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Title: Vascularization underlies differences in sexually selected skin coloration in a wild primate

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Abstract

Male reproductive competition can select for condition-dependent, conspicuous traits that signal some aspect of fighting ability and facilitate assessment of potential rivals. However, the underlying mechanisms that link the signal to a male's current condition are difficult to investigate in wild populations, often requiring invasive experimental manipulation. Here, we use digital photographs and chest skin samples to investigate mechanisms of a visual signal used in male competition in a wild primate, the red chest patch in geladas (*Theropithecus gelada*). We analyzed photographs collected during natural (*n*=144) and anesthetized conditions (*n*=38) to understand variability in male and female chest redness, and we used chest skin biopsies (*n*=38) to explore sex differences in gene expression. Male and female geladas showed similar average redness, but males exhibited a wider within-individual range in redness under natural conditions. These sex differences were reflected at the molecular level, with 10.5% of genes exhibiting significant sex differences in expression. Subadult males exhibited intermediate gene expression patterns between adult males and females, pointing to mechanisms underlying the development of the red chest patch. We found that genes more highly expressed in males were associated with blood vessel development and maintenance but not with androgen or estrogen activity. Together, our results suggest male gelada redness variability is driven by increased blood vessel branching in the chest skin, providing a potential link between male chest redness and current condition as increased blood circulation to exposed skin could lead to heat loss in the cold, high-altitude environment of geladas.

Keywords: visual signal, skin, gene expression, male competition, *Theropithecus gelada*

Introduction

Male reproduction is a zero-sum game that can lead to intense competition (Weir et al., 2011). Because actual fighting is energetically costly and exposes both the winner and loser to injury and infection, rival males can benefit from displaying their strength or condition using signals that allow for competition without costly engagement in fighting (Bradbury & Vehrencamp, 2011; Maynard Smith & Parker, 1976). The conspicuous male traits used for rival assessment (sexually selected signals) allow for conflict resolution at the lowest cost to both males (Maynard Smith, 1982; Maynard Smith & Harper, 2003, 1995), and are often condition-dependent, such that they reliably indicate some aspect of "quality" that predicts their ability to win a physical fight (i.e., body size, body condition, current health status) (Fisher, 1915; Penn & Számadó, 2020; Trivers, 1972; Zahavi, 1977). Because the expression of the signal is limited by a male's condition, these signals tend to be honest indicators of ability because only high-quality males can produce the strongest signals (Weaver et al., 2017).

While the functional consequences of signals can be observed noninvasively and are thus relatively well-studied, the mechanisms underlying these traits are much less well-understood. For example, although observational data demonstrate that higher ranking male drills (*Mandrillus leucophaeus*) exhibit redder lip and groin coloration than lower ranking males (Marty et al., 2009), identifying the causal mechanism of this difference would require invasive methods such as surgical implantation of slow-release hormone devices, social manipulation, or genetic manipulation, which are often not possible or ethical to conduct in wild populations (Emlen et al., 2012; Hau et al., 2000; Karubian et al., 2011). However, capture-and-release programs in wild populations allow tissue sample collection for genomic and transcriptomic

analyses (Tung et al., 2010), providing a minimally invasive technique to investigate the molecular correlates of signal mechanisms without experimental manipulation.

Here we investigated the mechanisms underlying a visual signal that may mediate male competition in a wild primate: the brilliant red chest patch in male geladas (*Theropithecus gelada*), a cercopithecine primate endemic to the highlands of Ethiopia (**Fig. 1)**. Geladas have a multi-tiered social system in which multiple 'reproductive units' (one dominant adult male, one or more subordinate adult males, up to a dozen adult females, and related offspring) associate to forage, rest, and move. The dominant "leader" male of units have the majority of the mating opportunities and display the reddest chests as compared to the subordinate "follower" males (with many fewer reproductive opportunities) and "bachelor" males living in all-male groups (with no reproductive opportunities) (Bergman et al., 2009; Bergman & Beehner, 2008). Because geladas live in large, fluid societies where males frequently congregate and forage with other males that they do not recognize individually (Bergman, 2010), the chest patch is hypothesized to be a sexually selected signal that mediates male rival assessment and allows males to "size up" others prior to engaging in conflict (Benítez, 2016). Bachelor males, which tend to be young adult males awaiting their chance to overthrow and replace leader males (Pappano & Beehner, 2014), are particularly attentive to leader male chest color. For example, across a one-year study, leaders with redder chests (after a vigorous ritualized "vocal display" accompanied by running, throwing rocks, climbing trees, or shaking branches) were less likely to be overthrown by bachelors than their less-red counterparts (Benítez, 2016).

Previous research conducted on the same population has shown that chest redness in geladas is mediated by increased blood flow with a concomitant increase in surface temperature. Specifically, redder chests are associated with (1) increased physical activity (Bergman et al.,

2009; DeLacey et al., 2022) and (2) higher chest skin surface temperature, whether measured using internal body heat or following the application of an external heat pack (DeLacey et al., 2022). The relationship with physical activity could be similar to skin flushing in humans during exercise, when skin blood flow increases to dissipate heat generated by muscle contractions (Kenney & Johnson, 1992). Further, the production of skin redness has been linked to characteristics of the blood vessels beneath the skin in humans and other primates where higher hemoglobin oxygen saturation increases skin redness in the short term (Changizi et al., 2006).

Sexually selected signals in males are often mediated by testosterone, a steroid hormone involved in regulation of reproductive function in male vertebrates (Ketterson & Nolan, 1999; Plant & Zeleznik, 2014) that is associated with reproductive benefits (Enstrom et al., 1997) and physiological costs (Muehlenbein et al., 2006; Muehlenbein & Bribiescas, 2005). High doses of circulating testosterone are known to dilate vascular networks and increase blood flow, providing an avenue for testosterone to work alongside a blood flow mechanism to influence chest redness in geladas (Molinari et al., 2002; Webb et al., 1999). Testosterone can alter gene transcription by: (1) binding to the androgen receptor (AR), (2) aromatizing to estradiol and then binding to estrogen receptor α (ERα) or β (ERβ), or (3) converting to 5α-reductase which binds to AR but cannot convert to estradiol (Hau & Wingfield, 2011). Local conversion of testosterone to estradiol is a particularly strong candidate for chest redness regulation based on the results of a similar study; in the closely related male rhesus macaque (*Macaca mulatta*), increases in testosterone increased both redness and blood flow in sex skin areas. Moreover, administration of an aromatase inhibitor (which prevents the conversion of testosterone to estradiol) decreased skin redness (Rhodes et al., 1997).

Therefore, to better understand molecular correlates of the chest patch signal mechanism, we collected chest skin biopsies from male and female geladas from a wild population in Ethiopia during non-lethal, immobilizations (hereafter "capture-and-release") to explore differences in gene expression local to the chest patch. Digital photographs complement this dataset to assess variability in male and female chest redness both in natural conditions and while under anesthesia. We tested the hypothesis that male chest redness is a condition-dependent signal used during rival assessment. We predicted that, when compared to females, males would have: (1) redder chests and a larger within-individual range in chest redness under natural conditions and while under anesthesia, (2) increased expression of genes associated with vascularization, and (3) increased expression of genes associated with androgen and estrogen regulation.

