Received: 12 January 2023 | Revised: 2023 | Accepted: 12 May 2023

DOI: 10.1111/mec.17026

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

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

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Funding information
Arizona State Univers Association; Leakey F Award Number: AWD0123 AWD015438; Nacey Magg Foundation; National Grant/Award Number: NGS-504098R-18, 8124-06 and NGS-8981-19; National Scienc Grant/Award Number: 0715179, BCS-1723228, 1723237, BCS-1732231, 0942, BCS-1340542, IOS-2255974, BCS-1854359; Si Xi; Stony Brook Unive Michigan; University

Handling Editor: Michael M. Ha

Abstract

Male reproductive competition can select for conspicuous
that signal some aspect of fighting ability
However, the underlying mechanisms that link
are difficult to investigate in wild popula
manipulation. Here, we use digital photogra
the mechanisms of a visual signal used in m
chest patch (*Theropithecus gelada*). We analysed photograph
ing natural ($n = 144$) and anaesthetized conditions ($n = 38$) and (
male and female chest redness ($n = 38$) to explore
sex differences in gene expression. Male and
redness, but males exhibited a larger ... within
ral conditions. These sex differences were
10.5% of genes exhibiting significant sex d
exhibited intermediate gene expression patt
pointing to mechanisms underlying the develo
that genes more highly expressed in males w
opment and maintenance but not with androge
results suggest male gelada redness variabi

branching in the chest skin, providing a pot[illegible] current condition as increased blood circul[illegible] loss in the cold, high-altitude environment of geladas.

KEYWORDS
gene expression, *Theropithecus gelada*, [illegible], signal

1 | INTRODUCTION

Male reproductive success is often determined by male-male competition [illegible] (2011). Because actual fighting is frequently costly and exposes both the winner and [illegible] rival males can benefit from displaying [illegible] using signals that allow for competitor assessment in fighting (Bradbury & Vehrencamp, 2011; Maynard Smith & Parker, 1976). The conspicuous male traits used for [illegible] assessment (sexually selected signals) [illegible] lowest cost to both [illegible] (Maynard Smith, 1982; [illegible] Harper, 1995, 2003), and are often condition-dependent [illegible] they reliably indicate some aspect of [illegible] ability to win a physical fight (e.g. [illegible] health status) (Fisher, 1915; Penn & Számadó, 2020; Trivers, 1972; Zahavi, 1977). Because the expression of [illegible] male's condition, these signals [illegible] because only high-quality males can produce [illegible] (Weaver et al., 2017).

While the functional consequences of [illegible] noninvasively and [illegible] underlying these traits [illegible] (2022) [illegible] although observational data demonstrate that [illegible] drills (*Mandrillus leucophaeus*) exhibit redder [illegible] than lower ranking [illegible] (2009) [illegible] mechanism of this difference would [illegible] as surgical implants or hormone [illegible] manipulation, genetic manipulation, [illegible] or ethical to conduct in [illegible] (2012; [illegible] et al., 2000; Karubian et al., 2011). However, [illegible] programs in wild populations [illegible] nomic and transcriptomic [illegible] (2016) [illegible] minimally invasive technique to [illegible] of signal mechanisms without exp[illegible]

Here, we investigated the mecha[illegible] that may mediate male competitio[illegible] red chest patch (*Theropithecus gelada*) [illegible] cine primate endemic to [illegible] (Figure 1). Geladas have a multi-level social system in which [illegible] (one dominant adult male, one or [illegible] up to a dozen adult females and related [illegible] and move. The dominant 'leader' [illegible] of the mating opportunities and disp[illegible] to the subordinate 'follower' ma[illegible]

opportunities) and 'bachelor' male groups [illegible] no reproductive opportunities [illegible] Bergman et al., 2009 [illegible] (2008) [illegible] geladas live in la[illegible] [illegible] frequently congregate and forage [illegible] (2010) [illegible] sexually selected signal [illegible] competition [illegible] (2016). Bachelor males, which tend [illegible] [illegible] (2014) [illegible] 1-leaders with [illegible] ritualized 'vocal display' [illegible] 'climbing' [illegible] (2016) [illegible] Previous research conducted on the [illegible] chest patch [illegible] is [illegible] (Bergman et al., 2009; DeLacey et al., 2022) and (2) higher [illegible] following the application of [illegible] relationship with physic[illegible] [illegible]

