Causes and Consequences of a Sexually Selected Signal, the Chest Patch, in Male Geladas

by

Patricia M. DeLacey

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in the University of Michigan
2023

Doctoral Committee:

Professor Jacinta C. Beehner, Chair
Professor Thore J. Bergman
Associate Professor Ben Dantzer
Assistant Professor Stacy Rosenbaum
Assistant Professor Noah Snyder-Mackler, Arizona State University

Patricia M. DeLacey

pdelacey@umich.edu

ORCID iD: 0000-0002-1124-3660

© Patricia M. DeLacey 2023

Dedication

In loving memory of my mom, Ginger Sasser DeLacey

For leading Girl Scouts camping trips, taking me to visit every historical site within driving distance of Southeastern Virginia, pointing out every bird and moon phase, teaching me to crab, and guiding me on nature walks around the farm and through the Blue Ridge Mountains. For the long Sundays spent at the library as you wrote your master's thesis and cozy nights reading on the couch. For inspiring my love of nature and fostering my academic curiosity.

For my dad, Bill DeLacey

For your love, support, patience, constant Harry Potter quotes, and mutual love of science.

For my older brother, John Patrick DeLacey

For your fun-loving mischief and always picking up the phone when I need a chat.

For my older sister, Ginny DeLacey Lewinski

For our constant banter and having a spot at the table and an Auggie hug ready for me whenever I come to visit.

For my partner, Peter Cerda

For your love and support through our dissertation-writing, job applications, and all the challenges and adventures yet to come.

Acknowledgements

"No (wo)man is an island entire of itself,[1]" rings true for my six years as a graduate student. I am grateful for the guidance and support I received from so many wonderful mentors, colleagues, and friends during my time at the University of Michigan. Thank you for making this journey possible and for helping me achieve a great amount of personal and professional growth throughout the process.

First and foremost, thank you to my advisor Dr. Jacinta Beehner and co-advisor Dr. Thore Bergman for your guidance, feedback, and overall enthusiasm for gelada research during my time as a graduate student. I know I have become a better scientific writer and researcher under your supervision. Jacinta, thank you also for your encouragement while I pursued science communication opportunities outside of my own research and recognizing my passion (dare I say, talent?) for sending comical March Mammal Madness update emails. I am deeply grateful to Thore and Jacinta for first hiring me in 2015 for a one-year position as the camp manager for the gelada site in the Simien Mountains National Park, Ethiopia. Thank you for trusting me with your field site as a bright-eyed 22-year-old one month out of college. That first year in Ethiopia was one of the most formative experiences of my life and I truly enjoyed every minute as camp manager.

Thank you to the rest of my committee members for your feedback and guidance through this project and bearing with me as I adapted my project to disruptions caused by the COVID-19

---

[1] John Donne, *Devotions Upon Emergent Occasions,* no. 17, pp. 108-9 (1959). Originally published in 1624.

pandemic and civil unrest in Ethiopia. I am grateful to Dr. Noah Snyder-Mackler for taking me in to collaborate on the chest skin biopsies RNA-Seq project (Chapter 3). I appreciate you for taking the time to teach me genomics laboratory techniques when I visited the University of Washington and for your patience and guidance during one-on-one zoom meetings during data analysis. Thank you to Dr. Stacy Rosenbaum for your instruction and writing feedback during the grant writing course which ultimately ended with a successful NSF-DDRIG application. Your kind encouragement and positive attitude was just what I needed from a mentor through the grant application process and during the early stages of the pandemic as we finished the grant writing course on zoom. Thank you to Dr. Ben Dantzer for your mentorship and feedback through joint "squirrelada" lab meetings, my preliminary exams, and prospectus. Your genuine enthusiasm for science is infectious. Your instruction in the "Biology of Sex Differences in the Brain and Behavior" Biopsychology seminar inspired me to purchase my own copy of "Evolution's Rainbow" by Joan Roughgarden and to write an article titled "Recognizing the "I" in Science: Dispelling assumptions about sexual selection across the animal kingdom" for *EquilibriUM* magazine.

I have been fortunate to have had excellent "lab sisters", Sharmi Sen and Sofia Carrera throughout my time as a grad student. It's been a joy to share an office with you. Thanks for being a friend and a shoulder to cry on when I'm feeling overwhelmed. It's been great to have peer-mentors to help brainstorm ideas, code in R, and practice talks. I'm looking forward to our Pride & Prejudice marathon to celebrate turning in our dissertations. I am overjoyed that we can all walk in the Rackham graduation together. I know you two will make excellent researchers and professors. Sofia – I still think you should consider using my daily Adele song limit that I imposed on you in the field in your everyday life.

Working with the Simien Mountains Gelada Research Project allowed me to receive mentorship and develop friendships with researchers beyond the University of Michigan. In addition to the co-PIs acknowledged above (Dr. Jacinta Beehner, Dr. Thore Bergman, Dr. Noah Snyder-Mackler), I am thankful for Dr. Amy Lu's writing feedback on my first two manuscripts and her mentorship when we overlapped in the Simiens. While visiting Noah's lab, it was a delight to overlap with Dr. Kenny Chiou and Dr. India Schneider-Crease and witness their constant stream of banter. Thank you to Kenny for helping me troubleshoot a new protocol in the lab and thank you to both India and Kenny for their writing feedback, mentorship, and willingness to hang out in Seattle during my visit. During my first year in Ethiopia, I was fortunate to make close friends with Dr. Rachel Perlman who I then later collaborated with while validating chest redness measurement protocols (Chapter 2). Rachel, your organizational skills and tenacity were essential as we struggled to validate several objective color measurement methods. I truly couldn't have gotten through that headache of a project without you, and I need to come visit you in New York ASAP (or more likely, as soon as I have a job that pays better than grad school stipends).

My time in Ethiopia while working with the Simien Mountains Gelada Research Project allowed me to form friendships that I know will last a lifetime. Thank you to the long-term research assistants, Eshete Jejaw, Ambaye Fanta, and Setey Girmay, for teaching me how to identify geladas, helping me improve my Amharic, and for encouraging me with an "Aye-zosh" when I could barely catch a breath walking up steep inclines in the high-altitude grasslands. Eshete, I loved your "gelada quizzes" when helping me learn to identify geladas even when you threw some trick questions in there. Ambaye, your quick wit always got a laugh out of me even on a day of numerous field frustrations. Setey, your positive attitude and constant song request for “Waka Waka” was always an asset to the field team. Thank you to Yeshi for your hard work around the house in Sankaber, cozy coffee ceremonies, and injera demonstration to us ferengis. Thank you to the scouts and their families for making me feel welcome in the Sankaber community, celebrating with us at holiday gibshas, and for helping us get the truck out of the mud on multiple occasions in the rainy season.

Outside of the Sankaber community, thank you to all my friends and colleagues in Debark, Gondar, and Addis Ababa. I greatly appreciated Shiferaw Asrat and Meseret Mulat for serving as the translator between me and the Gondar mechanic, helping us find propane when it was nowhere to be found, and for your friendship. I am thrilled to have seen the Limalimo Lodge develop from a pipe dream to a reality, and I cannot wait to visit as a tourist when I accumulate enough savings. Thank you to Tariku W. Yadete and Simahne Lalisa for helping me manage logistics while in Addis Ababa and navigate Ethiopian immigration bureaucracy. Even when I found myself crying into a scarf on the front porch of the immigration office after spending 8+ hours waiting in line trying to renew my residency permit, you calmly planned which offices to visit the next day and ultimately helped me get the permit I needed. None of this research would have been possible without the support of everyone at the Ethiopian Wildlife Conservation Authority (EWCA), particularly Ato Kumara Wakjira and Dr. Fanuel Kebede, or the support of the Simien Mountains National Park Warden Ato Abebaw Azanaw, and the staff, scouts, and guides of the Simien Mountains National Park.

Thank you also to the wonderful community in Ann Abor that helped me complete this dissertation. First, thank you to all the past and present Biopsychology graduate students who have made my time here so much more enjoyable. I always look forward to summer for another Biopsych Boppers kickball season as a fun time to reconnect after a busy academic year. Thank you for being so willing to dress up and attend a Harry Potter themed party at my house and for representing the Area at social events when I was a PGSA co-chair. Graduate school is certainly not always an easy time and I have been fortunate to have a lovely community of peer mentors to rely on. I strongly considered leaving after my second year. After chatting to a few kind and patient senior Biopsych students and doing some soul searching, I clearly decided to stay. I'm glad that I did, but if you're reading this and working on making the same decision – do what's right for your mental health and happiness.

Thank you to all my roommates during my time living on Division Street (Anne Sabol, Kris Harmon, Giovanni Román-Torres, Sofia Carrera, Tim Williams, Jeremiah Hauth, Mattison Rose, Rosie Bettle, Sammy Iliff, Katie Winner, and honorary housemate Tessa Swanson). I loved living in this drafty house with so many brilliant, silly friends despite the bat infestation (fondly named Bruce Wayne). I am grateful to have ridden out the pandemic with a squad of six people (quaran-queens) even though I used a beer pong table with duct tape holding the edge together as a desk for 6+ months. Our family dinners, group bike rides, movie nights, Halloween parties, and bonfires brought me so much joy and provided the break I needed from work. Thank you as well to my current roommates, Erin and Mena Davidson, for taking me in as a renter after I was hanging out so much during "Ladies Lifting League" sessions. You two are incredible people and roommates and I proud to call you friends. I've learned a lot of life lessons over the years with you all including Costco sales, bike repairs, and dog training. Thank you for putting up with my incessant movie quotes and Kingsley's general chaos when he was a puppy. Our little menagerie with Aster, Kingsley, and Clover is endlessly entertaining. Thanks for creating such a cozy home to both work and relax in.

Thank you also to all my friends outside of Ann Arbor who have been a wonderful support system. I have been lucky to stay in touch with my close-knit Duke friends from Maxwell House – Teresa Rosenberger, Kirsti Harlan, Jamie Kessler, Hannah Neville Oakley, John Bentley Davis, Zach Wiener, and Hailey Johnson. I'm grateful to have shared so many ridiculous and fun times over the years and to always have a friend to call when times are tough. No matter how successful all of you continue to be in life, I'll still giggle and think of all the nonsense we got up to when we were 19. Liz Babbitt – you are gas. Many a sost to you. As it as such, on to you. Liz, I'm glad you tackled me while I was doing yoga on day 3 of knowing each other. You're always just there in the thick of the nonsense with a hilarious, understanding look. Always live life brakes yellum.

I am thankful to have had good choice in friends even when I was 3 years old when I met Ali Reilly Tisdel and Emily Lewinski. Ali and Emily, I am so grateful to have been your friends from after pre-school playdates to adulthood. Glad we could share those awkward middle school years together – especially when I got tall, got braces, and started wearing glasses all at the same time. You two were also integral for helping me keep moving forward after my mom's death. Thanks for being there when I needed to talk as well as when I just needed to be distracted with silly high school antics. I know "Ms. Ginger" would be so proud of the women you two have become since the Troop 434 days. I'm excited to keep up the July vacation together for years to come.

In addition to all my lovely peers, I am grateful to have been scooped up by so many mother figures after my mom's death. Thank you especially to my godmother, Amy Hammond, for our long phone calls and for putting me up in your extra bedroom while I was transitioning from my position in Ethiopia and applying to graduate school. My mom had excellent taste in best friends, and I know she'd be so appreciative of you looking after her daughter. Thank you as well to all my friend's mothers – Pat Reilly, Doreen Lewinski, and Justina Karali – for mothering me when I needed it most towards the end of high school. Doreen, thank you for hosting so many holiday meals when my dad and I most needed it. I'm thankful to feel at home at so many different homes around Western Branch.