Materials and Methods

Study site and subjects

Data were collected from wild geladas in the Simien Mountains National Park, Ethiopia as part of the Simien Mountains Gelada Research Project (SMGRP). The SMGRP has collected behavioral, demographic, and hormonal data from a population of wild geladas since 2006 and began conducting annual capture-and-release campaigns in 2017 under the supervision of licensed veterinarians and veterinary technicians. A subset of adult geladas were anesthetized with Telinject blow darts (Telinject USA Inc.) containing ketamine (~7.5 mg/kg) and medetomidine (~0.04 mg/kg). Animals were monitored for temperature, pulse, and respiration every 10 min during data collection, and sedation was reversed with atipamezole (0.2 mg/kg). Individuals were monitored through recovery until they returned to their social unit. All data

were collected with permission from the Ethiopian Wildlife Conservation Authority, and all research was approved by the Institutional Animal Care and Use Committee (for the University of Michigan IACUC: PRO00008871 and Stony Brook University IACUC: 773805 for non-invasive work; and for the University of Washington IACUC: 4416-01 and Arizona State University IACUC: 20-1754R for the capture-and-release work) and followed all laws and guidelines in Ethiopia. This research conformed to the American Society of Primatologists/International Primatological Society Code of Best Practices for Field Primatology.

Photo collection, measurement, and analyses

To assess patterns in male and female chest redness in geladas, we used objective color measurement methods for digital photographs taken under natural conditions (i.e., while conducting daily activities like resting, grazing, or grooming) and while animals were under anesthesia. Under natural conditions, chest redness was measured from 144 digital chest photographs of adult males (n=24) and females (n=13) collected between 2008-2010 (range=2-16 photos per individual, mean=4). We excluded photos taken within 10 minutes of vigorous activity because chest redness increases with such activity in males (Bergman et al., 2009; DeLacey et al., 2022). Females do not exhibit these displays, so we did not exclude any photos from females. We only included photos taken in March and April of each year because: (1) this was the only dataset available for females, and (2) previous analyses have demonstrated that males exhibit seasonal trends in chest redness that could skew male results if selected over multiple seasons (Benítez, 2016). While the animals were under anesthesia, chest redness was measured from photos taken at the start of anesthetization for both males (n=20) and females

(n=18). For a subset of individuals (n=13 males and n=3 females) we paired the photo taken at the start of anesthetization with a second chest photo after a heat pack was applied to one side of the chest. We include a visualization of the within individual change in redness after temperature treatment in the supplementary material (**Fig. S1**), but we did not run statistical tests due to the small sample size.

For all photos, we also photographed a color standard, the X-Rite ColorChecker® Classic chart (hereafter, "ColorChecker chart"), to correct for variable light conditions by adjusting the color in the photograph to the known color levels in the chart squares. Although the digital camera brand and model was not consistent across all photographs, f-stop, shutter speed, and white balance settings remained consistent between chart and chest photos. JPEG format photos were analyzed in Adobe Photoshop (Adobe Inc. 2022) using color profiles in the RGB color space created in ColorChecker Camera Calibration (v2.2.0; X-Rite Inc.) software designed for use with the ColorChecker chart. We measured redness as the Red to Green Ratio (hereafter, "Red/Green") because the value in each RGB channel is only informative relative to values in the other channels (Bergman & Beehner, 2008). Detailed methodology and instructions for photo measurement can be found elsewhere (DeLacey et al., 2022).

To determine whether males are redder than females during natural conditions, we constructed a linear mixed-effect model (LMM) with chest redness as the outcome variable and sex as the predictor variable while including ID and camera brand as random effects (R packages *lme4* (Bates et al., 2015) and *lmerTest* (Kunzetsova et al., 2017)). Next, to assess whether males have a larger range in chest redness during natural conditions, we ran a linear regression model with the range in chest redness within an individual (maximum R/G - minimum R/G for each individual) as the outcome variable and the interaction between sex and camera brand as the

predictor variable. Lastly, to determine whether males are redder than females under anesthesia at baseline, we ran a linear regression model with chest redness as the outcome variable and camera brand and sex as the predictor variables.

Skin biopsy collection

We collected chest skin biopsies from 15 adult males, 15 adult females, 6 subadult males, and 2 subadult females during the annual SMGRP capture-and-release campaigns. Adulthood was determined by the eruption of the third molar which aligned with our phenotypic metrics of adulthood for known individuals (McNamara & Graber, 1975). Biopsies were collected with a 4mm punch biopsy tool, placed vertically over the chest patch skin and pressed and rotated in one direction to move the punch through the skin to the subcutis. The biopsy was then removed, placed in a 1.5 mL microcentrifuge tube with 0.5 mL RNAlater™, and frozen in liquid nitrogen within 6 hours. The resulting small wound was treated with antimicrobial aluminum aerosol bandaging to stop any bleeding and prevent infection. Upon arrival in the laboratory (still frozen in a liquid nitrogen vapor shipper), samples were stored at -80°C until RNA extraction.

RNA extraction, sequencing, and data processing

DNA and RNA were extracted from chest skin biopsies using TRIzol™ Reagent and the Zymo Quick-DNA/RNA™ Microprep Plus Kit (Zymo Research, Irvine, CA). We quantified RNA integrity (RIN) using a Fragment Analyzer 5200 (Agilent Technology, Inc., Santa Clara, CA). RNA-sequencing libraries were prepared using 200 ng of total RNA following a recently developed 3'-based protocol, TM3'seq (Pallares et al., 2020). Libraries were amplified with 16 PCR cycles. All other procedures followed the published protocol or manufacturer

recommendations. Libraries were combined in equimolar quantities and sequenced on one lane of an Illumina NovaSeq S4 flow cell (Illumina Inc.) of 100 bp single-end with an average of 2.17 million reads per sample mapping to the transcriptome. Reads were mapped to the *Macaca mulatta* reference assembly Mmul_10 (Warren et al., 2020) using kallisto (v0.43.1) (Bray et al., 2016). We chose the genome of *Macaca mulatta*, a closely related cercopithecoid, over the *Theropithecus gelada* genome (Tgel_1) that our team assembled (Chiou et al., 2022) because of the richer annotation of Mmul_10. Mmul_10 has 20% more annotated genes than Tgel_1, which is likely due to the fact that Mmul_10 was annotated using RNA-seq data from 13 tissues, while Tgel_1 was annotated using only two RNA-seq libraries derived from cultured fibroblasts. Given that our current study focuses on a more diverse population of cells from skin patches, we were concerned that relevant genes would be poorly annotated or missing in the gelada genome. Thus, to ensure that we were capturing the most relevant and complete set of chest-patch-expressed genes, we chose to map to the macaque genome. Indeed, we found that more of our reads were assigned to an annotated gene when we used Mmul_10 compared to Tgel_1. Further, given that all of our samples were collected from the same population, and the macaque represents shared outgroup, all samples are all equally evolutionarily distant from the macaque reference, and thus any reference genome alignment effect, if present, will be the same across all samples (both males and females).

Read count normalization

First, we removed reads mapping to 7 genes encoding ribosomal RNA subunits and hemoglobin genes to remove the influence of blood contamination on the skin biopsies. While investigating RNA integrity, we removed two samples with low RNA quality (retaining samples

with RQN > mean - 2 standard deviations), leaving a final sample size of 36 geladas (*n*=20 males, *n*=16 females). We then removed genes with low expression (median TPM < 10) for either males or females and 14 Y-chromosome genes (removing sex differences that are a product of their location on the male-specific Y-chromosome), which resulted in 10,212 detectably expressed genes for our downstream analysis. We normalized read counts using the *voom* function in the R package *limma* (Ritchie et al., 2015).