FIGURE 1 An adult male gelada (*Theropithecus gelada*) with a brilliant red chest patch. Photo by [illegible]

Sexually selected signals in [illegible] testosterone, a steroid hormone [illegible] tive function in male verte[illegible] 1999; [illegible] Plant & [illegible] 2014) [illegible], is associated [illegible] efits (Ens[illegible] 1997) [illegible] physiological [illegible] et [illegible] 2006; Muehlenbein [illegible] 2005) [illegible] culating testosterone are known [illegible] increase blood flow, providing a [illegible] alongside a blood flow mechanism [illegible] geladas (M[illegible] 2002; Webb[illegible] 1999). Testosterone [illegible] alter gene transcription by (1) b[illegible] (2) aromatizing to oestradiol and then binding to oestrogen receptor α (ERα) or β (ERβ) or (3) co[illegible] binds to AR but cannot convert to [illegible] 2014) [illegible] conversion of testosterone to oestradiol is a particularly strong candidate for chest redness regulat[illegible] study; in the closely r[illegible] (*Macaca mulatta*) [illegible] increases in testosterone increas[illegible] sex skin areas. Moreover, the [illegible] itor (which prevents the conversion [illegible] decreased skin redness (Rhodes et al., 1997).

Therefore, to better understand [illegible] the chest patch signal mechanism [illegible] from male and female geladas fro[illegible] during [illegible], immobilizat[illegible] to explore differences in gene e[illegible] Digital photographs complement t[illegible] male and female chest redness bo[illegible] under anaesthesia. We tested t[illegible] ness is a [illegible] signal used d[illegible] We predicted that, when compared [illegible] (1) redder chests i[illegible] ness under natural conditions [illegible] creased expression of genes asso[illegible] increased expression of genes as[illegible] gen regulation.

2 | MATERIALS AND METHODS

2.1 | Study site and subjects

Data were collected from wild ge[illegible] National Park, Ethiopia as part of the Simien Mountains Gelada Research Project (SMGRP). The SMGRP has collected [illegible] demographic and hormonal data [illegible] das since 2006 and began a[illegible] campaigns in 2017 under the supe[illegible] and veterinary technicians. A sub[illegible] thetized with Telinject blow dar[illegible] ketam[illegible] (~7.5 mg/kg) and ~0.04 mg/kg [illegible] were monitored for temperature, pulse [illegible] during data collection, and sedation [illegible]

2.2 | Photo collection, measure[illegible]

[illegible]

tion with a second chest photo after [illegible] side of the chest. We include a visu[illegible] change in redness after temperature [illegible] tary material (Figure S1), but we did not run sta[illegible] the small sample size.

For all photos, we also photograph[illegible] Rite ColorChecker chart (hereafter, [illegible] other channels (Bergman & Beehner, 2008) and instructions for photo measurement [illegible] (DeLacey et al., 2022).

To determine whether males are [illegible] ural conditions, we constructed [illegible] with chest redness as the outcome variable [illegible] tor variable while including ID [illegible] (R package lme4 (Bates et al., 2015) and lmerTest (Kunze[illegible] et al., 2017)). Next, to assess whether [illegible] chest redness during natural conditions [illegible] model with the range in chest red[illegible] R/G − minimum R/G for each individual [illegible] the interaction between sex and [illegible] able. Lastly, to determine whether [illegible] under anaesthesia at baseline, we [illegible] chest redness as the outcome var[illegible] the predictor variables.