Thank you to my family for your love and support through my entire life. My dedication above sums up the sentiment. I wouldn't be here finishing a Ph.D. program without my mom, Ginger Sasser DeLacey. Although she's no longer with us today, I know she would have been there cheering me on through the dissertation, worried sick while Sofia and I were evacuating from Ethiopia, and likely giving me writing comments in Mirado Black Warrior Pencil. Thank you to my dad, Bill DeLacey, for your love and encouragement through good times and bad. Your eternal optimism has always been inspiring. I feel prepared to attempt to fix my own sprinkler system (with many trips to Lowes) when I'm a homeowner one day. Thank you to my siblings, John Patrick and Ginny, who teased me relentlessly as a child but are now some of my closest friends. Thank you as well to the Sasser Family and Uncle Dan for your encouragement and kindness over the years. My Nana, Patricia Sasser, has always been a rock in my life. Your quick wit, tenacity, and charisma always brings a smile to my face. I'm looking forward to a road trip down to North Carolina to come visit and belatedly celebrate your 90th birthday.

Peter (now Dr. Peter Cerda), I love you and I am so proud of you for landing a job with U-M Library post-graduation. This past year and a half sharing the ups and downs of life with you have been some of the best times of my life so far. Thank you for all your encouragement as we defended our dissertations within 48 hours of one another. I'm excited to make a home here in Ann Arbor with you and for all the adventures we have ahead.

Table of Contents

Dedication ii

Acknowledgements iii

List of Tables xiv

List of Figures xv

List of Appendices xix

Abstract xx

Chapter 1 : Introduction 1

1.1 Background 1

1.1.1 Sexual selection & male signals 1

1.1.2 Sexually selected skin coloration in primates 4

1.1.3 Red skin coloration mechanisms in primates 6

1.1.4 Color vision in primates 7

1.2 Study site and subjects 9

1.3 Research questions and chapters 11

1.4 Significance of research 13

Chapter 2 : Assessing Male Gelada Chest Patches: Color Measurement and Physiological Mechanisms 15

2.1 Abstract 15

2.2 Introduction 16

2.3 Methods 20

2.3.1 Study site and subjects 20

2.3.2 Camera, color standard, and redness metrics 21

2.3.3 Data collection 23

2.3.4 Photo measurement 26

2.3.5 Data analyses 29

2.4 Results 31

2.4.1 Method consistency 31

2.4.2 Method accuracy 33

2.4.3 Activity and redness 34

2.4.4 Heat manipulation and redness 35

2.4.5 Redness and skin temperature 36

2.5 Discussion 37

2.6 Acknowledgements 43

2.7 Author contributions 43

2.8 Funding 43

Chapter 3 : Vascularization Underlies Differences in Sexually Selected Skin Coloration in a Wild Primate 45

3.1 Abstract 45

3.2 Introduction 46

3.3 Materials and methods 49

3.3.1 Study site and subjects 49

3.3.2 Photo collection, measurement, and analyses 50

3.3.3 Skin biopsy collection 52

3.3.4 RNA extraction, sequencing, and data processing 52

3.3.5 Read count normalization 53

3.3.6 Modeling the effect of sex on gene expression 53
3.3.7 Enrichment analyses 54
3.4 Results 55
3.4.1 Chest redness in male and female geladas 55
3.4.2 Sex differences in gene expression 56
3.4.3 Sex-biased genes involved in vascularization 58
3.5 Discussion 59
3.5.1 Limitations 63
3.5.2 Conclusion 64
3.6 Acknowledgements 64
Chapter 4 : The Relationship Between Chest Redness, Testosterone, and Tenure Length in Male Geladas (*Theropithecus gelada*) 66
4.1 Abstract 66
4.2 Introduction 67
4.3 Methods 70
4.3.1 Study site and subjects 70
4.3.2 Photo collection, measurement, and analyses 70
4.3.3 Fecal sample collection, extraction, and storage 71
4.3.4 Fecal androgen metabolite assay 72
4.3.5 Weather variables 73
4.3.6 Age, status, group size, and tenure length 73
4.3.7 Data analyses 74
4.4 Results 76
4.4.1 Across males of all statuses 76
4.4.2 Across leaders 78
4.5 Discussion 81

Chapter 5 : Conclusion.. 87

  5.1 Overview 87

    5.1.1 What are the causal mechanisms associated with redder chest patches? 88

    5.1.2 What are the functional consequences associated with redder chest patches?............ 90

  5.2 Limitations of study and future directions 91

  5.3 Concluding remarks 94

Appendix A 95

Appendix B 107

Bibliography 113

List of Tables

Supplementary Table 1. The Red values for the ColorChecker chart and Nix for each of the 24 ColorChecker chart squares. 95

Supplementary Table 2. The Green values for the ColorChecker chart and Nix for each of the 24 ColorChecker chart squares. 96

Supplementary Table 3. The Blue values for the ColorChecker chart and Nix for each of the ColorChecker chart squares. 98

Supplementary Table 4. Results of linear regression model for redness across light condition for Adobe Photoshop. 102

Supplementary Table 5. Results of linear regression model for redness across light condition for ImageJ. 103

Supplementary Table 6. Results of linear regression model for luminance across light condition for ImageJ. 104

Supplementary Table 7. Sample collection by year, sex, and age category for the 36 samples used in analyses 108

List of Figures

Figure 1. Outline of the following chapters that investigate the causes and consequences of variation in the red chest patch for leader male geladas. 12

Figure 2. Adult male gelada (Theropithecus gelada) in the Simien Mountains National Park, Ethiopia. Photo by R. Perlman. 18

Figure 3. Method consistency: Adobe and ImageJ micaToolbox across four lighting conditions for two colored squares on the X-Rite ColorChecker chart. Variation across lighting conditions for the “light skin” and “moderate red” colored squares for: **A)** Red/Green measured in Adobe. **B)** R-G Opponency ((LW – MW)/(LW + MW)) measured in ImageJ micaToolbox. **C)** Luminance ((LW+MW)/2) measured in ImageJ micaToolbox. Dotted lines represent the average values for each square for photos across all light conditions. Error bars represent the standard error of the mean. 32

Figure 4. Method accuracy: redness measurements from all three methods were significantly correlated with the Nix measurement. The relationship between the Nix chest redness values (Red/Green) and **A)** subjective redness scores averaged across all observers, **B)** Adobe Photoshop chest redness values (Red/Green), and **C)** ImageJ micaToolbox chest redness values (Red-Green Opponency (LW – MW)/(LW+MW)) 33

Figure 5. Activity and redness: male chests were redder immediately after a display (“post-display”) compared to “baseline” 34

Figure 6. Heat manipulation and redness: male chests were redder immediately after a heat application compared to before (“baseline”) 35

Figure 7. Redness and skin temperature: redder chests have higher skin surface temperatures. Chest patch surface temperature (°C) measured with a Raytek MX6 Phototemp Infrared Thermometer increases with chest redness (Red/Green) in leader males 36

Figure 8. Adult males had a wider range in chest redness within individuals compared to females under natural conditions. 56

Figure 9. 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. 56

Figure 10. Subadult males exhibited intermediate expression of sex-biased genes between adult males and adult females. Mean, normalized expression of the 1,068 sex-biased genes for each individual across all age categories 58

Figure 11. Genes more highly expressed in males were enriched for biological processes associated with angiogenesis. Points 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 of each subcategory. The solid red line represents the median standardized effect of sex for all genes not in angiogenesis-associated categories. 59

Figure 12. Across males, redder chests were associated with higher cumulative rainfall from the past 90 days and higher maximum temperatures. Leader males had redder chests compared to former leaders but not bachelors. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval. 76

Figure 13. Across males, higher fAMs were associated with higher cumulative rainfall from the past 90 days and younger males. Leader males had higher fAMs compared to follower males but not bachelors. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval. 77

Figure 14. For paired chest photo and fAM samples collected from the same individual within 60 days of one another, there was a positive but non-significant relationship between chest redness and fAMs. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval. 78

Figure 15. Across leaders, redder chests were associated with (i) higher maximum temperatures, (ii) higher cumulative rainfall from the past 90 days, (iii) a time when leaders were closer to being overthrown, and (iv) longer tenures (although this last one was a trend that did not reach significance). The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval. 79

Figure 16. Across leaders, higher fAMs were associated with (i) higher cumulative rainfall from the past 90 days, (ii) a timer period further from being overthrown, and (iii) shorter tenure lengths. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval... 80

Figure 17. Across leaders, for paired chest photos and fecal samples collected from the same individual within 60 days of one another, there was a positive but non-significant relationship between chest redness and fAMs. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval. 80

Figure 18. Within leaders, males with shorter tenures trended towards higher redness during their first 4 months than their last 4 months compared to their own overall average. Males with longer tenures trended towards lower redness during their first 4 months than their last 4 months compared to their own overall average.. 81

Supplementary Figure 1. The relationship between Nix and the ColorChecker chart for A) Red values, B) Green values, and C) Blue values of the 24 ColorChecker chart squares. ……..…..100

Supplementary Figure 2. Measuring chest patch skin surface temperature. One of the authors (TJB) using a Raytek MX6 PhotoTemp Infrared Thermometer ("temperature gun") to measure the surface temperature of an adult male's chest patch. All measurements were taken from 1-2 meters away. ……………………………………………………………………..………100

Supplementary Figure 3. Adobe measurement process. ……………………………….……101

Supplementary Figure 4. ImageJ micaToolbox measurement process.……………………..…101

Supplementary Figure 5. Within individual leader males following a display ("post-display") as compared to "baseline" conditions measured for: **A**) Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox. ……………………………………………………………………………………..105

Supplementary Figure 6. Within individual adult males, direct application of a heat pack to the chest as compared to measurements taken immediately prior for: **A)** Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox. ………………………………………………………………………………105

Supplementary Figure 7. Chest patch surface temperature (°C) measured with a Raytek MX6 PhotoTemp Infrared Thermometer compated with **A)** Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox. ……………………………………………………………………………..106

Supplementary Figure 8. **A)** An adult male gelada sitting with his head lowered over his chest patch on a cold morning. **B)** A reproductive unit of geladas huddling together with their chest patches turned inwards (photographs by Julie Jarvey). ………………………………..106

Supplementary Figure 9. Adult males and females show a similar pattern in change in redness between baseline and application of a heat pack directly to the skin. …………………………107

Supplementary Figure 10. Sample collection year was significantly associated with the first and second principal component of gene expression. …………………………………………...108

Supplementary Figure 11. The first principal component of gene expression by age category while controlling for year of sample collection. ………………………………………………109

Supplementary Figure 12. The results of the principal component analysis did not change between **A)** when we removed genes located on the Y chromosome and **B)** when we analyzed only those found on autosomes (both X-linked and Y-linked genes removed). ……………….110

Supplementary Figure 13. Genes more highly expressed in males were enriched for biological processes associated with blood pressure regulation and blood vessel maintenance. Beeswarm dots represent individual genes within the top 13 most represented subcategories of the blood pressure and blood vessel maintenance gene ontology category. 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 blood pressure regulation of blood vessel maintenance associated categories. ………………………………..111

Supplementary Figure 14. Expression of genes associated with ERα, ERβ, or AR did not correlate with chest redness at baseline while under anesthesia within males or within females. 112

List of Appendices

Appendix A: Supplementary Material Accompanying Chapter 2 95

Appendix B: Supplementary Material Accompanying Chapter 3 107

Abstract

In species with intense male competition, selection favors conspicuous signals that allow rival males to display their strength or condition, reliably indicating some aspect of "quality" that predicts their ability to win a physical fight. These sexually selected conspicuous signals ensure only evenly matched males engage in fights which resolves conflicts at the lowest cost to both males as fighting is energetically costly and exposes both the winner and loser to injury and infection. In primates, conspicuous red skin signals occur in several male catarrhine species with a strong reproductive skew towards dominant males. Red skin has been associated with aspects of male competition such as rank or rates of aggression in these species.