Modeling the effect of sex on gene expression

We used a mixed modeling approach with the R package *EMMREML* to quantify the effect of sex on gene expression while controlling for sample collection year, RNA extraction date, RNA concentration, and RNA quality (Akdemir & Godfrey, 2015). We used an identity matrix as the known covariance structure which is required for the *EMMREML* mixed modeling approach. To focus on gene expression of this putatively sexually selected trait, we only included adults in this model (*n*=14 adult males, *n*=14 adult females). We calculated the false discovery rate (FDR) for each gene using the R package *qvalue* (Storey et al., 2022). Genes that passed a threshold of a FDR of 20% were considered differentially expressed between the sexes: "male-biased" if they were more highly expressed in males and "female-biased" if they were more highly expressed in females. We then Z-transformed expression values for this set of male-biased and female-biased genes across all 36 individuals (*n*=14 adult males, *n*=14 adult females, *n*=6 subadult males, *n*=2 subadult females) and averaged the Z-transformed expression levels of these genes per individual to obtain a composite sex-biased gene expression score for each individual. Finally, we ran a linear regression model with expression score as the outcome variable and age

category (adult male, adult female, subadult male, subadult female) as the predictor variable to investigate whether subadults differed from adults in sex-biased gene expression.

Enrichment analyses

To test our prediction that males would exhibit increased expression of genes associated with vascularization, we conducted Gene Ontology (GO) enrichment analyses using the R package *topGO* to identify biological processes that were enriched in genes differentially expressed between males and females (Alexa & Rahnenfuhrer, 2020). We searched for terms related to angiogenesis, the formation of new blood vessels, including the phrase "angio" and removed those not involved in angiogenesis (i.e., "lymphangiogenesis"). We compared the standardized effect of sex for these angiogenesis genes with the standardized effect of sex for all other detectably expressed genes with a Kolmogorov-Smirnov test.

To test our prediction that males would have higher expression of genes associated with estrogen and androgen regulation, we used the Online Predicted Human Interaction Database (OPHID) to assess protein-protein interaction (PPI) networks associated with hormone receptor proteins of interest: ERα, ERβ, and AR (Brown & Jurisica, 2005). We used ENSEMBL orthology information queried through the R package *biomaRt* (Durinck et al., 2009) to identify one-to-one orthologs in the human genome, then filtered and reindexed our expression matrix to detectably expressed genes in the human genome. We retained a total of 8,255 human genes for analysis. Next we ran a query in OPHID to identify the set of human genes involved in PPI networks with each hormone receptor protein and used *biomaRt* to convert genes names from UniProt to Ensembl (The UniProt Consortium, 2021). We found the genes associated with PPI networks for ERα, ERβ, and AR and compared the standardized effect of sex for these genes

with the standardized effect of sex for all other detectably expressed genes with human orthologs with a Kolmogorov-Smirnov test.

Results

Chest redness in male and female geladas

Under natural conditions (i.e., not anesthetized), chest redness for males and females overlapped substantially, with males displaying only marginally redder chests than females (β=0.11, P=0.06, **Fig. 2a**). However, males had a wider range of chest redness within individuals compared to females (β=0.63, P=0.007, **Fig. 2b**). While under anesthesia, male geladas did not have redder chests than females (β=0.04, P=0.40, **Fig. 2c).**

Sex differences in gene expression

We then quantified how sex was associated with chest skin gene expression while including sample collection year in the model (year was significantly associated with both the first and second principal component of gene expression; PC1: year β=0.39, P=0.02; PC2: year β=-0.22, P=0.003, **Table S1, Fig. S2**). Males and females differed along the first principal component of gene expression while including year as a predictor variable (PC1: sex β=-0.39, P=0.01, **Fig. S3**; PC2: sex β=-0.13, P=0.049). The results of the principal component analysis did not change when we removed genes located on sex chromosomes and analyzed only those found on autosomes (**Fig. S4**).

At the level of the individual genes, we found that 10.5% of the 10,212 detectably expressed genes exhibited significant differential expression across males and females (*n*

genes=1,068, FDR<20%, **Fig. S5**), in a model controlling for the effects of RIN, RNA concentration, RNA extraction date, and year of sample collection. Of the 1,068 differentially expressed genes, 201 genes were female-biased and 867 genes were male-biased. The average standardized sex-bias gene expression level for each individual illustrates that subadult males exhibited an intermediate gene expression pattern that differed from adult males (β=-0.66, P=0.003) and adult females (β=0.45, P=0.04; **Fig. 3).** The sample size for subadult females (n=2) was too small to draw conclusions about sex-bias gene expression trends in this age category.

Sex-biased genes involved in vascularization

In line with our prediction that males would exhibit increased expression of genes associated with vascularization, we found that genes more highly expressed in males were enriched for biological processes associated with angiogenesis (K-S Test: D=0.19, P=7.25x10^{-7}; **Fig. 4**). Additionally, these genes in males were also enriched for biological processes associated with blood pressure regulation and blood vessel maintenance (K-S Test: D=0.19, P=2.59x10^{-5}; **Fig. S6**). Contrary to our predictions, we did not find evidence for increased expression of genes associated with estrogen or androgen regulation in males – including no evidence for indirect effects through protein-protein networks: there was no enrichment for genes involved in PPI networks for ERα (K-S Test: D=0.04, P=0.07), ERβ (K-S Test: D=0.05, P=0.17), or AR (K-S Test: D=0.04, P=0.46). For subset of individuals for which we had matched skin biopsies and chest redness at baseline while under anesthesia (n=10 males, n=8 females), we did not find a correlation between the average standardized expression level of genes involved in PPI networks for ERα, ERβ, or AR and chest redness within males or within females (**Fig. S7).**

Discussion

We sampled chest skin biopsies from wild geladas to directly measure putative mechanisms underlying a uniquely evolved sexual signal. We found that male and female geladas showed a substantial overlap in chest redness under natural and unmanipulated conditions, but males exhibited a wider within-individual range in baseline redness under natural conditions (**Figs. 2a-b)**. Further, subadults displayed an intermediate gene-expression pattern from adult males and adult females (**Fig. 3**). We also found sex differences in gene expression, where higher expression in males was associated with angiogenesis, blood pressure, and blood vessel maintenance, suggesting that blood flow and vascularization may underlie sex differences in this sexually-selected signal (**Fig. 4**). Contrary to our predictions, genes encoding proteins that interact with androgen or estrogen were not more highly expressed in males. Together, these results suggest that males may have more variable chest redness due to increased blood flow and blood vessel branching in the chest skin.

Chest photograph measurements revealed an overlap in redness between the sexes at baseline in both natural and anesthetized conditions, but males overall exhibited a wider within-individual range in redness. Selection on an ornamentation trait in one sex can create a correlated response in the opposite sex within a species (e.g., male and female coloration are highly correlated in passerines), suggesting that changes in one sex can be constrained by changes in the other sex (Dale et al., 2015; Poissant et al., 2010; Potti & Canal, 2011). In geladas, this overlap in chest redness between the sexes could simply be the result of a positive genetic correlation. Alternatively, as male and female color traits function differently, female chest redness in geladas could have continued to evolve under a different selective pressure (Dale et al., 2015; Tobias et al., 2012), and the overlap in redness could be caused by each sex using the chest patch

to communicate different signals. In males, chest redness varies among males by status (Bergman et al., 2009) and within males by activity level (DeLacey et al., 2022), suggesting the chest patch aids in male-male competition. Females have instead co-opted chest redness to communicate reproductive status through hormonal and blood signaling as they have the reddest chests late in gestation when estrogen levels and blood volume are the highest (Hytten, 1985; Roberts et al., 2017). In addition to chest color variation, gelada females exhibit sexual swellings consisting of cutaneous vesicles surrounding the chest region where vesicle turgidity varies across the ovarian cycle, suggesting sexual swellings work in tandem with chest redness to signal a different aspect of reproductive state (Roberts et al., 2017).