2.3 Skin biopsy collection

We collected chest skin biopsies from 15 adult males, 15 adult females, six subadult males and two subadult females [illegible] annual SMGRP capture campaigns. Adulthood was determined by the eruption of the [illegible] phenotypic metrics of adulthood [illegible] Jr & Gilbert, 1975). Biopsies were collected [illegible] opsy tool, placed vertically over [illegible] and rotated in one direction to [illegible] the subcutis. The biopsy was then [illegible] centrifuge tube with 0.5 mL RNAlater™ [illegible] within 6 h. The resulting small [illegible] aluminium aerosol bandaging to [illegible] fection. Upon arrival in the laboratory [illegible] vapour shipper), samples were stored at −80°C [illegible]

2.4 RNA extraction, sequencing and data processing

DNA and RNA were extracted from chest skin biopsies using TRIzol™ Reagent and Direct-zol™ RNA MiniPrep [illegible] Kit (Zymo Research, Irvine, CA) [illegible] using a Fragment Analyzer 5200 (Agilent [illegible]) Clara, CA). RNA-seq libraries were prepared [illegible] of total RNA following the [illegible] TM3'seq (Pallares et al., 2020). Libraries were [illegible] PCR cycles. All other procedures followed the manufacturer recommendations. Libraries were [illegible] molar quantities and sequenced on a [illegible] S4 flow cell (Illumina) [illegible] 2.17 million reads per sample [illegible] were mapped to the Macaca mulatta reference assembly Mmul_10 [illegible]

[illegible] (Wickham, 2020) using kallisto (Bray et al., 2016) (v0.44.0). We chose the Macaca mulatta [illegible] elsewhere [illegible] related [illegible] coid, the Theropithecus gelada genome (Tgel_1) tha[illegible] assembled [illegible] (2022) [illegible] Mmul_10 has 20% more annot[illegible] RNA-seq data from 13 tissues [illegible] only two RNA libraries derived from [illegible] tion, and the macaque represents a [illegible] all equally evolutionarily distant [illegible] thus any reference genome alignment [illegible] same across all samples (i.e., both [illegible]

2.5 Read count normalization

[illegible] voom function in limma (Ritchie et al., 2015) [illegible]

2.6 Modelling the effect of s[illegible] gene expression

[illegible] We used a mixed modelling approach [illegible] EMMREML [illegible] RNA [illegible] (RQN) [illegible] 2015) [illegible] matrix as the known [illegible] EMMREML [illegible] modelling appro[illegible] qvalue (Storey [illegible] 2022) [illegible] expressed between [illegible] more highly expressed in males and 'female-biased' if it was more highly expressed in females. We then compared expression values for these sex-biased genes between adult males across all 36 individuals ($n = 14$ adult males, $n = 14$ adult females, $n = 6$ subadult males, $n = 2$ subadult females) through the Z-transformed expression levels of the genes in each individual to obtain a composite sex-expression score for each individual. Finally, we ran a linear regression model with the expression score as the outcome variable and sex category (adult male, adult female, subadult male, subadult female) as the predictor, which allowed us to be able to investigate whether subadults differ from adults in sex-biased gene expression.

2.7 | 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 topGO package to identify biological processes that were enriched in genes differentially expressed between males and females (Alexa & Rahnenführer, 2020). We searched for terms related to angiogenesis, the formation of new blood vessels, including the [illegible] involved in angiogenesis (i.e. [illegible]). We compared the standardized effect of sex for [illegible] to the standardized effect of sex for all [illegible] with a Kolmogorov–Smirnov test.

To test our prediction that males would [illegible] of genes associated with oestrogen regulation, we used the Online Predicted Human Interaction Database (OPHID) to create protein–protein interaction (PPI) networks for the hormone receptor proteins ERα, ERβ and AR (Brown & Jurisica, 2005). We used ENSEMBL orthology through the biomaRt package (Durinck et al., 2009) to identify one-to-one orthologs in the human genome, and restricted our expression matrix to detectably expressed genes with orthologs [illegible] 8255 [illegible] (OPHID [illegible]) PPI networks [illegible] biomaRt [illegible] UniProt Consortium, 2021). We found the genes in the networks for ERα, ERβ and AR and compared the effect of sex for these genes with that for all other detectably expressed genes with a Kolmogorov–Smirnov test.

3 | RESULTS

3.1 | Chest redness in males and females

Under natural conditions (i.e. not [illegible]), males [illegible] females [illegible]. We [illegible] marginally [illegible] redder [illegible] ($\beta = 0.11$, $p = 0.06$; Figure 2a). However, adult males had a wider range in redness within individuals ($\beta = 0.27$, $p = 0.007$; Figure 2b). When [illegible] under anaesthesia [illegible] ($\beta = 0.04$, $p = 0.40$; Figure 2c).