This research examines a putatively sexually selected trait used in rival assessment, the brilliant red chest patch in male geladas (*Theropithecus gelada*). Although previous research has established variation in chest redness across males, little is known about the mechanisms underlying chest redness or the functional consequences associated with a red chest patch. I aim to answer the following question: *what are the causes and consequences of variation in the red chest patch for leader male geladas?* To achieve this, I use long-term demographic, hormonal, photogrammetric, and climatic data accompanied by transcriptomic and photogrammetric data collected during annual capture-and-release events from a population of wild geladas living in the Simien Mountains National Park, Ethiopia.

In Chapter 2, I first sought to identify the best method of objective color measurement for working with gelada chest skin redness. I compared the accuracy and consistency of multiple objective color measurement methods and selected the Adobe Photoshop method for all subsequent color measurement analyses based on the results. I then examined physical correlates of chest redness and found that redder chests were associated with increased physical activity and higher chest skin surface temperature. In Chapter 3, I tested whether male and female geladas exhibit differences in (1) chest redness under natural, free-ranging conditions and while under anesthesia during a capture-and-release program, and (2) chest skin gene expression patterns. While both males and females overlapped substantially in chest redness, males exhibited a wider within-individual range in chest redness under natural conditions. I detected sex differences in chest skin gene expression where higher expression in males was associated with angiogenesis, blood pressure, and blood vessel maintenance. Together, the results from Chapters 2 and 3 provide 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 Chapter 4, I investigated whether higher circulating androgens predict redder chests and found a weak positive relationship between chest redness and fecal androgen metabolites (fAMs) suggesting circulating androgens may work alongside a blood flow mechanism to increase chest redness. Lastly, I assessed whether chest redness predicts how long an adult male gelada is the dominant male in a reproductive unit. I found that leaders with redder chests trend towards longer tenures. This potentially suggests that a redder chest wards off rival males and thus indirectly contributes to reproductive success by allowing leaders a longer time to sire more offspring. Together, these chapters advance our understanding of a unique sexually selected signal of male competitive ability in a non-human primate.

Chapter 1 : Introduction

1.1 Background

1.1.1 Sexual selection & male signals

Competition within the sexes has selected for elaborate weaponry or conspicuous signals to assess rivals' condition and fighting ability (Andersson, 1994; Darwin, 1871). As there is typically a male-biased operational sex ratio where more males are ready to mate at any given time compared to females, selection often favors these traits within males to aid in male-male competition (Weir et al., 2011). However, fighting is energetically costly and exposes both the winner and loser to the risk of injury, infection, or death. Displays allow individuals to resolve conflict so that competition only occurs between evenly matched competitors (Maynard Smith, 1982; Maynard Smith & Harper, 2003, 1995).

Organisms signal competitive ability in multiple modalities including vocalizations as in the common toad (*Bufo bufo*; Davies & Halliday, 1978), visual assessment as with the blue scrotal color in vervet monkeys (*Chlorocebus pygerythrus;* Gerald, 2001), olfactory signals as with musth in African elephants (*Loxodonta africana;* Poole, 1989), or tactile signals as with web vibrations in the funnel-web building spider (*Agelenopsis aperta*; Riechert, 1978). These signals increase the fitness of the signaler by altering the behavior of the receiver in a favorable way (i.e., deterring challenges from unevenly matched males; Maynard Smith & Harper, 2003, 1995). However, male displays are only an evolutionary stable strategy if the signal delivers reliable information to the receiver (rival male) allowing them to decide whether to engage in a fight (Maynard Smith & Harper, 2003, 1995). So how do receivers ensure that senders are conveying accurate information about their own fighting ability? There are several mechanisms for maintaining signal honesty: individual recognition, index signals, and condition-dependent signals.

Individual recognition, or the ability to identify a conspecific based on individually distinctive characteristics, provides a mechanism for rival assessment as repeated interactions build up associations with each individual's competitive ability (Dale et al., 2001; Tibbetts & Dale, 2007). Individual recognition has been established in a wide variety of taxa including invertebrates (Karavanich & Atema, 1998), fish (Höjesjö et al., 1998), birds (Dale et al., 2001), reptiles (Olsson, 1994), and mammals (Sayigh et al., 1999). A key prerequisite of individual recognition, specialized face-learning abilities, has been demonstrated in paper wasps (*Polistes fuscatus*, (Sheehan & Tibbetts, 2011), and primates including humans (Parr et al., 2000; Pascalis & Kelly, 2009). Non-human primates often use individual recognition in competitive contexts. Male bonnet macaques (*Macaca radiata*) recruit allies that outrank themselves and their opponent when forming coalitions, which requires recognition of others as well as their associated rank (Silk, 1999). Chacma baboons (*Papio hamadryas ursinus*) even extend recognition beyond individuals to kinship relationships as demonstrated by stronger reactions to playback experiments with call sequences mimicking dominance rank reversals between families than within families (Bergman et al., 2003). However, as individual recognition requires repeated interactions, it is more likely to be employed in species with groups composed of a small number of individuals (Tibbetts & Dale, 2007).

In large groups where individual recognition is more difficult, conspicuous signals that indicate competitive ability can supplement or replace social knowledge (Bergman & Sheehan, 2013). In these cases, alternate mechanisms maintain the reliable link between signal and male fighting ability. Mechanisms that maintain signal honesty can come with an efficacy cost (a cost necessary to clearly transmit the information), or with both an efficacy cost and a strategic cost (a cost associated with the reliability of the signal) (Maynard Smith & Harper, 2003, 1995). Index signals require only an efficacy cost and generate honesty through a mechanistic link between signal intensity and a given trait (Maynard Smith & Harper, 1995; Számadó, 2011). In red deer stags (*Cervus elaphus*), the length of the vocal tract determines roar resonance frequencies (the formant) which correlate with body weight, age, and reproductive success (Reby & McComb, 2003). Territorial marks such as frequently countermarking other males as in mice (*Mus domesticus*) or scratching as high as possible on trees to indicate size as in tigers (*Pathera tigris*) can also act as index signals tied to quality that are not energetically costly to produce (Rich & Hurst, 1999; Thapar, 1986).

Condition-dependent signals include both an efficacy cost and strategic cost as males adjust their signal expression intensity according to their body condition where low-quality males are less able to bear the fitness costs for signaling compared to high-quality males (Fisher, 1915; Trivers, 1972; Zahavi, 1977); (reviewed in Penn & Számadó, 2020). In an experimental demonstration of condition-dependent signaling, food-supplemented male field crickets (*Gryllus campestris*) showed increases in body condition accompanied by increases in calling rate and number of females attracted (Holzer et al., 2003). Similarly, male blue grosbeaks (*Guiraca caerulea*) with the bluest plumage have the largest body size and maintain the largest and highest quality territories (Keyser & Hill, 2000). In these cases, body condition dictates differential investment into signaling where only high-quality males can afford to invest energy into signals of high quality.

1.1.2 Sexually selected skin coloration in primates

Although many primates rely on individual recognition for social communication, strong sexual selection pressures have produced conspicuous skin coloration in both males and females to aid in sociosexual communication (Bradley & Mundy, 2008; Dixson, 2012). Attention-grabbing red, white, or blue skin occurs in prominent areas such as the face (e.g., mandrills, snub-nosed monkey), genitals (e.g., vervet monkeys, drills), or perineal region (e.g., chimpanzees, bonobos) (Bradley & Mundy, 2008). White and blue skin is due to structural coloration, where organized arrays of collagen fibers coherently scatter short wavelength light (Prum & Torres, 2004), whereas red skin is generally produced by blood flow to highly vascularized tissue (Changizi et al., 2006). This section will specifically focus on sexually selected red skin as this information is most relevant to the gelada chest patch.

The role of red bare skin in primate communication has been under speculation since Darwin's observations on human blushing in *The Expression of the Emotions in Man and Animals.*

> "Blushing is the most peculiar and the most human of all expressions. Monkeys redden from passion, but it would require an overwhelming amount of evidence to make us believe that any animal could blush." (Darwin, 1872b)

Darwin further mused on the role of red skin in aggressive contexts following a zookeeper's observation of facial reddening in a rhesus macaque (*Macaca mulatta*) following an aggressive encounter with a conspecific.

> "Mr. Sutton has often observed the face of the Macacus rhesus, when much enraged, growing red. As he was mentioning this to me, another monkey attacked a rhesus, and I saw its face redden as plainly as that of a man in a violent passion." (Darwin, 1872a)

Darwin's early observations on monkey skin redness following "passion" or when "enraged" allude to red skin functioning as a sexually selected signal. In a later essay, "Sexual

Selection in Relation to Monkeys", Darwin attributes the brightly colored hindquarters in several primate species and facial coloration in mandrills to a function as a sexual ornament particularly for males to attract females (Darwin, 1876). More recently, the role of red bare skin on the head, chest, or genitals (also known as "sexual skin") in primates has been attributed to both attracting mates (intersexual selection) and displaying competitive ability to same sex rivals (intrasexual selection) (Bradley & Mundy, 2008; Dixson, 2012).

Among primates, conspicuous red skin is most common in catarrhine primates with a few notable exceptions in platyrrhines (Dixson, 2012; Moreira et al., 2019). In female cercopithecines, red skin has been linked to intersexual selection where skin redness is attractive to males. Indeed, both male rhesus macaques (*Macaca mulatta*) and male Japanese macaques (*Macaca fuscata*) prefer redder female faces in choice paradigm experiments (Higham et al., 2011; Pflüger et al., 2014). Further, red skin can indicate aspects of fertility varying with the timing of the fertile period (rhesus macaques: Dubuc et al., 2009; mandrills: Setchell et al., 2006) or pregnancy (Japanese macaques: Rigaill et al., 2015). In contrast, in male cercopithecines, red skin coloration has most often been associated with aspects of male-male competition such as dominance rank or rates of aggression. Higher rank is associated with redder lip and groin coloration in drills (*Mandrillus leucophaeus*: Marty et al., 2009), redder face and genital coloration in mandrills (Setchell & Dixson, 2001), redder lip color during the mating season in black-and-white snub-nosed monkeys (*Rhinopithecus bieti*: Grueter, Zhu, et al., 2015), and lower penile redness in vervet monkeys (*Chlorocebus pygerythrus*: Greenberg et al., 2022). Evidence suggests skin redness mediates aggressive interactions in male rhesus macaques, where males with more similar face redness are more likely to interact aggressively than those with dissimilarly colored faces (Petersdorf et al., 2017), and mandrills as threats and fights are more frequent between similarly colored males compared to dissimilarly colored males (Setchell & Wickings, 2005).

Although female preference for brighter male coloration has been demonstrated in mandrills when controlling for rank (Setchell, 2005), it remains difficult to parse apart the individual impact of coloration and dominance on male attractiveness as dominance is correlated with reproductive success in these taxa (Winters et al., 2019). The above species have multimale-multifemale or polygynous mating systems with a strong reproductive skew towards dominant males (Dixson, 2012). In these species, male competition likely exerts a stronger selective pressure than attractiveness to females as males are unable to gain proximity to reproductive females if they cannot first attain high dominance status (Winters et al., 2019). Thus, it is more likely that male competition favored the selection of a male red skin signal in these species.

1.1.3 Red skin coloration mechanisms in primates

Most studies have attributed hormones, vascular mechanisms, or a combination of the two as the underlying mechanism for redness in male primates. Castration causes red sexual skin to fade in rhesus macaques (Vandenbergh, 1965), rump color to decrease in size and color in hamadryas baboons (*Papio hamadryas*) (Zuckerman & Parkes, 1939), and red perineal skin to fade in patas monkeys (*Erythrocebus patas*) (A. F. Dixson, 1983). In mandrills, gaining alpha status increases testicular size, circulating testosterone, and the reddening of the skin on the face and genitals (Setchell & Dixson, 2001). An early study on mandrills suggests a vascular mechanism for facial redness based on anecdotal evidence that while under anesthesia, if the nose is pinched or squeezed it becomes pale briefly until the blood flows back to the compressed area (Hill, 1955). A more in-depth study on captive male rhesus macaques found that sexual skin blood flow increased in animals treated with testosterone while blood flow in non-sexual skin areas were unchanged suggesting hormones and vascular mechanisms work in tandem (Rhodes et al., 1997).

