonstrate a heightened response to stress compared to both *C. polykomos* and *C. angolensis palliatus*. This idiosyncrasy reflects *P. badius* historic lack of resilience to environmental fluctuations, particularly in the context of intense anthropogenic change. Future work should highlight differences in stress between species and populations of *Piliocolobus* to better understand the underpinnings and variations of this unfortunate idiosyncrasy. Moreover, given the presence of secondary sexual traits among colobus monkeys, future endeavors should reassess differences in FA severity across sex.

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**Tables**

Table 2.1 Sample details

Species	N Male	N Female	N Unknown (X)	Total N
<i>Ptilocolobus badius</i>	40	21	2	63
<i>Colobus polykomos</i>	9	8	0	17
<i>Colobus angolensis palliatus</i>	10	11	2	23

Table 2.2 Landmarks

Landmark	Midline / Bilateral	Description
1, 13	Bilateral	Most inferior point of masseter muscle attachment
2, 14	Bilateral	Most lateral point of zygomatic foramen/foramina
3, 15	Bilateral	Most superior point of supraorbital foramen
4, 16	Bilateral	Most inferior point of premaxillary – maxillary suture
5, 17	Bilateral	Most lateral and posterior point of palatine foramen
6, 18	Bilateral	Most anterior point of zygomatic – maxillary suture
7, 19	Bilateral	Most medial point of zygomatic – frontal suture
8, 20	Bilateral	Most medial point of infraorbital foramina
9, 21	Bilateral	Most lateral point of infraorbital foramina
10, 22	Bilateral	Most lateral point of incisive foramen/foramina
11	Midline	Most inferior and middle extent of nasal bone juncture
12	Midline	Most anterior point of anterior nasal spine

Table 3.1 ANOVA output for bilateral asymmetry for all specimens (before isolating FA)

Effect	DF	SS	MS	Rsq	F	Z	Pr(>F)
individual	102	0.984	0.010	0.0117	0.987	-0.105	0.545
side	1	82.175	82.175	0.976	8405.250	9.163	<b>0.001</b>
individual x side	102	0.997	0.010	0.0119			
Total	205	84.156					

Table 3.2 ANOVA output for bilateral asymmetry for *Piliocolobus badius*

Effect	DF	SS	MS	Rsq	F	Z	Pr(>F)
individual	62	0.469	0.008	0.00911	0.942	-0.244	0.573
side	1	50.521	50.521	0.981	6294.367	8.628	<b>0.001</b>
individual x side	62	0.498	0.008	0.00967			
Total	125	51.487					

Table 3.3 ANOVA output for bilateral asymmetry for *Colobus polykomos*

Effect	DF	SS	MS	Rsq	F	Z	Pr(>F)
individual	16	0.095	0.0059	0.00693	1.067	0.0312	0.431
side	1	13.494	13.494	0.987	2428.857	2.986	<b>0.001</b>
individual x side	16	0.0889	0.0056	0.00650			
Total	33	13.					

Table 3.4 ANOVA output for bilateral asymmetry for *Colobus angolensis palliatus*

Effect	DF	SS	MS	Rsq	F	Z	Pr(>F)
individual	22	0.0969	0.0044	0.00522	1.087	0.218	0.534
side	1	18.377	18.377	0.999	4533.095	4.225	<b>0.001</b>
individual x side	22	0.0892	0.0041	0.00480			
Total	45	18.562					

Table 3.5 ANOVA output for FA component for all specimens

Effect	DF	SS	MS	Rsq	F	Z	Pr(>F)
species	3	102.630	34.210	0.987	2541.511	3.46	<b>0.001</b>
sex	2	0.036	0.018	0.00035	1.334	0.962	0.149
species : sex	3	0.034	0.011	0.00032	0.835	-0.519	0.704
Residuals	95	1.279	0.013	0.0123			
Total	102	103.978					