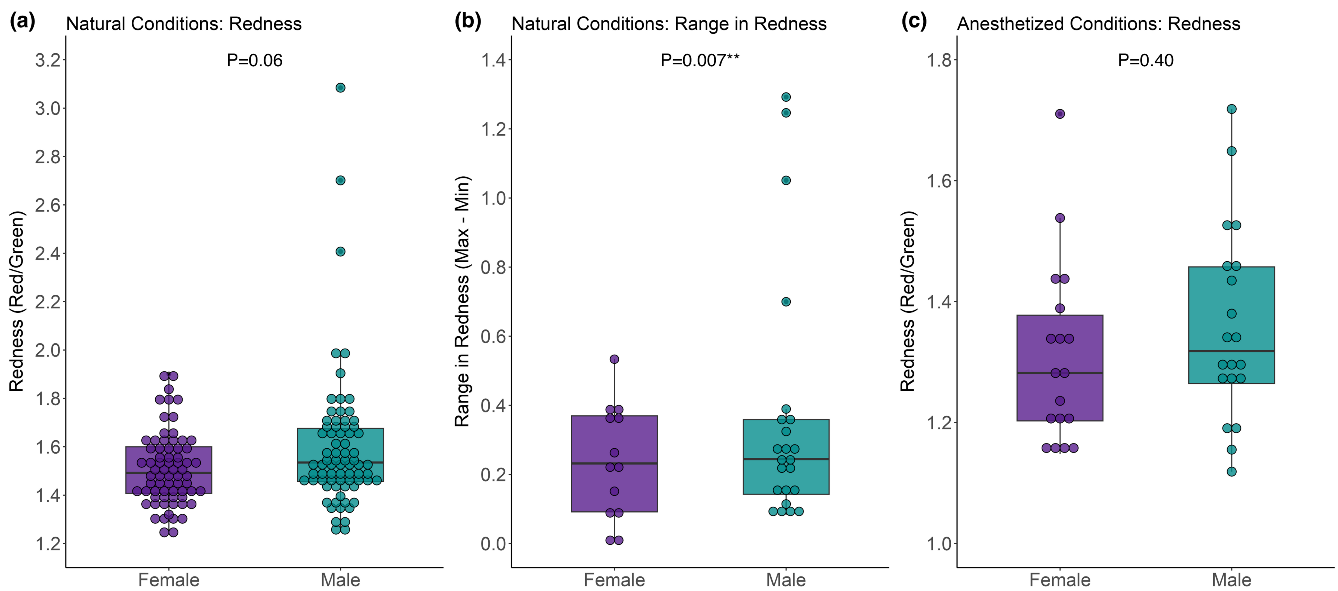

FIGURE 2 (a) Males and females overlap substantially in chest redness, [illegible] chests than females (Linear mixed model, $\beta = 0.11$, $p = 0.06$). (b) Adult males had a wider range [illegible] compared to females under natural conditions ($\beta = 0.27$, $p = 0.007$). (c) [illegible] baseline while under anaesthesia. Adult males did not have redder chests [illegible] (Linear regression model, $\beta = 0.04$, $p = 0.40$).

3.2 | Sex differences in gene expression

We then quantified how sex was associated with gene expression while controlling for [illegible]. Sex was significantly associated with both the first [illegible] component of gene expression (PC1: β = 0.39, p = 0.02; PC2: β = −0.22, p = 0.03; Table S1, Figure S2). Males and females [illegible] along the first principal component [illegible] controlling for year (PC1: [illegible] Figure S3; PC2: β = −0.13, p = 0.49). The results of the [illegible] did not change when we removed [illegible] somes and analysed only [illegible] (Figure S4).