Upon entering a target cell, testosterone can alter gene transcription in several ways including (1) directly binding to the androgen receptor (AR), (2) converting to estradiol through the action of the enzyme aromatase and then binding to estrogen receptor α (ERα) or estrogen receptor β (ERβ), or (3) converting to 5-reductase which binds to AR but cannot convert to estradiol (Hau & Wingfield, 2011). Thus, there are other factors in addition to circulating testosterone levels influencing an androgen-mediated signal such as higher density or activity of ERα or ERβ in the chest skin as androgens are readily aromatized to estrogens in skin tissue (Zouboulis et al., 2007). In rhesus macaques, the administration of both estradiol and testosterone increased redness in males and the administration of an aromatase inhibitor (which prevents testosterone from converting to estradiol) decreased redness suggesting that testosterone only indirectly controls skin redness in the local conversion to estradiol (Rhodes et al., 1997).

1.1.4 Color vision in primates

When assessing a visual signal, researchers must consider the visual system of the signal receiver (e.g., conspecifics in the case of socio-sexual signals). The prevalence of bare red skin in primates stands out amongst the generally dull coloration of other mammalian species (Bradley & Mundy, 2008; Caro et al., 2021), but this distinction is less surprising taking into consideration that primates are the only eutherian mammals with trichromatic color vision while most eutherians are dichromatic (red-green colorblind) or monochromatic (colorblind) (Surridge et al., 2003). Color vision varies greatly within the primate order where all catarrhines and one platyrrhine genus, *Alouatta* (howler monkeys), are “routine trichromats”, with very little variation between species in spectral sensitivities, the specific light wavelengths to which the cone cells of the retina respond (SWS (short wavelength-sensitive), MWS (medium wavelength-sensitive), and LWS (long wavelength-sensitive) peaks at 430 nm, 535 nm, and 562 nm respectively (Jacobs, 2008; Jacobs et al., 1996; Jacobs & Deegan, 1999). Most platyrrhines and some strepsirrhines are dichromatic where they have a single X-linked LWS/MWS cone gene, with between two and six alleles with spectral sensitivity peaks between 535-562 nm range, and an autosomal SWS cone which leads to a visual system where all males are dichromatic and balancing selection maintains a polymorphism where about half of the females are trichromatic (Jacobs, 2008; Veilleux et al., 2016). In the remaining the remaining platyrrhines and strepsirrhines, both sexes are dichromatic or in the case of some nocturnal primates, they are colorblind due to loss of the functionality of SWS cone genes (Jacobs et al., 1996). Of course, there are a few notable variations including the aye-aye (*Daubentonia*) in which examination of the SWS opsin gene revealed a spectral sensitivity at 406 nm which is ~20 nm closer to the ultraviolet region of the spectra than most primates (Melin et al., 2012).

The unique ability for trichromatic color vision in some primates prompts the question of how and why this trait evolved. Molecular evidence suggests the ancestral primate species were dichromats (only SWS and LWS pigments; Hunt et al., 1998), and that trichromacy evolved through the duplication of the LWS pigment (Carvalho et al., 2017). The duplication of the LWS pigment allowed a mutational drift between the two copies eventually generating two spectrally distinct isoforms sensitive around the 530 nm peak (MWS pigment) or 560 nm (LWS pigment) (Carvalho et al., 2017). Several competing hypotheses exist for the selective pressures leading to the evolution of primate color vision. The frugivory hypothesis, where color vision provides an advantage in the ability to detect conspicuously colored fruits against a dappled background foliage that varies randomly in luminosity (Mollon, 1989), was the first to garner support in the nineteenth century (Jacobs, 2007). Although the frugivory hypothesis has been supported with several behavioral experiments (Caine & Mundy, 2000; Leonhardt et al., 2009; Smith et al., 2003), there is not clear support for a foraging advantage under natural conditions (Melin et al., 2009; Veilleux et al., 2016) or a heterozygote superiority (Fedigan et al., 2014), which would be expected as part of the balancing selection maintaining the polymorphism in platyrrhines.

An alternative has also been proposed; the folivory hypothesis suggests that trichromatic primates should have an advantage in selecting young edible leaves based on their redness (Dominy & Lucas, 2001; Lucas et al., 1998, 2003). This hypothesis is supported by data from *Aloutta,* the most folivorous platyrrhines and the only routine trichromats in the parvorder (Dominy & Lucas, 2001). However, the highly folivorous wooly spider monkey (*Brachyteles arachnoides*) has a relative low frequency of trichromatic individuals in their populations which weakens support for the folivory hypothesis (Talebi et al., 2006). Beyond foraging selective pressures, the detection of predators (Pessoa et al., 2014)or socio-sexual signals (Changizi et al., 2006) have been posed as playing a role in the evolution of color vision. Within catarrhines, experimental evidence supports that individuals attend to red sexual skin in mate choice (Waitt et al., 2003), but trichromatic vision only evolved once in this parvorder while red sexual skin evolved at least four times in this group independently (Bradbury & Vehrencamp, 2011). Therefore, it is more likely that an ecological or predation selective color vision first selected for color vision, and the ability was later co-opted for socio-sexual communication.

1.2 Study site and subjects

All data were collected in the Sankaber region of the Simien Mountains National Park, Ethiopia as part of the Simien Mountains Gelada Research Project, which has been collecting behavioral and demographic data on a population of habituated geladas since 2006. The Simien

Mountains National Park encompasses an Afroalpine grassland ecosystem consisting of undulating grassland plateaus, scrublands, and Ericaceous forests (Puff & Nemomissa, 2005).

Geladas (*Theropithecus gelada*) are a cercopithecoid monkey endemic to the Ethiopian highlands (elevations between 2,350 and 4,440 m above sea level; Yalden et al., 1977) and the only extant species of their genus. The gelada diet primarily consists of graminoid (grass and sedge) leaves for most of the year (Fashing et al., 2014; Hunter, 2001), but expands to underground foods such as roots, corms, and tubers during the dry season (Hunter, 2001). In gelada societies, dozens of modular "reproductive units" travel and forage together in "bands". Each reproductive unit comprises of a dominant "leader" male and 1-2 adult females and their offspring (Snyder-Mackler, Beehner, et al., 2012). About one-third of these units also contain one or more "follower" males that may gain some reproductive opportunities (Snyder-Mackler, Alberts, et al., 2012). At the periphery of the reproductive units, "bachelor" males form another type of core group (the "all-male group"). Bachelor males lack reproductive opportunities until they overthrow a leader and gain access to that unit's females (or join a unit as a subordinate follower; (Pappano & Beehner, 2014).

There are two putative sexually selected signals among male geladas: a loud call emitted during aggressive displays and the chest patch, an hour-glassed shaped patch of bare red skin on the chest (Benítez et al., 2016; Bergman & Beehner, 2008). Previous research has established that leader males with the highest quality loud calls have the longest tenures (Benítez et al., 2016). On the other hand, chest patch redness is a plastic trait where adults have redder chests than subadults and leader males have the reddest chests compared to followers and bachelors (Bergman et al., 2009; Bergman & Beehner, 2008). One prominent hypothesis for multimodal signaling is that signals that develop over long periods of time may be good indicators of a male’s long-term genetic quality, whereas more-plastic, flexible signals may be better indicators of a male’s current condition (Candolin, 2003). Thus, loud calls and chest redness may signal different aspects of male “quality” where loud calls indicate a male’s genetic quality while chest patch color reliably indicates a male’s current condition (Benítez, 2016).

1.3 Research questions and chapters

For condition-dependent signals, males must adjust their signal intensity according to their body condition which indicates that the production of the signal is physiologically costly. Although previous research established variation in chest redness across males, little is known about the underlying mechanisms of chest redness associated with this putatively condition-dependent signal. **In this thesis, I focus on the following question: what are the causes and consequences of variation in the red chest patch for leader male geladas?** With respect to “causes”, I will examine the mechanisms that are associated with (and thought to mediate) variation in chest redness both within and between leader males. With respect to “consequences”, I will examine the fitness outcomes associated with (and thought to result from) having a red chest patch.

I address these questions in three chapters that are published (Chapter 2), under peer-review (Chapter 3), or in preparation for submission for peer-review (Chapter 4). Although I am the first author for all chapters, each chapter is a collaborative effort with many co-authors including committee members Dr. Thore Bergman, Dr. Noah Snyder-Mackler, and Dr. Jacinta Beehner. For all remaining chapters, I will use the pronoun “we” instead of “I” to denote this collaborative effort. Each chapter is outlined below (**Fig. 1**).

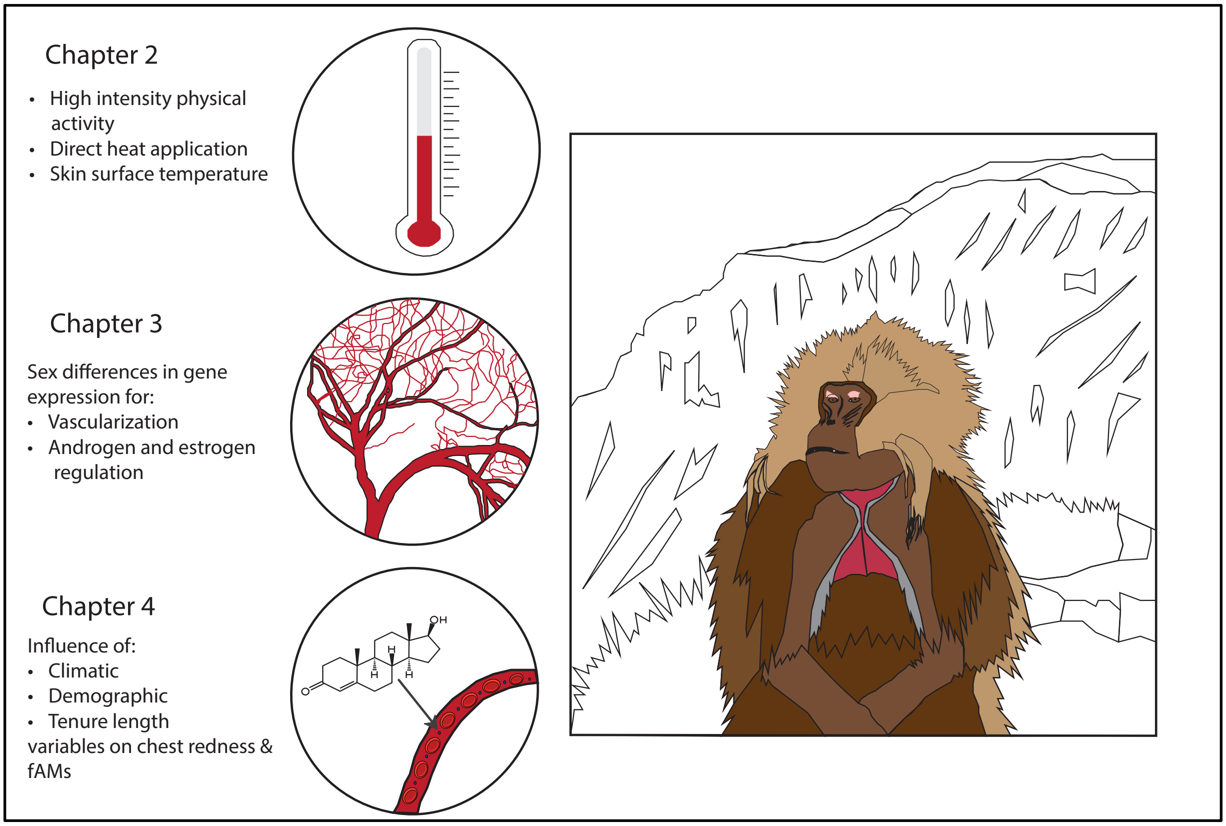


Figure 1. Outline of the following chapters that investigate the causes and consequences of variation in the red chest patch for leader male geladas.