Table 3.6 Pairwise Procrustes variances in FA component by species

Species	Variance
<i>Piliocolobus badius</i>	0.0158
<i>Colobus polykomos</i>	0.0103
<i>C. angolensis palliatus</i>	0.00770



Table 3.7 Pairwise absolute differences between variance in FA component by species

	<i>Piliocolobus badius</i>	<i>Colobus polykomos</i>	<i>C. angolensis palliatus</i>
<i>Piliocolobus badius</i>	0	0.00551	0.00812
<i>Colobus polykomos</i>	0.00551	0	0.00260
<i>C. angolensis palliatus</i>	0.00812	0.00260	0

Table 3.8 Pairwise ratios in variance in FA component by species

	<b>Denominator</b>		
<b>Numerator</b>	<i>Piliocolobus badius</i>	<i>Colobus polykomos</i>	<i>C. angolensis palliatus</i>
<i>Piliocolobus badius</i>	1	1.534	2.052
<i>Colobus polykomos</i>	0.652	1	1.338
<i>C. angolensis palliatus</i>	0.487	0.748	1

Table 3.9 Pairwise P-values for difference in variance in FA component by species

	<i>Piliocolobus badius</i>	<i>Colobus polykomos</i>	<i>C. angolensis palliatus</i>
<i>Piliocolobus badius</i>	1	0.201	<b>0.035</b>
<i>Colobus polykomos</i>	0.201	1	0.525
<i>C. angolensis palliatus</i>	0.035	0.525	1

Table 3.10 Pairwise Procrustes variances in FA by sex

<b>Sex</b>	<b>Variance</b>
Female	0.0186
Male	0.0196

Table 3.11 Pairwise absolute differences between variance in FA by sex

	Male	Female
Male	1	0.00120
Female	0.00120	1

Table 3.12 Pairwise ratios in variance in FA component by sex

	<b>Denominator</b>	
<b>Numerator</b>	Male	Female
Male	1	1.065
Female	0.939	1

Table 3.13 Pairwise P-values for difference in variance in FA component by sex

	Male	Female
Male	1	0.781
Female	0.781	1

**Figures**

Figure 3.1



Figure 3.1: *C. angolensis palliatus* cranium

Figure 3.2



Figure 3.2: *C. polykomos* cranium

Figure 3.3

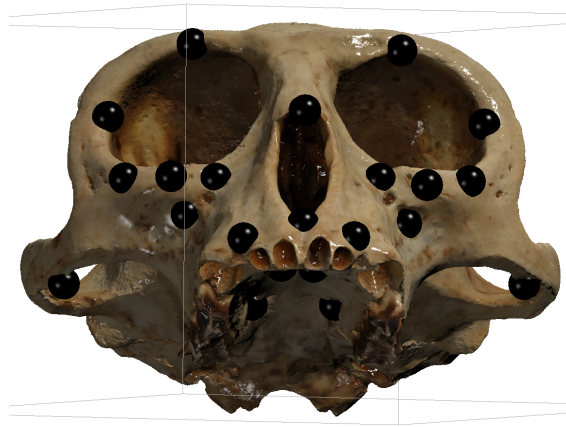
Figure 3.3: *P. badius* cranium with landmarks