At the level of the individual gene, we found that 10.5% of the 10,212 detectably expressed genes exhibited significant differential expression across males and females (n = 1068, FDR [illegible]; Figure S5), in a model controlling for [illegible], RNA concentration, RNA extraction date and year of sample collection. Of the 1068 differentially expressed [illegible] biased and 867 [illegible] sex-biased gene expression level for [illegible] subadult males exhibited an intermediate [illegible] when compared to adult males (β = −0.66, p = 0.003) and adult females (β = 0.45, p = 0.04; Figure 3). The sample size for subadult females (n = 2) was too small to draw [illegible] pression trends in this age category.

3.3 | Sex-biased genes involved in vascularization

In line with our prediction that [illegible] pression of genes associated with [illegible] genes more highly expressed in [illegible] cal processes associated [illegible] p = 7 × 10^-5 [illegible]; Figure 4). Additionally, these [illegible] were also enriched for biological pro[illegible]

[illegible] regulation and [illegible] $p = 2 \times 10^{-5}$; Figure S6). Contrary to our predi[illegible]

[illegible] skin biopsies and chest redness at b[illegible]

[illegible] and chest redness [illegible] Figure S7 [illegible]

4 | DISCUSSION

[illegible] mechanisms underlying [illegible]

[illegible] expression, where higher expression [illegible] angiogenesis, blood pressure and bl[illegible] [illegible] vascularization [illegible] and vascula[illegible] ferences in this s[illegible] (Figure 4) [illegible]

[illegible] chest photograph measurements [illegible] redness between the sexes at baseline [illegible] aesthetized conditions, but males o[illegible] individual range in redness. Select[illegible] in one sex can create a correlated [illegible] within a species (e.g. male and fem[illegible] related in passerines), suggesting [illegible] constrained by changes [illegible] 2015; [illegible] et al., 2010; Potti [illegible] 2011). [illegible] geladas, this [illegible] redness between the sexes could sim[illegible] tive genetic correlation. Alternati[illegible] traits function differently, female[illegible] have continued to evolve under anot[illegible] et al., 2015; Tobias et al., 2012), and the overlap in [illegible] be caused by each sex using the ch[illegible] different signals. In males, chest [illegible] status (Bergman et al., 2009) and within males by [illegible] (DeLacey et al., 2022), suggesting the chest p[illegible] [illegible] females [illegible]

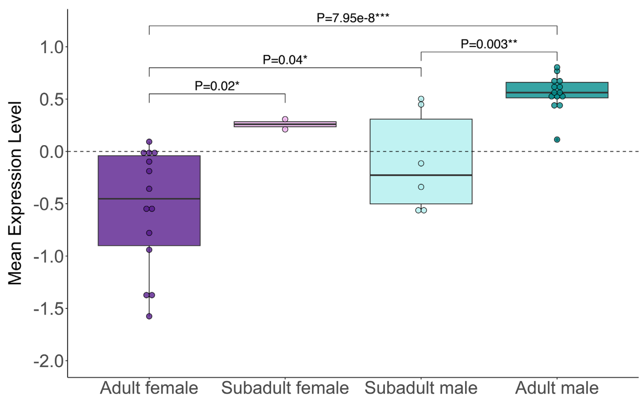

FIGURE 3 Subadult males exhibited intermediate expression of sex-biased genes between adult males (Linear regression model, β = −0.66, p = 0.003) and adult females (Linear regression model, β = 0.45, p = 0.04). Mean, normalized expression of the 1068 sex-biased genes for each individual [illegible] age category.