The sex difference in gelada chest skin gene expression aligns with findings in humans where a wide variety of tissues exhibit small effects of sex on gene expression (Lopes-Ramos et al., 2020; Oliva et al., 2020). However, small expression changes have been shown to have large phenotypic effects, particularly in the manifestation of disease (Khramtsova et al., 2019). Within primates, sex-biased gene expression has also been detected in rank-related genes, immune regulation, and aging in wild baboons through blood sampling (Anderson et al., 2021; Lea et al., 2018). The magnitude of sex-biased gene expression has been shown to increase across development with the greatest differences in adult tissue (Mank et al., 2010; Perry et al., 2014), and differences are particularly exaggerated in sexually dimorphic tissues such as elaborated weaponry (Zinna et al., 2018). Subadult geladas showed an intermediate pattern of gene expression between that of adult males and females suggesting that gene expression differences increase at sexual maturity when sexually selected signals develop for mate acquisition. Further, an analysis of an avian clade that found the degree of sexual selection predicts the proportion of male-biased gene expression (Harrison et al., 2015). This finding is consistent with gelada chest

skin, as we found more genes that were more highly expressed in males compared to genes that were more highly expressed in females in this species with a high male reproductive skew.

Male geladas expressed genes associated with angiogenesis, blood pressure regulation, and blood vessel maintenance more highly than females. The mechanism of increased blood vessel branching in the chest skin may indicate chest redness is a condition-dependent signal where the differential costs of signaling based on current body condition inhibit low-quality males from investing in the signal (Grafen, 1990; Penn & Számadó, 2020). We propose energy balance and heat loss as possible costs associated with producing a red chest. Male geladas may develop more extensive blood vessel branching in the skin compared to females through engaging in vocal displays (a behavior females do not exhibit). Post-display chest redness increases with display rate per hour in gelada males (Benítez, 2016) which suggests that after frequent activity has built up vascular networks, an instance of increased blood flow will prompt a larger increase in chest redness. Among males, leaders spend less time resting, more time engaging in low-intensity aggression, and produce more calls per vocal display bout compared to bachelors (Benítez et al., 2016; Perlman, 2021). The physical effort required to engage in aggression and vocal displays may contribute to ensuring only high-quality males in good body condition have red chests (if it is difficult to break the link between exertion and vascularization). Further, redder chests have higher surface skin temperatures which indicates the increased blood flow to this area may also result in heat loss in the cold, high-altitude environment of the Simien Mountains (DeLacey et al., 2022). These potential constraints could provide an avenue for chest redness to communicate current body condition to potential rivals.

Contrary to our predictions, males did not have increased expression of genes associated with androgen and estrogen regulation in the chest skin. Although this result could simply

indicate that androgen and estrogen regulation are not important to sex differences in chest redness, it could also (1) indicate both males and females use the same androgen and estrogen regulation pathways in the chest skin or (2) be a product of sequencing skin biopsies in particular as sex-biased genes have tissue-specific expression profiles (Lopes-Ramos et al., 2020; Yang et al., 2006). Perhaps we would detect more sex differences in expression in brain regions involved in the regulation of hormone secretion rather than the target tissue (Becker et al., 2007). Additionally, we measured the expression of genes that interact with estrogen and androgen receptors, but circulating hormones such as testosterone or changes in androgen receptor density may play a larger role in regulating redness in primates (Dixson, 1983; Rhodes et al., 1997; Setchell & Dixson, 2001). As yet, no relationship has been identified between testosterone and chest redness in adult male geladas (DeLacey and Beehner, unpublished data). This may be because this putative signal is not testosterone dependent, or because we are only able to measure fecal androgen metabolite levels (capturing an averaged level of the hormone over the past day) rather than actual circulating testosterone levels. Further, we may not detect a relationship between testosterone and chest redness because estrogens directly regulate chest redness and testosterone only indirectly influences redness through aromatization to estrogens.

Limitations

We only collected skin biopsies from the chest to limit the number of biopsies collected per individual during the capture-and-release. However, comparing chest skin gene expression to gene expression in a non-sexual skin area would allow us to assess (1) whether higher male expression associated with blood flow and vascularization is unique to chest skin biopsies and (2) whether both male and female chests are hotspots for expression of androgen and estrogen

related genes. While we were able to identify interesting sex differences, we were not able to assess gene expression differences between male status categories because anesthetizing a leader male to collect a biopsy sample would put them at risk of losing their status. Lastly, we restricted our male chest photo dataset to only photos taken in March and April to match our more limited female chest photo dataset. This facilitated a more direct comparison between the sexes, but may have reduced our ability to identify more within-individual variation, particularly in males.

Conclusion

Here, we provide mechanistic evidence that gelada chest redness is linked to increased blood flow near the surface of the skin, specifically through increased blood vessel branching in the chest skin in males. Although we did not detect a sex difference in chest skin gene expression related to androgen and estrogen regulation, future research into the correlation between fluctuations in fecal testosterone metabolites and chest redness within males will help determine whether another aspect of hormonal regulation is involved in chest redness signaling. Going forward, we hope to better understand whether increased angiogenesis creates a current-condition signal for males in the cold, high-altitude environment of the Ethiopian highlands by leveraging biomarkers of energy balance or heat loss.

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References

Akdemir, D., & Godfrey, O. U. (2015). *EMMREML: Fitting Mixed Models with Known Covariance Structures*. R package version 3.1, https://cran.r-project.org/web/packages/EMMREML/index.html

Alexa, A., & Rahnenfuhrer, J. (2020). *topGO: Enrichment Analysis for Gene Ontology.* R package version 2.50.0.

Anderson, J. A., Johnston, R. A., Lea, A. J., Campos, F. A., Voyles, T. N., Akinyi, M. Y., Alberts, S. C., Archie, E. A., & Tung, J. (2021). High social status males experience accelerated epigenetic aging in wild baboons. *eLife,* 10. https://doi.org/10.7554/eLife.66128

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Becker, J. B., Berkley, K. J., Geary, N., Hampson, E., Herman, J. P., & Young, E. (2007). *Sex Differences in the Brain: From Genes to Behavior.* Oxford University Press.

Benítez, M. E. (2016). *Sizing up strangers: sexual selection and vocal signals in gelada males* [Doctoral dissertation, University of Michigan]. UMich Campus Repository. https://hdl.handle.net/2027.42/135766

Benítez, M. E., Le Roux, A., Fischer, J., Beehner, J. C., & Bergman, T. J. (2016). Acoustic and

temporal variation in gelada (*Theropithecus gelada*) loud calls advertise male quality. *International Journal of Primatology*, 37(4-5), 568–585. https://doi.org/10.1007/s10764-016-9922-0

Bergman, T. J. (2010). Experimental evidence for limited vocal recognition in a wild primate: implications for the social complexity hypothesis. *Proceedings of the Royal Society B*, 277, 3045–3053. https://doi:10.1098/rspb.2010.0580

Bergman, T. J., & Beehner, J. C. (2008). A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). *Biological Journal of the Linnean Society*, 94(2), 231–240. https://doi.org/10.1111/j.1095-8312.2008.00981.x

Bergman, T. J., Ho, L., & Beehner, J. C. (2009). Chest color and social status in male geladas (*Theropithecus gelada*). *International Journal of Primatology*, 30(6), 791–806. https://doi.org/10.1007/s10764-009-9374-x

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication: Vol. 2nd Edition*. Sinauer Associates.