Figure 3.4

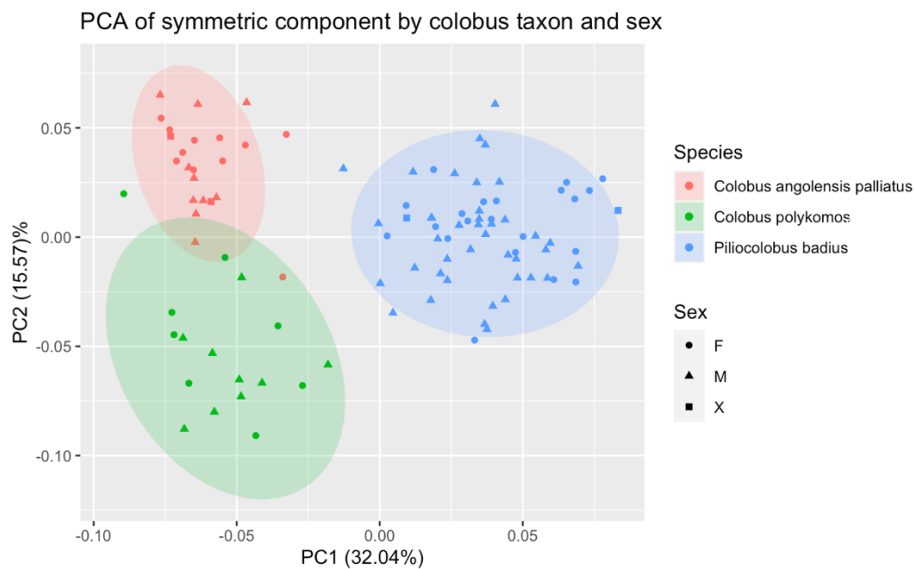


Figure 3.4: PCA of symmetric component of shape variation by colobus taxon and sex. PC1 likely describes differences in shape variation between *Piliocolobus* and *Colobus*, PC2 likely describes differences within *Colobus*.

Figure 3.5

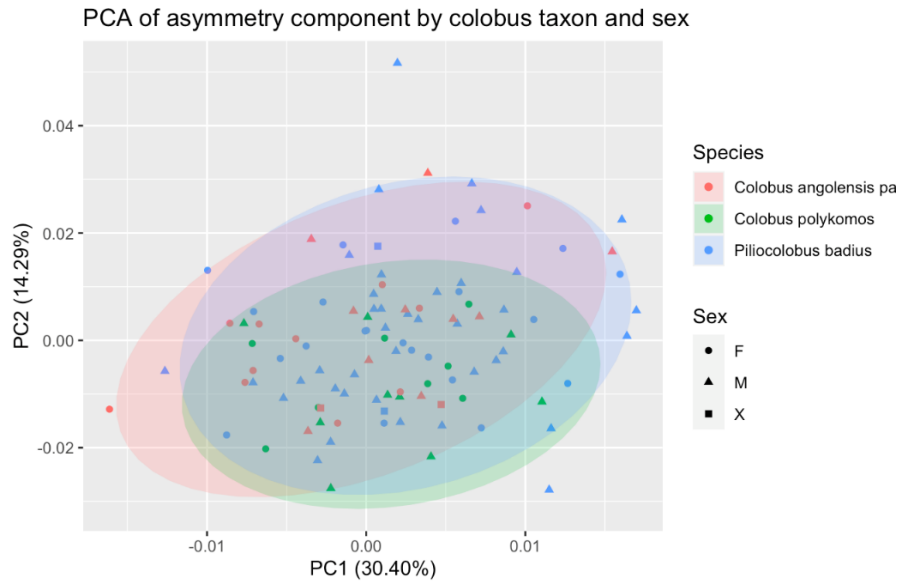


Figure 3.5: PCA of asymmetry component of shape variation by colobus taxon and sex, used as a diagnostic to proceed with further analysis. Data is centered at (0,0) and principal components are difficult to discern. Many *P. badius* individuals fall outside confidence ellipses. These specimens are relatively more asymmetric than other specimens.