In **Chapter 2,** we had two primary objectives: (1) to compare multiple methods of color measurement for working with color in wild animal populations, and (2) to examine the physical correlates of chest redness in male geladas. We validated and compared two digital photography color measurement methods: Adobe Photoshop, the method used in previous gelada studies but updated with current software, and the micaToolbox designed for use with ImageJ which incorporates the visual system of the animal. For physical correlates, we hypothesized that chest redness in male geladas is linked to blood flow in the skin similarly to how skin blood flow correlates with sexual skin redness in male rhesus macaques (Rhodes et al., 1997). We examined three variables that should be associated with increased blood flow to the chest patch: high-intensity physical activity, direct heat application, and higher skin surface temperatures.

In **Chapter 3,** we used tissue samples collected from male and female geladas during non-lethal, capture-and-release events to better understand molecular correlates of the chest patch signal mechanism. Building upon our findings from Chapter 2, we investigated whether males have increased expression of genes associated with vascularization or with androgen and estrogen regulation. We complement this dataset with digital photographs to assess variability in male and female chest redness both in natural conditions and while under anesthesia.

In **Chapter 4**, we explored correlates of chest redness, correlates of fecal androgen metabolites (hereafter, "fAMs"), and the relationship between chest redness and fAMs both across males and within leaders. Contrary to our predictions in Chapter 3, males did not have increased expression of genes associated with androgen and estrogen regulation in the chest skin. However, we measured the expression of genes that interact with estrogen and androgen receptors but circulating hormones such as testosterone may play a larger role in regulating redness in primates (A. F. Dixson, 1983; Setchell & Dixson, 2001). Using long-term data, we test the influence of demographic (status, age, group size), climatic (maximum temperature, minimum temperature, cumulative rainfall), and reproductive fitness proxy (tenure length) variables on chest redness and fAMs. Lastly, we test whether higher chest redness is associated with higher fAMs for individuals with chest photo and fecal metabolite samples collected within 60 days of one another.

1.4 Significance of research

This dissertation adds to the body of literature surrounding condition-dependent signals of male competitive ability. To our knowledge, this is the most complete dataset, from mechanism to maintenance to function, harnessed towards understanding a sexually selected signal of male competitive ability in a non-human primate. Humans are one of the only other primate species to form enormous group aggregations where individual recognition is limited. Although humans have well-developed individual recognition and complex social knowledge, humans routinely interact with and must make decisions about strangers (Schmidt & Cohn, 2001). The results of the proposed research will help us understand traits that are communicated via signals vs. traits that are communicated via individual identity. Geladas are one of the few non-human primates to routinely navigate interactions with “strangers” as they can form loose aggregations (“communities”) of up to 1,200 individuals (Snyder-Mackler et al., 2012). Thus, in addition to investigating chest color as a sexually selected signal, this research will lead to a better understanding of the social and ecological environments that favor the evolution of signals relative to social knowledge.

Chapter 2 : Assessing Male Gelada Chest Patches: Color Measurement and Physiological Mechanisms[2]

2.1 Abstract

Selective pressures have favored conspicuous coloration across a wide variety of taxa. A particularly striking example of conspicuous coloration is the brilliant red chest patch of male geladas (*Theropithecus gelada*), a species of cercopithecine monkey found in the high-altitude regions of Ethiopia. Previous research found that gelada chest patch redness increases with age (adult vs subadult), social status ("leader" vs non-leader), and mating opportunities (number of adult females), but the mechanism mediating changes in redness has not yet been examined. First, we validated and compared multiple color measurement methods (Adobe Photoshop, micaToolbox designed for use with ImageJ, and a subjective measure using the human eye). Second, we demonstrated that chest patch redness is positively associated with high-intensity physical activity, the application of a heat pack directly to the chest skin, and higher chest skin surface temperatures. Together, these results suggest that increases in chest redness are mediated by increased blood flow to this area with a concomitant increase in surface temperature. Further research is needed to understand both the energetic costs associated with redness and how other males respond to variation in the signal.

---

[2] Published in *Mammalian Biology*. February 2022. https://doi.org/10.1007/s42991-021-00211-5

2.2 Introduction

Conspicuous coloration has been favored by natural and sexual selection in a wide variety of both animals and plants. Colorful signals are exhibited in a diversity of contexts, including warning (aposematic) coloration (Exnerová et al., 2003; Lev-Yadun, 2001; Siddiqi et al., 2004), mimicry (Rettenmeyer, 1970), predator confusion (Cuthill et al., 2005), camouflage (Stevens & Merilaita, 2011), sexual selection (Houde, 2019; Keyser & Hill, 2000), and species discrimination (Kingdon, 1980; Uy et al., 2009). Among mammals, the primate order tends to be particularly colorful (Bradley & Mundy, 2008; Caro, 2005), exhibiting a range of pelage and skin colors that has been associated with phylogenetic relatedness (Bell et al., 2021; Winters et al., 2020), individual identity (Parr et al., 2000), sexes (Mourthe et al., 2019), developmental stages (Hendershott et al., 2019), sexual signals (Higham, Pfefferle, et al., 2013; Rigaill et al., 2019), degrees of UV protection (Jablonski & Chaplin, 2010), as well as ambient temperature and humidity (Kamilar & Bradley, 2011).

In primates, some of the most conspicuous color variation is associated with sexual signaling. Many primates have colorful sexual skin on their faces (mandrills: Setchell et al., 2006; rhesus macaques: Waitt et al., 2006), genitals (vervet monkeys: Gerald, 2001; drills: Marty et al., 2009, and perineal region (chimpanzees: Deschner et al., 2004; baboons: Gesquiere et al., 2007; macaques: Higham et al., 2012; bonobos: Douglas et al., 2016). In female primates, sexual skin coloration can vary with female reproductive status (Dubuc et al., 2009) and fecundity (Dubuc, Winters, et al., 2014). In males, colored skin appears to be selected by mechanisms of both inter- and intra-sexual selection, perhaps depending on the relative strength of different mechanisms in different systems. For example, in some species, male color expression has been linked to female mate choice (*Eulemur fulvus* sub-species: (Cooper & Hosey, 2003; rhesus macaques: Dubuc, Allen, et al., 2014b), whereas in others it has been linked more commonly to signaling of male social status to other males (geladas: Bergman et al., 2009; vervet monkeys: Gerald, 2001; black-and-white snub-nosed monkey: Grueter et al., 2015; mandrills: Setchell & Dixson, 2001).

A particularly striking example of primate coloration is the brilliant red chest patch of male geladas (*Theropithecus gelada,* a species of cercopithecine primate; **Fig. 2**). The signal may have been under selection for its role in mediating male-male contests. Gelada male contests can be extremely costly (Maynard Smith, 1982), and since their large, fluid societies ensure that males often encounter and interact with rival males they do not recognize individually (Bergman, 2010), gelada males would benefit from an external signal of strength or condition. In gelada societies, dozens of modular "reproductive units" travel and forage together in "bands". Each reproductive unit comprises a dominant "leader" male and 1–12 adult females and their offspring (Snyder-Mackler, Beehner, et al., 2012). Approximately one-third of these units also contain one or more subordinate "follower" males that may gain some reproductive opportunities (Snyder-Mackler, Alberts, et al., 2012). At the periphery of the reproductive units, "bachelor" males form another type of core group (the "all-male group"). Bachelor males lack reproductive opportunities until they overthrow a leader and gain access to that unit's females (or join a unit as a subordinate follower; Pappano & Beehner, 2014).

Figure 2. Adult male gelada (*Theropithecus gelada*) in the Simien Mountains National Park, Ethiopia. Photo by R. Perlman.

Previous research has shown that adult males have redder chests than subadult males (Bergman & Beehner, 2008), that leader males have redder chests than bachelor or follower males (Bergman et al., 2009), and that leader males with more adult females in their groups have redder chests than those with fewer females in their groups (Bergman et al., 2009). Specifically, this research proposed that redder chests in leader males may deter takeover attempts from bachelor males. Since males cannot rely on experience from previous encounters to guide each individual rival assessment, gelada males should benefit from a signal that reliably indicates condition and/or fighting ability (Bergman & Sheehan, 2013; Grueter, Isler, et al., 2015). Indeed, bachelor males are less likely to challenge males with the reddest chests, and leaders with the reddest chests are more likely to maintain their leader status across the subsequent year (Benítez, 2016).

We had two primary objectives for this manuscript. Our first goal was to compare multiple methods of color measurement for working with color in wild animal populations. We compared two digital photography color measurement methods: Adobe Photoshop (Adobe Inc. 2021; hereafter "Adobe"), the method used in previous gelada studies (Bergman et al., 2009; Bergman & Beehner, 2008) but updated with current software, and the micaToolbox (Empirical Imaging 2021) designed for use with ImageJ (Schneider et al., 2012) which incorporates the visual system of the animal (hereafter "ImageJ micaToolbox"). Specifically, we assessed: (1) whether Adobe and ImageJ micaToolbox methods were consistent across lighting conditions ("Method consistency"), and (2) whether subjective redness scores judged by human observers, Adobe, and ImageJ mica- Toolbox match values directly measured from the skin in anesthetized animals using a NixTM Mini Color Sensor (Nix Sensor Ltd. 2021; "Method accuracy").

Our second goal was to examine the physical correlates of chest redness in male geladas. We hypothesized that chest redness in male geladas is linked to blood flow in the skin, similarly to how skin blood flow correlates with sexual skin redness in male rhesus macaques (Rhodes et al., 1997). We examined three variables that should be associated with increased blood flow to the chest patch: high-intensity physical activity, direct heat application, and higher skin surface temperatures. First, we predicted that leader male chest redness would increase after vigorous physical activity that increases blood flow ("Activity and redness"), such as male displays—a ritualized loud-call followed by running, throwing rocks, climbing trees, or shaking branches (hereafter "display"; Benítez et al., 2016). These displays (and their quality and frequency) are thought to deter bachelors who might otherwise challenge leader males (Benítez, 2016). Second, we predicted that the application of heat to the skin (Heinonen et al., 2011) would increase chest red- ness in anesthetized male geladas ("Heat manipulation and redness"). Third, we predicted that leader males with redder chests would have higher surface skin temperature (presumably as a result of increased skin blood flow; "Redness and skin temperature").

2.3 Methods

2.3.1 Study site and subjects

Field data were collected from wild geladas in the Simien Mountains National Park (SMNP), Ethiopia as part of the Simien Mountains Gelada Research Project (SMGRP). The SMGRP has collected behavioral, demographic, and hormonal data on a population of geladas in the SMNP since 2006, and has conducted annual immobilization campaigns (hereafter, "darting") since 2017. Each year, 20–50 adult male and female geladas are anesthetized with Telinject blowdarts (Telinject USA Inc.) containing ketamine (7.5 mg/ kg) and medetomidine (0.04 mg/kg). While the animal is anesthetized, we collect a variety of data, including blood, a skin biopsy, a muscle biopsy, hair, microbiome swabs, chest photos, tooth metrics, and morphological measurements. This is conducted under the supervision of licensed veterinarians and veterinary technicians who monitor the animal's temperature, pulse, and respiration every 10 min. Following data collection, sedation is reversed with atipamezole (0.2 mg/kg). Individuals are monitored during recovery until they return to their social units. During the study period, on average, individuals lifted their heads 18 min after reversal and started walking 42 min after reversal. Every animal successfully returned to their unit by the end of the day. All data were collected with permission from the Ethiopian Wildlife Conservation Authority, and all research was approved by the Institutional Animal Care and Use Commit- tee (for the University of Michigan for non-invasive work: IACUC: PRO00008871; and for the University of Washington IACUC: A120397 and Arizona State University IACUC: 20-1754R for the darting 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.