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*, 34(5), 525–527. https://doi.org/10.1038/nbt.3519

Brown, K. R., & Jurisica, I. (2005). Online predicted human interaction database. *Bioinformatics*, 21(9), 2076–2082. https://doi:10.1093/bioinformatics/bti273

Changizi, M.A., Zhang, Q., Shimojo, S. (2006). Bare skin, blood, and the evolution of primate colour vision. *Biology Letters,* 2:217-221. https://doi.org/10.1098/rsbl.2006.0440

Chiou, K. L., Janiak, M. C., Schneider-Crease, I. A., Sen, S., Ayele, F., Chuma, I. S., Knauf, S., Lemma, A., Signore, A. V., D'Ippolito, A. M., Abebe, B., Haile, A. A., Kebede, F., Fashing, P. J., Nguyen, N., McCann, C., Houck, M. L., Wall, J. D., Burrell, A. S., … Snyder-Mackler, N. (2022). Genomic signatures of high-altitude adaptation and chromosomal polymorphism in geladas. *Nature Ecology & Evolution*. https://doi.org/10.1038/s41559-022-01703-4

Dale, J., Dey, C. J., Delhey, K., Kempenaers, B., & Valcu, M. (2015). The effects of life history and sexual selection on male and female plumage colouration. *Nature*, 527, 367–370. https://doi.org/10.1038/nature15509

DeLacey, P. M., Perlman, R. F., Sen, S., Schneider-Crease, I., Chiou, K. L., Lemma, A., Ayele, F., Higham, J. P., Lu, A., Snyder-Mackler, N., Beehner, J. C., & Bergman, T. J. (2022). Assessing male gelada chest patches: color measurement and physiological mechanisms. *Mammalian Biology.* https://doi.org/10.1007/s42991-021-00211-5

Dixson, A. F. (1983). Observations on the evolution and behavioral significance of "sexual skin" in female primates. In J.S. Rosenblatt, R.A. Hinde, C. Beer, & M.-C. Busnel (Eds.), *Advances in the Study of Behavior* (Vol. 13, pp. 63–106). Academic Press.

Durinck, S., Spellman, P. T., Birney, E., & Huber, W. (2009). Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nature Protocols*, 4(8), 1184–1191. https://doi.org/10.1038/nprot.2009.97

Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I., & Lavine, L. C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons.

Science, 337(6096), 860–864. https://doi.org/10.1126/science.1224286

Enstrom, D. A., Ketterson, E. D., & Val Nolan, J. R. (1997). Testosterone and mate choice in the dark-eyed junco. *Animal Behaviour*, 54(5), 1135–1146. https://doi.org/10.1006/anbe.1997.0555

Fisher, R. A. (1915). The evolution of sexual preference. *The Eugenics Review*, 7(3), 184–192.

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology*, 144(4), 517–546. https://doi.org/10.1016/S0022-5193(05)80088-8

Harrison, P. W., Wright, A. E., Zimmer, F., Dean, R., Montgomery, S. H., Pointer, M. A., & Mank, J. E. (2015). Sexual selection drives evolution and rapid turnover of male gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, 112(14), 4393–4398. https://doi.org/10.1073/pnas.1501339112

Hau, M., Wikelski, M., Soma, K. K., & Wingfield, J. C. (2000). Testosterone and year-round territorial aggression in a tropical bird. *General and Comparative Endocrinology*, 117(1), 20–33. https://doi:10.1006/gcen.1999.7390

Hau, M., & Wingfield, J. C. (2011). Hormonally-regulated trade-offs: evolutionary variability and phenotypic plasticity in testosterone signaling pathways. In *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs* (pp. 349–361). Oxford Academic.

Hytten, F. (1985). Blood volume changes in normal pregnancy. *Clinics in Haematology*, 14(3), 601–612. https://doi.org/10.1016/S0308-2261(21)00496-3

Karubian, J., Lindsay, W. R., Schwabl, H., & Webster, M. S. (2011). Bill coloration, a flexible signal in a tropical passerine bird, is regulated by social environment and androgens. *Animal Behaviour*, 81(4), 795–800. https://doi.org/10.1016/j.anbehav.2011.01.012

Kenney, W. L., & Johnson, J. M. (1992). Control of skin blood flow during exercise. *Medicine & Science in Sports & Exercise*, 24(3), 303–312. https://doi.org/10.1249/00005768-199203000-00005

Ketterson, E. D., & Nolan, V., Jr. (1999). Adaptation, Exaptation, and Constraint: A Hormonal Perspective. *The American Naturalist*, 154(S1), S4–S25. https://doi:10.1086/303280

Khramtsova, E. A., Davis, L. K., & Stranger, B. E. (2019). The role of sex in the genomics of human complex traits. *Nature Reviews Genetics*, 20(3), 173–190. https://doi.org/10.1038/s41576-018-0083-1

Kunzetsova, A., Brockhoff, P., & Christensen, R. (2017). lmerTest package: Tests in linear mixed effect models. *Journal of Statistical Software*, 82, 1–26. https://doi.org/10.18637/jss.v082.i13

Lea, A. J., Akinyi, M. Y., Nyakundi, R., Mareri, P., Nyundo, F., Kariuki, T., Alberts, S. C., Archie, E. A., & Tung, J. (2018). Dominance rank-associated gene expression is widespread, sex-specific, and a precursor to high social status in wild male baboons. *Proceedings of the National Academy of Sciences of the United States of America*, 115(52), E12163–E12171. https://doi.org/10.1073/pnas.1811967115

Lopes-Ramos, C. M., Chen, C.-Y., Kuijjer, M. L., Paulson, J. N., Sonawane, A. R., Fagny, M.,

Platig, J., Glass, K., Quackenbush, J., & DeMeo, D. L. (2020). Sex Differences in Gene Expression and Regulatory Networks across 29 Human Tissues. *Cell Reports*, 31(12), 107795. https://doi.org/10.1016/j.celrep.2020.107795

Mank, J. E., Nam, K., Brunström, B., & Ellegren, H. (2010). Ontogenetic complexity of sexual dimorphism and sex-specific selection. *Molecular Biology and Evolution,* 27(7), 1570–1578. https://doi.org/10.1093/molbev/msq042

Marty, J. S., Higham, J. P., Gadsby, E. L., & Ross, C. (2009). Dominance, Coloration, and Social and Sexual Behavior in Male Drills *Mandrillus leucophaeus*. *International Journal of Primatology,* 30(6), 807. https://doi.org/10.1007/s10764-009-9382-x

Maynard Smith, J. (1982). *Evolution and the theory of games.* Cambridge University Press. https://doi.org/10.1017/CBO9780511806292

Maynard Smith, J., & Harper, D. (2003). *Animal Signals*. Oxford University Press.

Maynard Smith, J., & Harper, D. G. C. (1995). Animal Signals: Models and Terminology. *Journal of Theoretical Biology*, 177(3), 305–311. https://doi.org/10.1006/jtbi.1995.0248

Maynard Smith, J., & Parker, G. A. (1976). The logic of asymmetric contests. *Animal Behaviour,* 24(1), 159–175. https://doi.org/10.1016/S0003-3472(76)80110-8

McNamara, J. A., Jr, & Graber, L. W. (1975). Mandibular growth in the rhesus monkey (*Macaca mulatta*). *American Journal of Physical Anthropology*, 42(1), 15–24. https://doi.org/10.1002/ajpa.1330420104

Molinari, C., Battaglia, A., Grossini, E., Mary, D. A. S. G., Vassanelli, C., & Vacca, G. (2002). The effect of testosterone on regional blood flow in prepubertal anaesthetized pigs. *The Journal of Physiology*, 543(Pt 1), 365–372. https://doi:10.1113/jphysiol.2002.022756

Muehlenbein, M. P., & Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. *American Journal of Human Biology* 17(5), 527–558. https://doi.org/10.1002/ajhb.20419