Figure 3.6

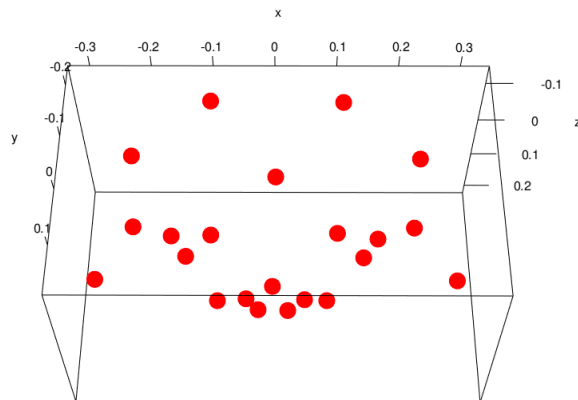


Figure 3.6: Average Procrustes landmarks for *P. badius*

Figure 3.7

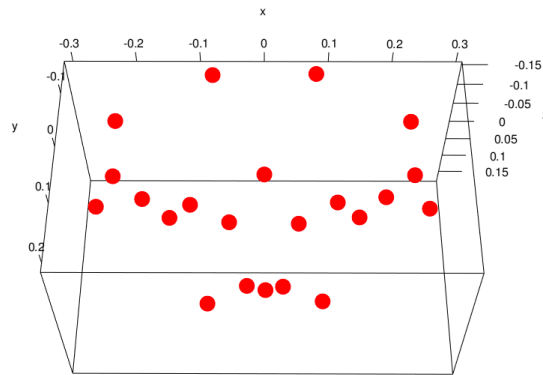


Figure 3.7: Average Procrustes landmarks for *C. polykomos*

Figure 3.8

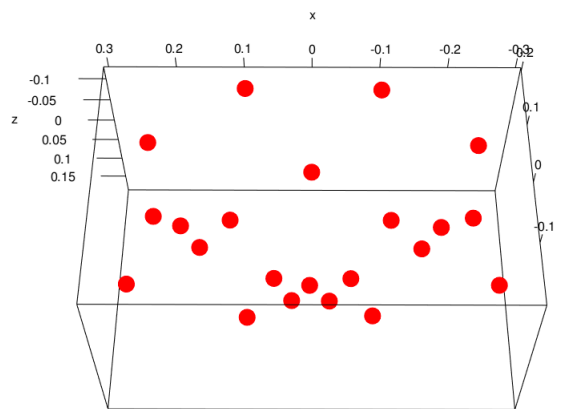


Figure 3.8: Average Procrustes landmarks for *C. angolensis palliatus*

Figure 3.9

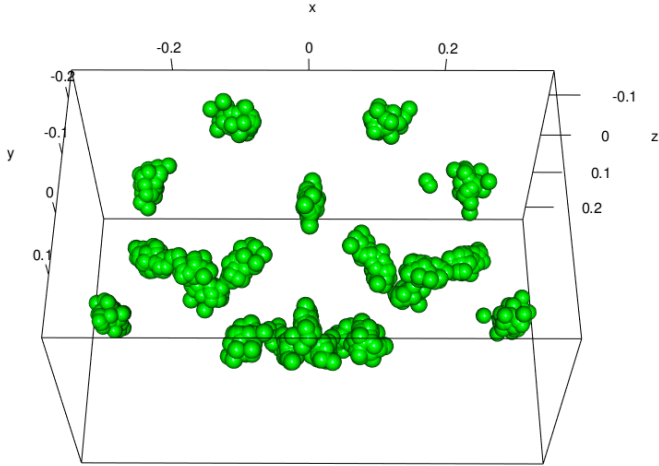


Figure 3.9: All Procrustes landmarks for *P. badius*

Figure 3.10

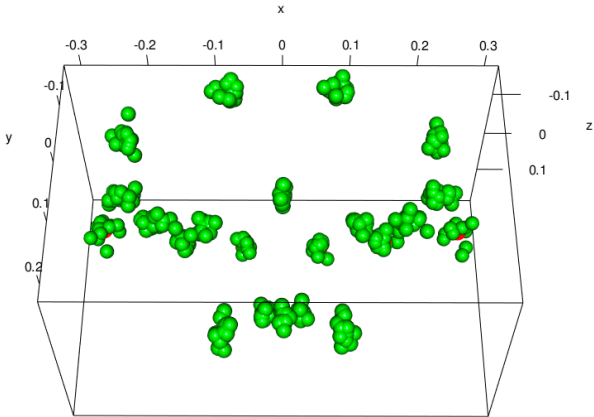


Figure 3.10: All Procrustes landmarks for *C. polykomos*