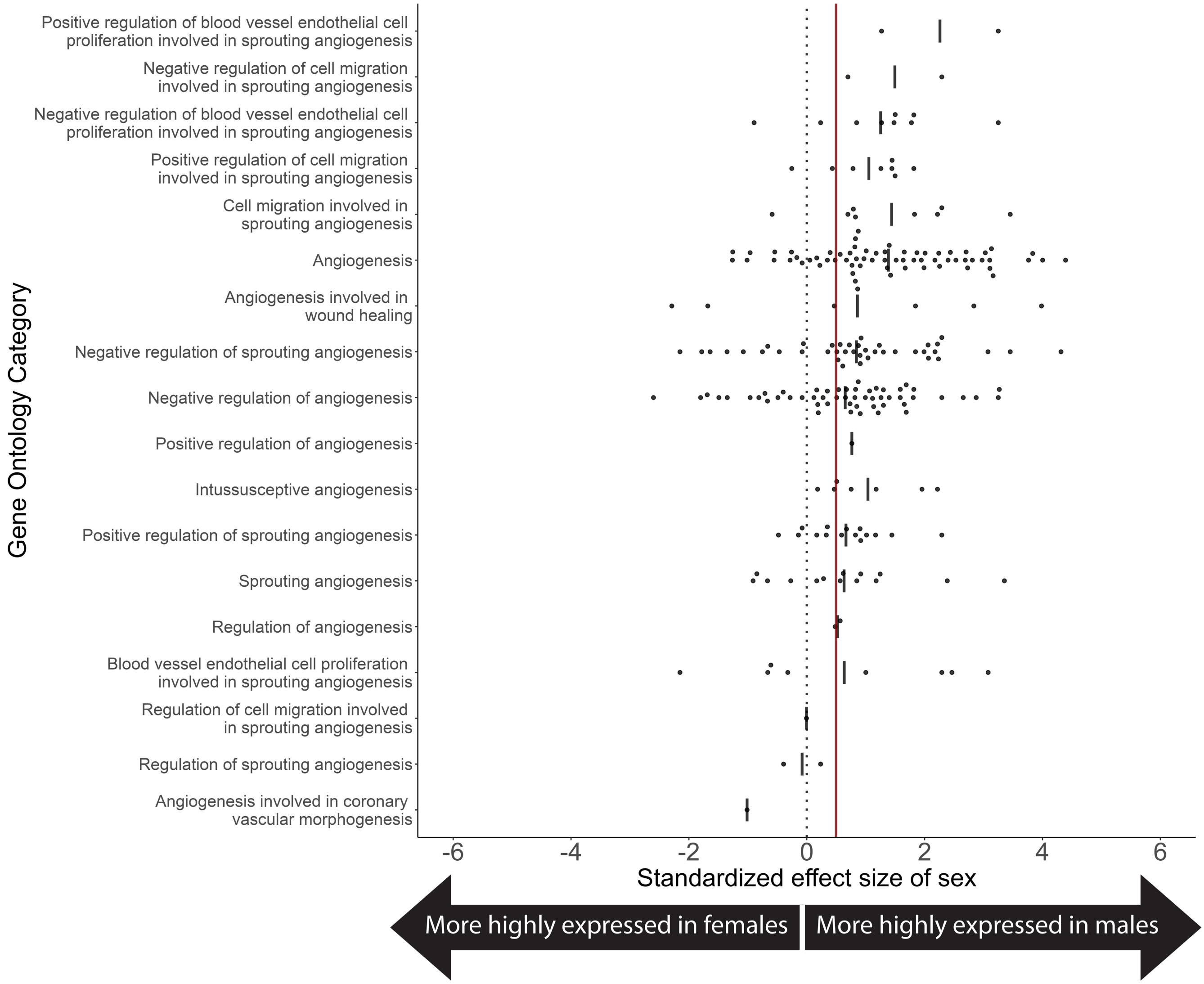

FIGURE 4 Genes more highly expressed in males were enriched for biologi
Smirnov D = 0.19, p = 7 × 10[-7]). Beeswarm dots represent individual genes within e
ontology category, and the solid vertical lines reflect the average
line represents the median standardized effect size of sex for all g

to communicate reproductive status through hormonal and blood sampl
signalling as they have the redd (Anderson et al., 2021; Lu et al., 2018). The ... of
oestrogen levels and blood 1985; ... gene expression has been shown to i
Roberts et al., 2017). In addition to chest ... variation ... 2010; ... Pen ... du
females exhibit sexual swellings ... 2014), ... differences ... sex ... l
surrounding the chest region when ... 2018) ... c
the ovarian cycle, suggesting se... Sub... swellings ... d
chest redness to signal a differ... between ... e n
(Roberts et al., 2017). expression differences increase a
The sex difference in gelada chest skin ... gene expression ... a c
with findings in humans where a wide variety of ... le
effects of sex on gene expression ... 2020; ... 2015) ... n (Ha
et al., 2020). However, small expression changes ... have been shown ... with gela
to have large phenotypic effects, ... e
disease (Khramtsova et al., 2019). ... were more highly expressed in
expression has also been ... reproductive skew.