2.3.2 Camera, color standard, and redness metrics

Photographs from the redness and skin temperature dataset were taken with a Nikon COOLPIX 8700, and photographs from all other datasets (method consistency, method accuracy, activity and redness, and heat manipulation and redness) were taken with a Nikon D3200 camera. The camera settings should matter more than the make and model of the camera itself for the implementation of these methods. Although RAW file format is preferred to minimize image processing from the camera and to allow the generation of images that are linear with respect to radiance (Troscianko & Stevens, 2015), we collected all photos in JPEG file format. JPEG format was necessary to maintain consistency across datasets, particularly because our long-term data- base photos were collected in JPEG format. We also note that the primary issue with the use of JPEGs as opposed to RAW images is one of loss of pattern resolution due to lossy compression, which is particularly important when measuring fine pattern details (e.g., the pattern on a butterfly's wing), but which should be less important here (Stevens et al., 2007). To overcome the obstacle of assessing color under variable light conditions, we incorporated the use of a color standard, the X-Rite ColorChecker® Classic chart (hereafter, "ColorChecker chart") to adjust the color in the photograph to the known color levels in the chart squares. The ColorChecker chart is included in the same photo as the gelada ("simultaneous method") for the darting dataset while individuals were anesthetized and in a consecutive photo directly after the photo of the gelada ("sequential method") for all other datasets (Bergman & Beehner, 2008; Higham, 2006).

Redness metrics differ slightly between Adobe and ImageJ micaToolbox measurements because the two sets of software utilize different color spaces. For Adobe, we used the Adobe RGB (1998) ICC (International Color Consortium) color profile, which uses RGB color space. Similar to previous studies of redness in geladas, we used 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). For ImageJ micaToolbox, we converted measurements to the color space of the *Papio* (baboon) visual system as a proxy for the visual system of *Theropithecus* as the *Theropithecus* visual system has not yet been characterized but spectral sensitivity (the specific light wavelengths to which the cone cells of the retina respond) is highly conserved across cercopithecine monkeys (Jacobs & Deegan, 1999). After conversion to the *Papio* visual system, ImageJ micaToolbox measurements were recorded in LW (long wavelength), MW (medium wavelength), and SW (short wavelength) channels. We used Red-Green Opponency (hereafter, "R-G Opponency" (LW − MW)/(LW + MW)) as the redness metric for ImageJ micaToolbox measurements similar to other primate studies (Dubuc, Winters, et al., 2014). In our method consistency inves- tigation, we also calculated luminance ((LW + MW)/2), an achromatic measurement of brightness, from ImageJ micaToolbox measurements (Osorio & Vorobyev, 2005). We validated luminance along with redness because it has been hypothesized that redness varies with blood oxygenation (redder skin has more oxygenated blood) while luminance varies with skin blood flow (higher blood flow creates darker, less luminous skin), suggesting both may be important metrics for characterizing variation in gelada chest patch color (Changizi et al., 2006; Stephen et al., 2009).

2.3.3 Data collection

Method consistency

For the first step of our validation process, we created a validation set of photos following protocols outlined previously for the field (Bergman & Beehner, 2008). In brief, the photos of the subject were followed immediately (within 1–2 min) by a second photo of the ColorChecker chart ensuring the ambient light conditions had not changed and that the f-stop and shutter speed of the camera were consistent between the two photos (the sequential method). To simulate this process, we took two photos of the ColorChecker chart: in the first photo, the ColorChecker chart simulated the subject, and in the second photo the ColorChecker chart simulated the color standard. To ensure consistency across lighting conditions, validation photos were taken at various angles and across four light conditions: (1) backlit, (2) cloud, (3) shade, and (4) full sun (*n*=10 photo sets per condition; 40 photos total).

Method accuracy

We measured chest patch color on anesthetized geladas using a Nix™ Mini Color Sensor (Nix Sensor Ltd.), a small device that blocks out ambient light, provides its own standardized light source, scans a surface, and records the color in a variety of color spaces (e.g., sRGB, CIELAB). We chose the Nix™ Mini Color Sensor (hereafter, "Nix") because the compact design is useful for field research, and the Nix accurately reproduced published sRGB values when measuring a ColorChecker chart directly (Appendix A: Fig. S1). The Nix measures represent our "gold standard" for color measures because our own Nix values on the ColorChecker chart almost perfectly reproduced the values from the ColorChecker chart itself (R^2=0.99; **Appendix**

A: Tables S1–S3, **Fig. S1**). Although the Nix represents our "gold standard" for this validation, it is not useful for routine use because animals must be anesthetized first. The annual SMGRP dartings provided us with opportunities to measure chest color more directly to better assess the accuracy of our methods. We placed the Nix on three different spots on the chest patch and recorded three scans. To directly compare the Nix to the color measurement methods, we took digital photographs of each subject's chest patch and a ColorChecker chart (Bergman & Beehner, 2008).

As an alternative metric to the Adobe and ImageJ mica- Toolbox methods, we assessed the utility of subjective redness scores. Four authors (PMD, RFP, JCB, TJB) independently ranked 44 photos collected from a subset of the darted geladas (11 males and 11 females, *n* = 1–3 photos per ID). Individuals were represented in the dataset in three possible temperature manipulation conditions: (1) baseline, (2) heat pack application, or (3) ice pack application. Each photo was considered a separate data point for subjective redness as authors were blinded to the individual's ID when they viewed the chest photo and scored each photo from 1 (palest) to 5 (reddest). Each photo was given an average subjective redness score compiled from scores across the four observers.

Activity and redness

To assess the impact of activity level on chest patch redness, we analyzed gelada chest photos collected between 2017 and 2018 from leader males under free-moving conditions (i.e., non-darting photos). In total, we analyzed 13 sets of photos across 7 males, each set comprising one chest photo (and the corresponding ColorChecker chart) taken "post-display" and a complementary "baseline" photo taken within the same month (Sep 2017–Aug 2018). Post-display photos were taken within 10 min of vigorous display activity. Baseline photos were taken when the individual was conducting daily activities like resting, grazing, or grooming at least 10 min after a vigorous display activity. Since previous analyses found that chest redness shows a moderately seasonal pattern (Benítez, 2016), we matched each post-display photo to a baseline photo taken within the same month (± 30 days).

Heat manipulation and redness

We examined whether a heat pack applied to the chest patches of 15 anesthetized male geladas increased chest redness. These data were collected during the 2017–2018 darting season. We took two chest photos within the same frame as the ColorChecker chart (simultaneous method). The first photo was taken at the beginning of the anesthetization. Subsequently, we applied a heat pack on one side of the chest for 1 min and took another photo of that side of the chest with the ColorChecker chart. Each heat pack was activated directly before application, ensuring consistency in the heat condition across study subjects.

Redness and skin temperature

To assess whether chest redness is associated with chest skin surface temperature, we used a Raytek MX6 PhotoTemp Infrared Thermometer (Fluke Process Instruments; hereafter, “temperature gun”) to measure chest skin temperature (°C) non-invasively from a distance of 1-2 m (**Appendix A: Fig. S2**). Previous findings indicate daily minimum and maximum ambient temperatures do not impact chest redness (P. DeLacey et al., 2019). However, to minimize the impact of fluctuations in ambient temperature and seasonality, data were collected in the morning between 8:00 and 11:00 am within a 1-month window. These data were collected in 2007 from 14 adult leader males.

2.3.4 Photo measurement

Adobe measurements

Our previous Adobe method utilized a software called inCamera Plugin (PictoColor Software Inc.) which creates a color profile that adjusts the color in the photograph to the known color levels in each square of the chart (Bergman et al., 2009; Bergman & Beehner, 2008). However, because the inCamera Plugin is no longer available in the most recent versions of Adobe Photoshop, we validated the Adobe method with new software, ColorChecker Camera Calibration (X-Rite Inc.), that is compatible with Adobe Photoshop 2021. We analyzed JPEG photos in Adobe Photoshop 2021 (22.1.0 release) using color profiles created in ColorChecker Camera Calibration (v2.2.0), software designed for use with the ColorChecker chart.

First, each ColorChecker chart photo was processed in ColorChecker Camera Calibration to create a color profile, which was then used to adjust the color in the accompanying chest photo. We converted JPEG images of the Color- Checker chart to TIFF files to be compatible with the software. Second, we uploaded each TIFF image and aligned the software's 24 square grid with our ColorChecker chart image. Third, we selected "Create Profile" to create an ICC color profile. These profiles automatically save to a folder that is accessible to Adobe Photoshop. Color standard photos where there was "clipping", when the light levels (0–255) for the RGB channels reach the upper limits of the camera (255), were discarded (Stevens et al., 2007).

After creating the profiles, we measured the photographs in Adobe Photoshop following previous protocols established for geladas (Bergman & Beehner, 2008). We assigned and converted the color profile specific to that photo. Following color correction, we used the "rectangular marquee" tool to select an area of the chest (minimum 500 pixels) and recorded the mean of the Red, Green, and Blue channels of the selected area using the "histogram" tool (**Appendix A: Fig. S3**). Since skin color is not uniform across the chest patch, we developed protocols for selecting areas of the chest to maintain consistency across measurements. We selected the flattest area possible on the lateral corner of the chest patch while avoiding areas of dirt or dry, flaking skin. We also avoided measuring medial areas of the chest because the chest rounds inward creating a convex surface with inconsistent lighting.

ImageJ micaToolbox measurements

The micaToolbox (Empirical Imaging 2021), designed for use with ImageJ (Schneider et al., 2012), offers a free and open-source software suite to calibrate non-linear digital images and convert images to the visual system (i.e., cone-catch values) of a wide variety of animals to measure color, luminance, and pattern information (Troscianko & Stevens, 2015). The micaToolbox was initially designed for use with RAW file formats only. A recent update, however, allows the use of JPEG images (i.e., non-linear images) which expands its utility for long-term field sites such as ours that may only have stored JPEG images for analysis (van den Berg et al., 2020). JPEG photos were analyzed in ImageJ (v1.52a) with the micaToolbox (v2.0.2) (Schneider et al., 2012; Troscianko & Stevens, 2015).

To accurately quantify color in a JPEG image, an extra transformation step is necessary to linearize the image (van den Berg et al., 2020). To create a linearization model in ImageJ, we used a high-quality JPEG image of the ColorChecker chart. Using the micaToolbox, we specified six gray standard reflectance values (squares 19–24), which creates numerous model linearization curves for the camera using a range of different equations. We selected the best fitting model based on the highest coefficient of determination (R^2) between the measured pixel values and the standard reflectance values of the six gray standards of the ColorChecker chart (in this case, the JT Linearisation model). We applied the selected model to all JPEG images, and for each image we generated a "multispectral image", a stack of images taken at different ranges of wavelengths, specifying the "white" square (91.57% standard reflectance) and "black" square (3.22% standard reflectance) as the gray standards for each image. The gray standards can be selected either in the same photo or in a different photo, allowing for use of either the simultaneous or sequential method.

Next, we converted the image using a cone-catch model, which corrects for both the camera's spectral sensitivity (i.e., the camera's relative efficiency to detect light) and the visual system of the study animal. We used micaToolbox to characterize the Nikon D3200 camera sensitivity using a photo of the ColorChecker chart which we then applied to all images (van den Berg et al., 2020). *Papio* cone-catch sensitivities were calculated using a rhodopsin template, developed by Govardovskii et al. (2000) and adapted for use in primates (Osorio & Vorobyev, 2008), based on peak spectral sensitivities of Guinea baboons (*Papio papio*) ($\lambda_{\max}$ = 565.5, 536.5, 428; Bowmaker et al., 1991).