Muehlenbein, M. P., Cogswell, F. B., James, M. A., Koterski, J., & Ludwig, G. V. (2006). Testosterone correlates with Venezuelan equine encephalitis virus infection in macaques. *Virology Journal*, 3, 19. https://doi.org/10.1186/1743-422X-3-19

Oliva, M., Muñoz-Aguirre, M., Kim-Hellmuth, S., Wucher, V., Gewirtz, A. D. H., Cotter, D. J., Parsana, P., Kasela, S., Balliu, B., Viñuela, A., Castel, S. E., Mohammadi, P., Aguet, F., Zou, Y., Khramtsova, E. A., Skol, A. D., Garrido-Martín, D., Reverter, F., Brown, A., … Stranger, B. E. (2020). The impact of sex on gene expression across human tissues. *Science*, 369(6509). https://doi.org/10.1126/science.aba3066

Pallares, L. F., Picard, S., & Ayroles, J. F. (2020). TM3'seq: A Tagmentation-Mediated 3' Sequencing Approach for Improving Scalability of RNAseq Experiments. G3, 10(1), 143–150. https://doi.org/10.1534/g3.119.400821

Pappano, D. J., & Beehner, J. C. (2014). Harem-holding males do not rise to the challenge: androgens respond to social but not to seasonal challenges in wild geladas. *Royal Society Open Science*, 1(1), 140081. https://doi.org/10.1098/rsos.140081

Penn, D. J., & Számadó, S. (2020). The Handicap Principle: how an erroneous hypothesis

became a scientific principle. *Biological Reviews of the Cambridge Philosophical Society*, 95, 267–290. https://doi.org/10.1111/brv.12563

Perlman, R. F. (2021). *The energetics of male reproductive strategies in geladas.* [Doctoral Dissertation, Stony Brook University].

Perry, J. C., Harrison, P. W., & Mank, J. E. (2014). The ontogeny and evolution of sex-biased gene expression in Drosophila melanogaster. *Molecular Biology and Evolution*, 31(5), 1206–1219. https://doi:10.1093/molbev/msu072

Plant, T. M., & Zeleznik, A. J. (Eds.). (2014). *Knobil and Neill's Physiology of Reproduction*. Academic Press.

Poissant, J., Wilson, A. J., & Coltman, D. W. (2010). Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution: International Journal of Organic Evolution*, 64(1), 97–107. https://doi.org/10.1111/j.1558-5646.2009.00793.x

Potti, J., & Canal, D. (2011). Heritability and genetic correlation between the sexes in a songbird sexual ornament. *Heredity*, 106(6), 945–954. https://doi.org/10.1038/hdy.2010.142

Rhodes, L., Argersinger, M. E., Gantert, L. T., Friscino, B. H., Hom, G., Pikounis, B., Hess, D. L., & Rhodes, W. L. (1997). Effects of administration of testosterone, dihydrotestosterone, oestrogen and fadrozole, an aromatase inhibitor, on sex skin colour in intact male rhesus macaques. *Journal of Reproduction and Fertility*, 111(1), 51—57. https://doi.org/10.1530/jrf.0.1110051

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, 43(7), e47. https://doi.org/10.1093/nar/gkv007

Roberts, E. K., Lu, A., Bergman, T. J., & Beehner, J. C. (2017). Female reproductive parameters in wild geladas *(Theropithecus gelada*). *International Journal of Primatology,* 38(1), 1–20. https://doi.org/10.1007/s10764-016-9939-4

Setchell, J. M., & Dixson, A. F. (2001). Changes in the secondary sexual adornments of male mandrills (*Mandrillus sphinx*) are associated with gain and loss of alpha status. *Hormones and Behavior*, 39(3), 177–184. https://doi.org/10.1006/hbeh.2000.1628

Storey, J. D., Bass, A. J., Dabney, A., & Robinson, D. (2022). *qvalue: Q-value estimation for false discovery rate control.* R package version 2.30.0, http://github.com/jdstorey/qvalue

The UniProt Consortium (2021). UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Research,* 49(D1), D480–D489. https://doi.org/10.1093/nar/gkaa1100

Tobias, J. A., Montgomerie, R., & Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1600), 2274–2293. https://doi.org/10.1098/rstb.2011.0280

Trivers, R. L. (1972). Parental investment and sexual selection. In B. G. Campbell (Ed.), *Sexual selection and the descent of man, 1871-1971* (pp. 136–179). Routledge.

Tung, J., Alberts, S. C., & Wray, G. A. (2010). Evolutionary genetics in wild primates:

combining genetic approaches with field studies of natural populations. *Trends in Genetics*, 26(8), 353–362. https://doi.org/10.1016/j.tig.2010.05.005

Warren, W. C., Harris, R. A., Haukness, M., Fiddes, I. T., Murali, S. C., Fernandes, J., Dishuck, P. C., Storer, J. M., Raveendran, M., Hillier, L. W., Porubsky, D., Mao, Y., Gordon, D., Vollger, M. R., Lewis, A. P., Munson, K. M., DeVogelaere, E., Armstrong, J., Diekhans, M., … Eichler, E. E. (2020). Sequence diversity analyses of an improved rhesus macaque genome enhance its biomedical utility. *Science*, 370(6523), eabc6617. https://doi.org/10.1126/science.abc6617

Weaver, R. J., Koch, R. E., & Hill, G. E. (2017). What maintains signal honesty in animal colour displays used in mate choice? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1724), 20160343. https://doi.org/10.1098/rstb.2016.0343

Webb, C. M., McNeill, J. G., Hayward, C. S., de Zeigler, D., & Collins, P. (1999). Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation*, 100(16), 1690–1696. https://doi.org/10.1161/01.CIR.100.16.1690

Weir, L. K., Grant, J. W. A., & Hutchings, J. A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *The American Naturalist*, 177(2), 167–176. https://doi.org/10.1086/657918

Yang, X., Schadt, E. E., Wang, S., Wang, H., Arnold, A. P., Ingram-Drake, L., Drake, T. A., & Lusis, A. J. (2006). Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Research*, 16(8), 995–1004. https://doi.org/10.1101/gr.5217506

Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology*, 67(3), 603–605. https://doi.org/10.1016/0022-5193(77)90061-3

Zinna, R., Emlen, D., Lavine, L. C., Johns, A., Gotoh, H., Niimi, T., & Dworkin, I. (2018). Sexual dimorphism and heightened conditional expression in a sexually selected weapon in the Asian rhinoceros beetle. *Molecular Ecology*, 27(24), 5049–5072. https://doi.org/10.1111/mec.14907

Author Contributions

N.S.-M., T.J.B., J.C.B. and P.M.D. conceived the research and designed the study. S.S., I.A.S.-C., K.L.C., A. Lemma, F.A., A. Lu, T.J.B., J.C.B., and N.S.-M. collected field gelada samples and data, facilitated by A. A. H. P.M.D. performed photo analyses. P.M.D., K.L.C., and N.S.-M. generated genomic data. P.M.D. and N.S.-M. performed genomic analyses. P.M.D., J.C.B., and N.S.-M. wrote the paper. All authors revised and approved the final manuscript.

Conflicts of interest

On behalf of all the authors, the corresponding author states that there is no conflict of interest.

Data Accessibility Statement

Data and code are available at https://github.com/GeladaResearchProject/DeLacey_et_al_chest_skin_transcription_2023

Benefit-Sharing Statement

We developed a research collaboration with scientists from Ethiopia who have assisted us with gelada capture-and-release campaigns. All collaborators are included as co-authors. The results of the research will be shared with the Simien Mountains National Park staff and guides in the local communities surrounding the park.

Figures

Figure 1. An adult male gelada (*Theropithecus gelada*) with a brilliant red chest patch. Photo by E. Roberts.