Male geladas expressed genes associated with angiogenesis, blood pressure regulation and blood vessel maintenance more highly than females. The mechanism of in[illegible] the chest skin may indicate [illegible] signal where the differential c[illegible] body condition [illegible] (Grafen, 1990; Penn & Számadó, 2020). We propose [illegible] balance and heat loss as possibl[illegible] a red chest. Male geladas may [illegible] sel branching in the skin compar[illegible] in vocal displays (a behaviour [illegible] chest redness increases with disp[illegible] (Benítez, 2016), which suggests that, aft[illegible] built up vascular networks, an i[illegible] prompt a larger increase in chest [illegible] spend less time resting, more [illegible] gression and produce more calls [illegible] bachelors (Benítez, 2016; Pérez [illegible], 2021). The phys[illegible] required to engage in aggression and vocal displays may contribute to ensuring [illegible] in good body condition have red chests (if it is difficult to [illegible] between exertion and vascularization). Further, redder chests have higher surface skin temperatures which indicate that the in[illegible] also result in heat [illegible] Simien Mountains [illegible] (2022). [illegible] could provide an avenue for ches[illegible] body condition to potential rival[illegible].

Contrary to our predictions, [illegible] pression of genes associated wit[illegible] tion in the chest skin. Although [illegible] androgen and oestrogen regulat[illegible] ences in chest redness, it could [illegible] males use the same androgen and [illegible] the chest skin or (2) be a produ[illegible] ticula[illegible] sex-biased gene expression profiles (Lopes-Ramos et al., 2020; Yang et al., 2006). Perhaps, [illegible] detect more sex differences in e[illegible] in the regulation of hormone sec[illegible] sue (Beck et al., 2007). Additionally, we [illegible] of genes that interact with oestr[illegible] circulating hormones such as test[illegible] receptor density may play a lar[illegible] mates (Dixson, 1983; Rhodes et al., 1997; Setchell & Dixson, 2001). As yet, no relationship has been identified between testosterone and chest redness in adult male[illegible] (DeLacey and Beehner, unpublished data). This may be bec[illegible] tosterone dependent, or because [illegible] androgen metabolite levels (captu[illegible] mone over the past day) rather th[illegible] levels. Further, we may not de[illegible] one and chest redness because o[illegible] redness and testosterone only ind[illegible] aromatization to oestrogens.

4.1 | Limitations

We were only able to collect [illegible]

[illegible]

5 | CONCLUSION

[illegible]

AUTHOR CONTRIBUTIONS

[illegible] revised and approved the final [illegible]

ACKNOWLEDGEMENTS

[illegible] Jarvey, Levi Morris, Tara Regan, Ca[illegible]

Sloan, Megan Gomery, Laura Morrissey, ... Babbitt, Maddie Melton and Anna Cryer). This work was supported by the National Science Foundation (BCS-0715179, BCS-1732231, BCS-1723237, BCS-0919..., BCS-1723228, IOS-155... and BCS-1854359), the Leakey Foundation, the ... Foundation Baldwin Award (AWD012312), the National Geographic Society (NGS-8100-06, NGS-8991-11, NGS-... and NGS-50409R-18), the Fulbright Scholars Program, Nacey Maggioncalda Foundation, Sigma Xi, the University of Michigan, Arizona State University, University of Washington and Stony Brook University.

CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

Data and code are available at https://github.com/PGelada/Project_DeLacey_et_al_chestpatch_2023_trans

BENEFIT-SHARING STATEMENT

We developed a research collaboration with scientists from Ethiopia who have assisted us with every ... 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.

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How to cite this article: DeLacey, P. M., Sen, S., Diekhans, M., Chiou, K. L., Lemmon, ... Creasey, ... Lu, A., Bergman, T. J., Beehner, J. C., ... (2023). Vascularization underlies ... selected skin coloration in a wild ... *Molecular Ecology*, *32*, 4401–441[illegible]. https://doi.org/10.1111/mec[illegible]