Lastly, we used the rectangle tool in ImageJ to select the chest patch as the region of interest (ROI) and measured the mean and standard deviation of each channel in the ROI. The polygon selections tool in ImageJ can be used to select a larger region (e.g., the entire chest patch), but to ensure direct comparison between methods, we selected the same rectangular area of the chest to measure in both the Adobe and ImageJ methods. We then recorded the long wavelength (LW), medium wavelength (MW), and short wavelength (SW) values (**Appendix A: Fig. S4**).

2.3.5 Data analyses

All statistical analyses were performed in R version 4.0.2. Linear models were run in the R package lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). Inter-rater reliability was assessed in the R package irr (Gamer et al., 2019). Plots were constructed with R packages ggplot2 (Wickham et al., 2016) and ggpubr (Kassambara, 2020).

Method consistency

Following Bergman and Beehner (2008), we measured the “light skin” and “moderate red” squares in each set of photos (these square names are not our own, but assigned by the ColorChecker chart and maintained in this publication for easy reference), which span the range of color observed in gelada male chest patches. For each of the two squares, we ran linear regression models, with redness as the outcome variable and light condition (cloud, backlit, shade, sun) as the predictor variable to quantify variation in measurements between light conditions. Redness was quantified as Red/Green for the Adobe method and R-G Opponency for the ImageJ micaToolbox method. We also ran two separate linear regression models with luminance as the outcome variable and light condition as the predictor variable for the “light skin” and “moderate red” squares for the ImageJ micaToolbox method. One cloud condition data point was discarded from all datasets because the f-stop and shutter speed differed between the paired set of photos.

Method accuracy

To compare measurements with the Nix "gold standard", we ran three separate linear regression models with the Nix values as our independent variable to the (1) subjective redness scores, (2) redness measured with Adobe, and (3) redness measured with ImageJ micaToolbox to determine which metric was nearest to the values obtained from the Nix. All independent variables were standardized with a z-score before running linear regression models to enable comparison across different redness metrics. Inter-rater reliability was assessed using a two-way mixed, consistency, average-measured intra-class correlation (hereafter, "ICC") (Hallgren, 2012; McGraw & Wong, 1996) with an ICC of 0.75–1.0 (Cicchetti, 1994) required to confirm a high degree of agreement in redness ratings.

Activity and redness

To assess whether redness increased after post-display activity for leader males, we ran a paired *t*-test of redness between baseline and post-display activity levels measured with Adobe. We assessed normality with Quantile–Quantile plots and a Shapiro–Wilk normality test for baseline and post-display redness. A Shapiro–Wilk test revealed that both "baseline" ($W(13) = 0.92$, $p = 0.248$) and "post- display" ($W(13) = 0.898$, $p = 0.124$) redness variables are normally distributed, and we proceeded with a parametric paired t-test.

Heat manipulation and redness

To examine whether our experimental application of a heat pack to the chest increased male chest redness, we ran a paired t-test of redness between photos from the same male taken at baseline and after heat application. We assessed normality with Quantile–Quantile plots and a Shapiro–Wilk normality test for baseline and heat manipulation redness. A Shapiro–Wilk test revealed that both baseline ($W(15) = 0.95$, $p = 0.528$) and heat application ($W(15) = 0.89$, $p = 0.068$) redness variables are normally distributed, and we proceeded with a parametric paired t-test. One individual was excluded from analyses as an outlier because the baseline redness value was over 1.5*IQR above the mean and over 2 SD above the mean.

Redness and skin temperature

To identify whether leader males with redder chest patches have higher skin surface temperatures, we ran a linear regression model of chest temperature (outcome variable) as a function of chest redness.

2.4 Results

2.4.1 Method consistency

For Adobe Photoshop, the sole difference in measurements across light conditions was the "light skin" square measured in the backlit condition ($b = 0.052$, SE = 0.005, $t = 7.620$, $p < 0.001$; **Fig. 3A; Appendix A: Table S4**). Note that this difference is of small magnitude (the difference in means across light conditions is only 6.5% of the difference between the two color patches).

For ImageJ micaToolbox, there were several significant differences across light conditions; in particular, the “light skin” square measured in the backlit condition ($b = -0.008$, SE = 0.001, $t = -5.767$, $p < 0.001$) and the “moderate red” square measured in the shade ($b = -0.009$, SE = 0.004, $t = -2.285$, $p = 0.030$) and sun conditions ($b = -0.010$, SE = 0.004, $t = -2.487$, $p = 0.019$; **Fig. 3B; Appendix A: Table S5**). Some differences were significant but also of small magnitude (the largest difference in means across light conditions is only 10.9% of the difference between the two squares). Luminance measured with ImageJ micaToolbox did not vary by light condition for either the “light skin” or “moderate red” square ($p > 0.05$; **Fig. 3C; Appendix A: Table S6**).

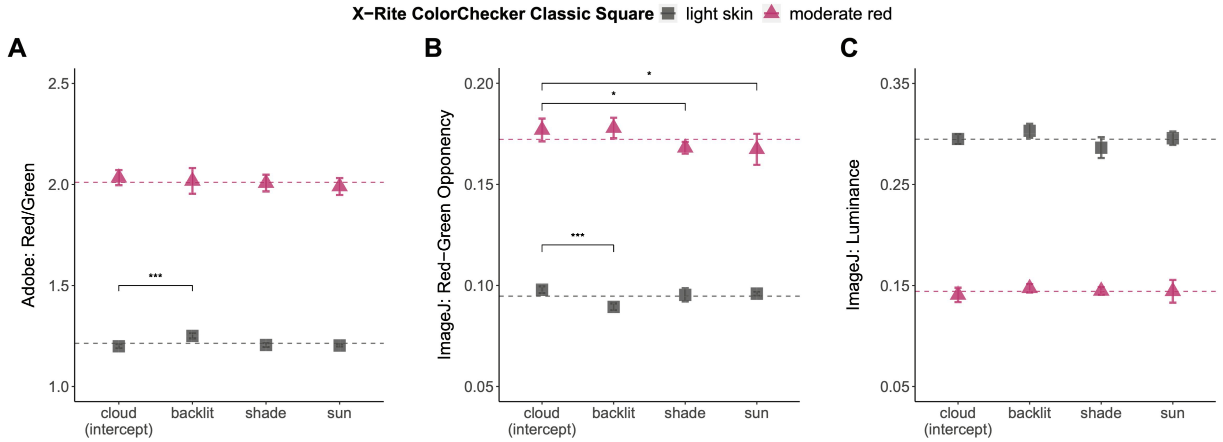


Figure 3. Method consistency: Adobe and ImageJ micaToolbox across four lighting conditions for two colored squares on the X-Rite ColorChecker chart. Variation across lighting conditions for the “light skin” and “moderate red” colored squares for: **A)** Red/Green measured in Adobe. **B)** R-G Opponency ((LW – MW)/(LW + MW)) measured in ImageJ micaToolbox. **C)** Luminance ((LW+MW)/2) measured in ImageJ micaToolbox. Dotted lines represent the average values for each square for photos across all light conditions. Error bars represent the standard error of the mean.

2.4.2 Method accuracy

Redness measures from all three methods were highly correlated with the Nix scores with the closest association between the subjective redness scores ($F(1,42) = 103.7$, $R^2 = 0.71$, $p < 0.001$, **Fig. 4A**), followed by the Adobe chest redness values ($F(1,42) = 36.75$, $R^2 = 0.47$, $p < 0.001$, **Fig. 4B),** followed by the ImageJ micaToolbox chest redness values ($F(1,42) = 20.73$, $R^2 = 0.33$, $p < 0.001$, **Fig. 4C**). Note that although statistics are calculated with z-scores, we plot the raw values in our figures below. Our method validation suggests that Adobe Photoshop (or subjective scores) are best suited for chest redness scores under field conditions. For all remaining results, the figures represent Adobe results (for figures employing ImageJ micaToolbox, see **Appendix A: Figs. S5–7**). Inter-rater reliability assessment confirmed that authors had a high degree of agreement in subjective redness ratings, ICC = 0.942.

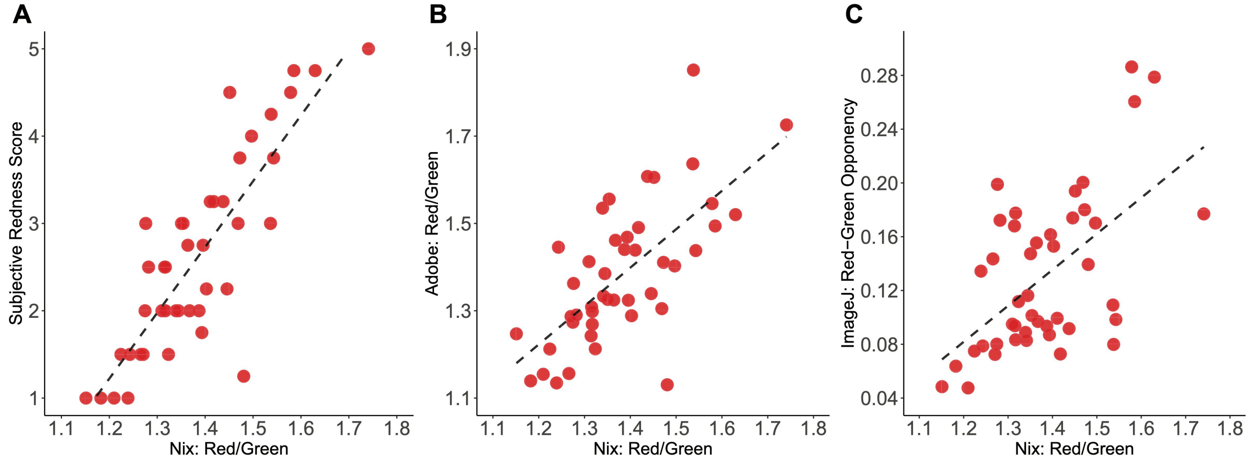


Figure 4. Method accuracy: redness measurements from all three methods were significantly correlated with the Nix measurement. The relationship between the Nix chest redness values (Red/Green) and **A)** subjective redness scores averaged across all observers, **B)** Adobe Photoshop chest redness values (Red/Green), and **C)** ImageJ micaToolbox chest redness values (Red-Green Opponency (LW – MW)/(LW+MW))

2.4.3 Activity and redness

For the paired baseline and post-display chest patch values, recent displays by males ("post-display") produced redder chest patches for the same males when compared to their own "baseline" photos (paired *t*-test: $t = 2.58$, $df = 12$, $p = 0.024$, **Fig. 5**; ImageJ results did not demonstrate redder ($p = 0.26$) or lower luminance ($p = 0.97$) chest patches for males immediately after a display, **Appendix A: Fig. S5**).