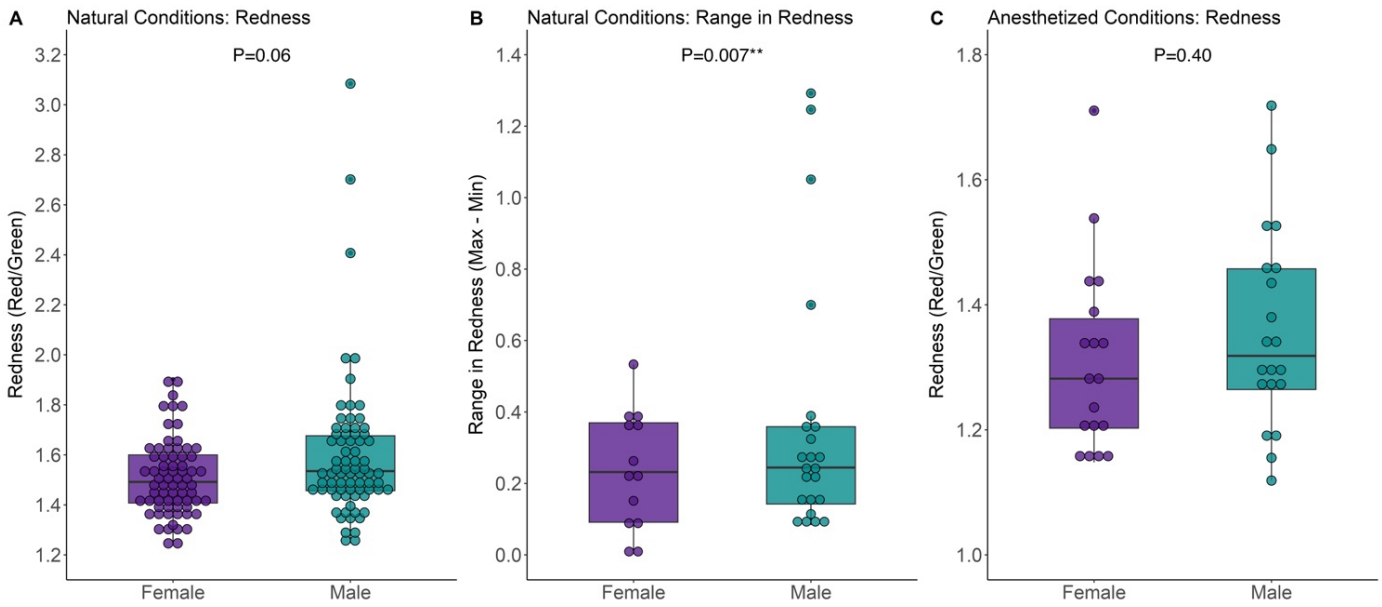


Figure 2. A) Males and females overlap substantially in chest redness under natural conditions where males show marginally redder chests than females (Linear mixed-effect model, β=0.11, P=0.06). **B) Adult males had a wider range in chest redness within individuals compared to females under natural conditions** (Linear regression model, β=0.63, P=0.007). **C) Minimal sex differences in chest redness at baseline while under anesthesia**. Adult males did not have redder chests than females while under anesthesia prior to heat application (Linear regression model, β=0.04, P=0.40).

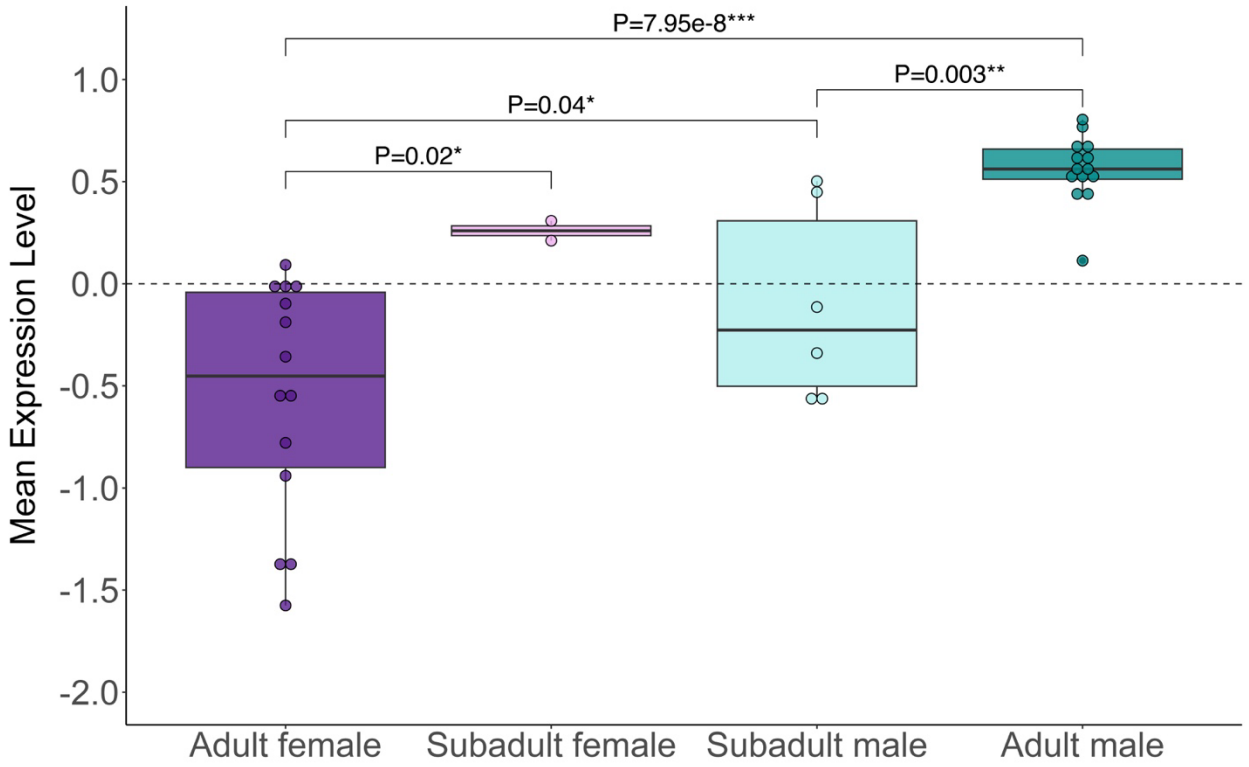


Figure 3. Subadult males exhibited intermediate expression of sex-biased genes between adult males (Linear regression model, β=-0.66, P=0.003) **and adult females** (Linear regression model, β=0.45, P=0.04). Mean, normalized expression of the 1,068 sex-biased genes for each individual across all age categories.