Figure 3.11

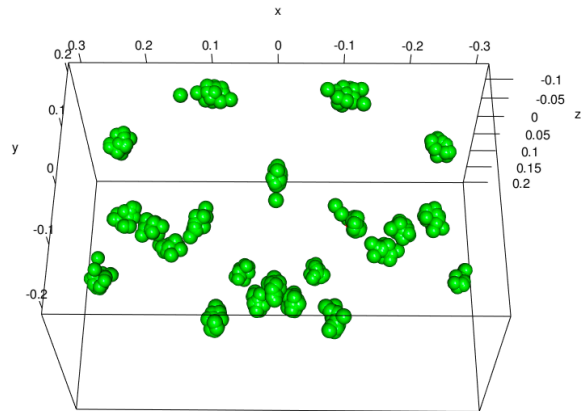


Figure 3.11: All Procrustes landmarks for *C. angolensis palliatus*

Figure 3.12

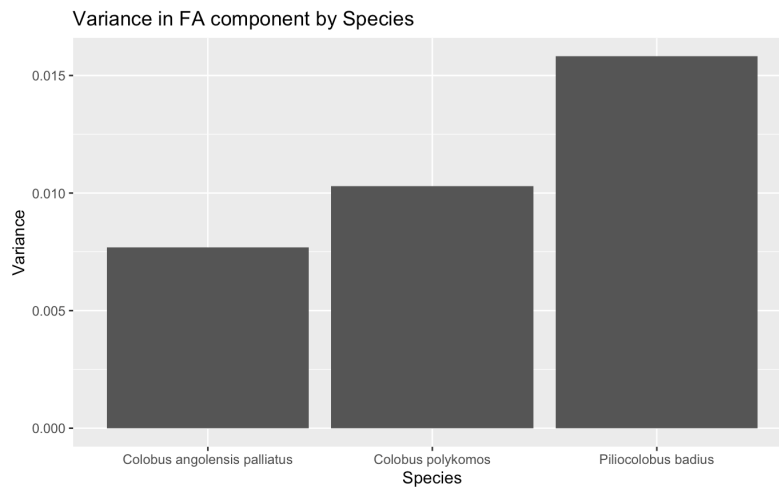


Figure 3.12: Visualization of FA component variance by species. There are significant differences between *C. angolensis palliatus* and *P. badius*.



Figure 3.13: Visualization of FA component variance by species

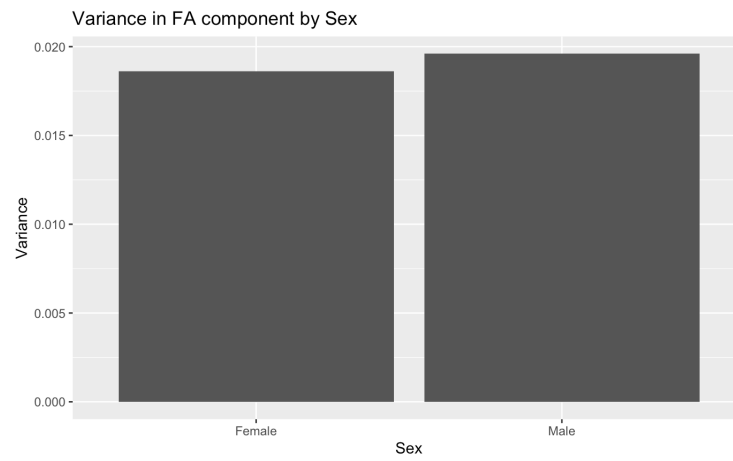


Figure 3.13: Visualization of FA component variance by sex, there is no significant difference.