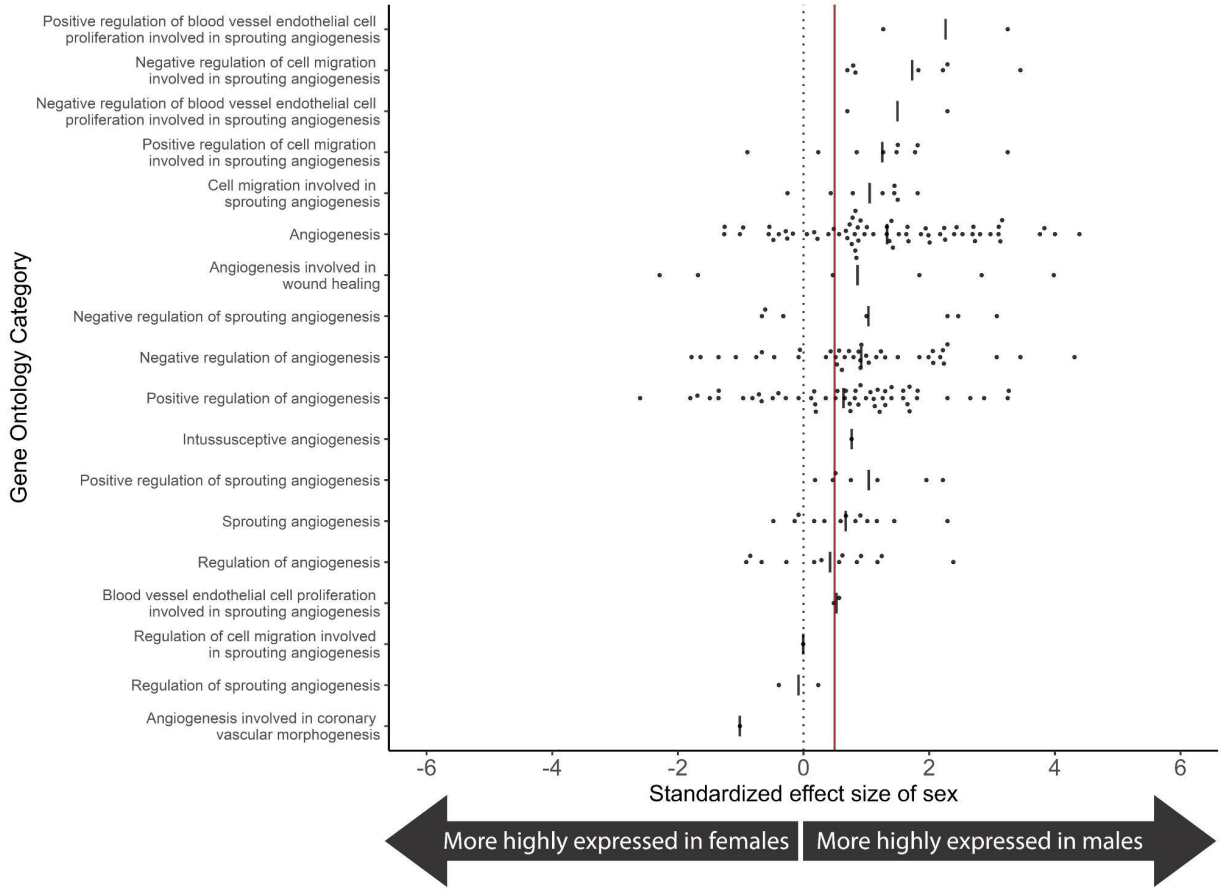
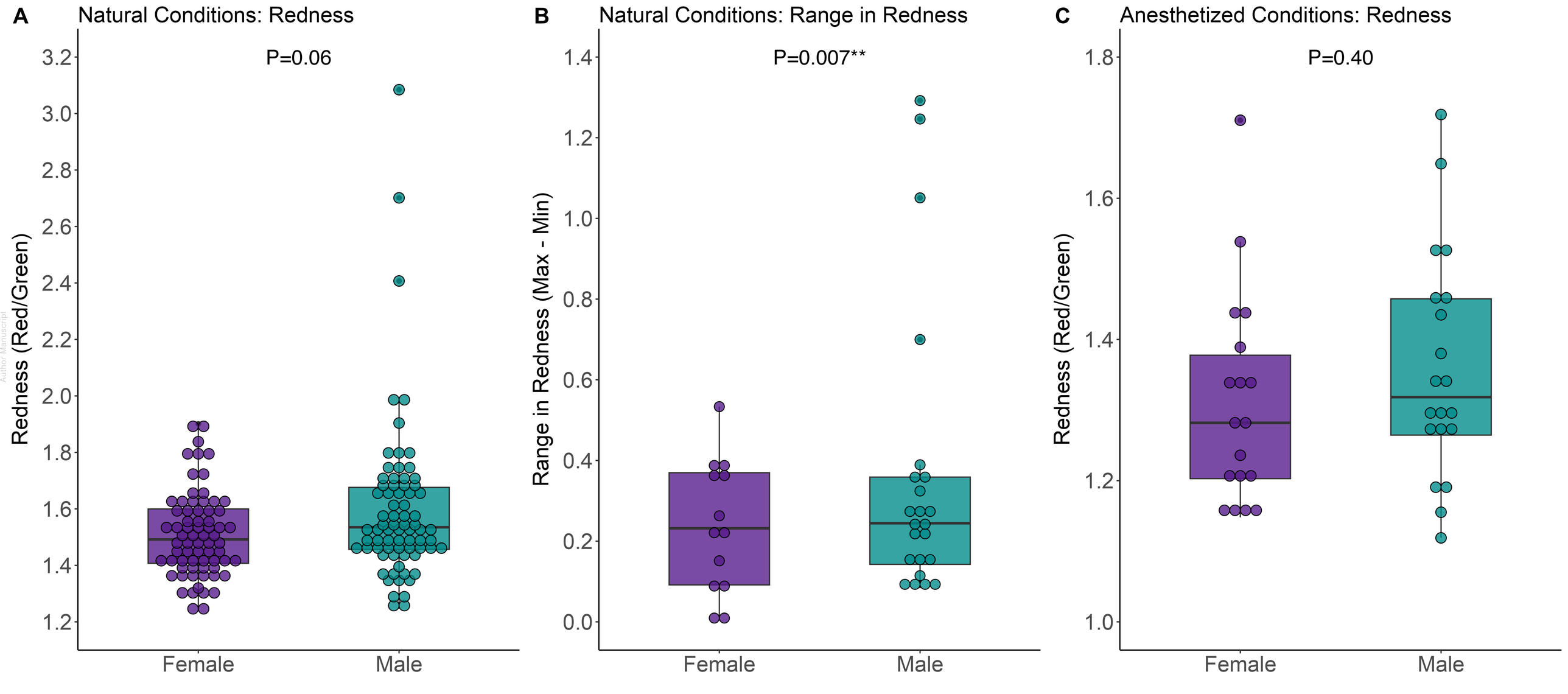


Figure 4. Genes more highly expressed in males were enriched for biological processes associated with angiogenesis (Kolmogorov-Smirnov test, D=0.19, P=7.25x10^{-7}). Beeswarm dots represent individual genes within each subcategory of the angiogenesis gene ontology category, and the solid vertical lines reflect the average standardized effect of sex for genes for each subcategory. The solid red line represents the median standardized effect size of sex for all genes not in angiogenesis-associated categories.

MEC_17026_fig1.jpg



P=7.95e-8***
P=0.003**
P=0.04*
P=0.02*
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Mean Expression Level
Adult female
Subadult female
Subadult male
Adult male

Gene Ontology Category

Positive regulation of blood vessel endothelial cell proliferation involved in sprouting angiogenesis
Negative regulation of cell migration involved in sprouting angiogenesis
Negative regulation of blood vessel endothelial cell proliferation involved in sprouting angiogenesis
Positive regulation of cell migration involved in sprouting angiogenesis
Cell migration involved in sprouting angiogenesis
Angiogenesis
Angiogenesis involved in wound healing
Negative regulation of sprouting angiogenesis
Negative regulation of angiogenesis
Positive regulation of angiogenesis
Intussusceptive angiogenesis
Positive regulation of sprouting angiogenesis
Sprouting angiogenesis
Regulation of angiogenesis
Blood vessel endothelial cell proliferation involved in sprouting angiogenesis
Regulation of cell migration involved in sprouting angiogenesis
Regulation of sprouting angiogenesis
Angiogenesis involved in coronary vascular morphogenesis

-6 -4 -2 0 2 4 6

Standardized effect size of sex

More highly expressed in females | More highly expressed in males