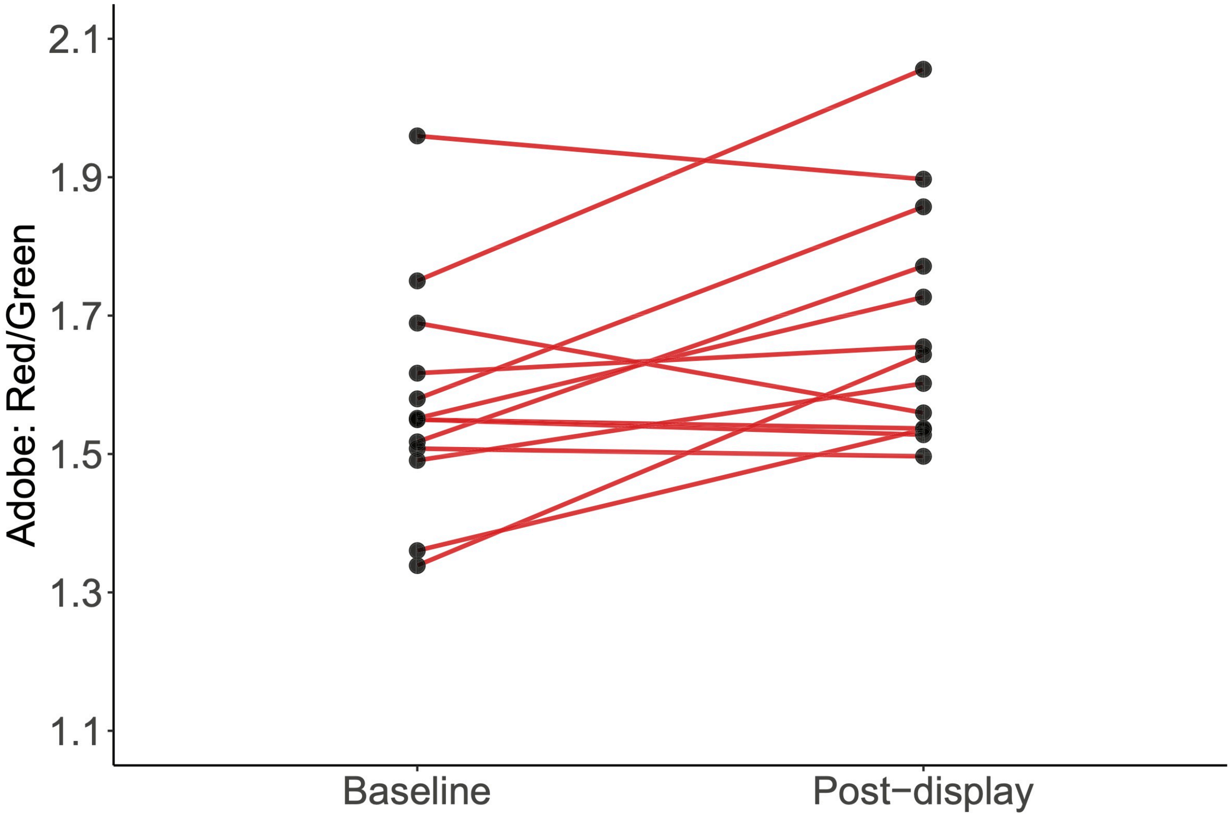


Figure 5. Activity and redness: male chests were redder immediately after a display ("post-display") compared to "baseline"

2.4.4 Heat manipulation and redness

Within individuals, chest redness (Red/Green measured in Adobe) increased after direct application of a heat pack to the chest for 1 min compared to baseline conditions (paired t-test: *t* = 2.41, *df* = 14, *p* = 0.03, **Fig. 6**; ImageJ results also found that chest redness increased after direct application of a heat pack to the chest (*p* = 0.02), but chest luminance did not change between baseline and heat application (*p* = 0.8), **Appendix A: Fig. S6**).

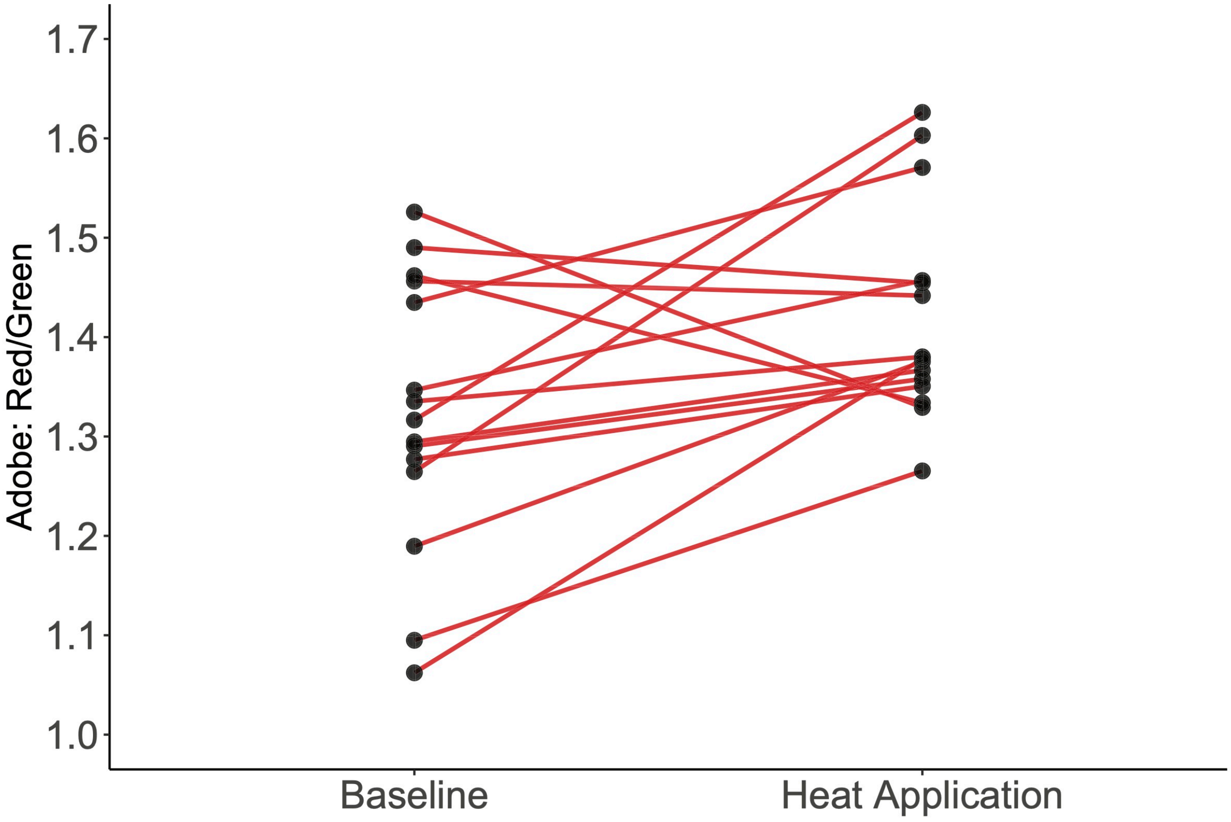


Figure 6. Heat manipulation and redness: male chests were redder immediately after a heat application compared to before ("baseline")

2.4.5 Redness and skin temperature

Chest skin surface temperature increased with chest redness (Red/Green) in adult males as measured with Adobe Photoshop ($b = 4.016$, SE = 1.526, $t = 2.632$, $p = 0.022$), **Fig. 7**; ImageJ results also indicated that chest skin surface temperature increased with chest redness ($p = 0.03$) and trended towards decreasing with luminance ($p = 0.09$), **Appendix A: Fig. S7**).

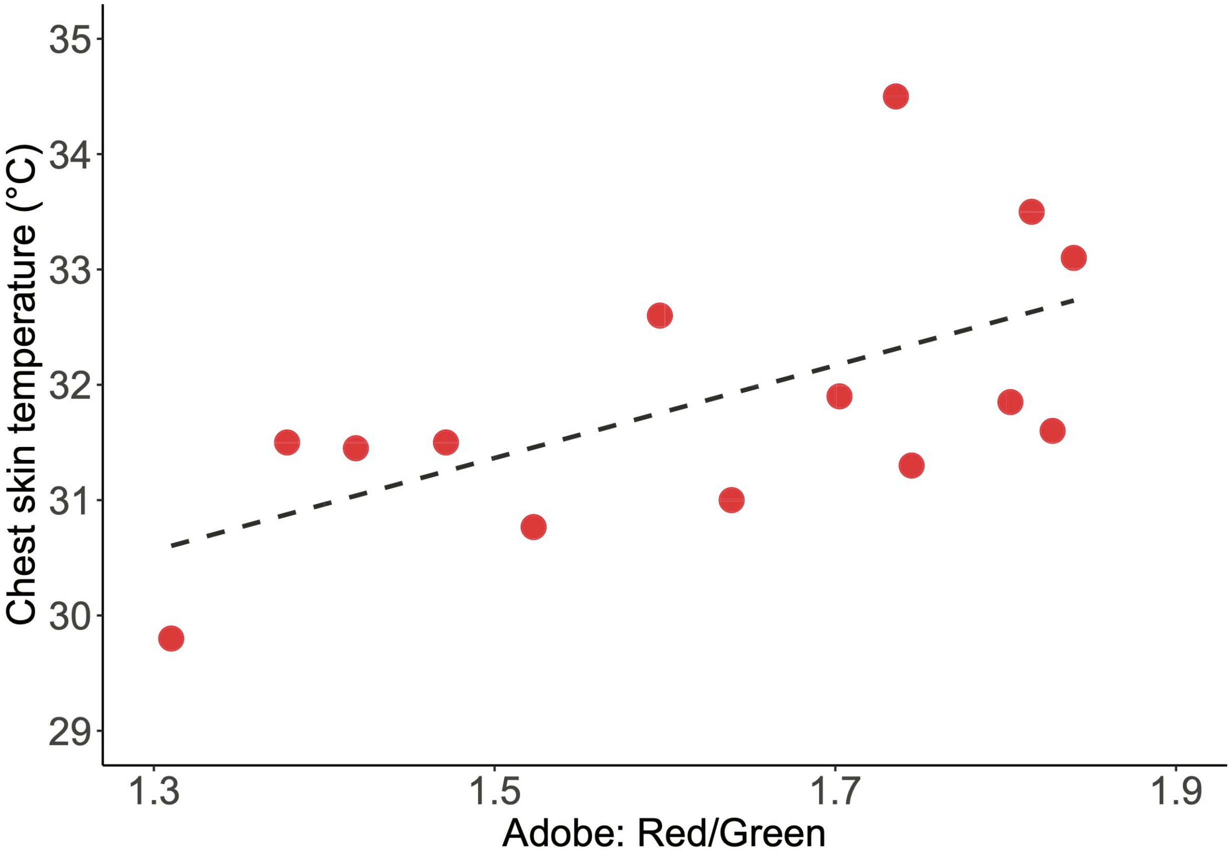


Figure 7. Redness and skin temperature: redder chests have higher skin surface temperatures. Chest patch surface temperature (°C) measured with a Raytek MX6 Phototemp Infrared Thermometer increases with chest redness (Red/Green) in leader males

2.5 Discussion

We validated two methods, Adobe and ImageJ micaToolbox, across four lighting conditions (**Fig. 3**) and in comparison to Nix redness measurements taken directly on the chest of immobilized geladas (**Fig. 4**). We used the Adobe method to show that increased chest redness is associated with increased activity, local application of a heat pack, and higher skin surface temperature (**Figs. 5, 6 and 7**). Together, these results suggest that one of the main sources of an increase in chest redness in gelada males derives from increased blood flow with a concomitant increase in surface temperature. This finding elucidates for the first time a mechanism by which color variation is produced and maintained in geladas, and also a mechanism that can link variation in the signal to variation in individual condition.

The complexities of a sexually selected system increase the importance of minimizing measurement errors. One of our goals was to help researchers choose appropriate methods for working with color in the wild. Our results suggest that the Adobe method performed slightly better in terms of consistency across light conditions and in comparison to the Nix direct skin measurements. Both Adobe and ImageJ micaToolbox methods were fairly consistent across a wide range of light conditions and correlated with the Nix redness measurements. Although redness measurements for backlit light conditions varied from other light sources for both methods, the differences were small (**Fig. 3**). Among the metrics compared to Nix, ImageJ micaToolbox had the weakest correlation (**Fig. 4**). This could be attributed to the ImageJ micaToolbox method only using two gray standards from each paired ColorChecker chart rather than all 24 squares (including the “light skin” square and “moderate red” square that are close in color to the chest patch) as the Adobe method does. In addition, our use of JPEG images could have contributed to the relatively poor performance of ImageJ micaToolbox as the software was only recently adapted for use with JPEGs and functions best with RAW file formats (van den Berg et al., 2020). Finally, it is possible that, by correcting for the visual system of the subject, the ImageJ micaToolbox results are capturing something that is biologically relevant to the geladas but is not apparent to us (or detected by the other methods that were developed for the human visual system).

In our study, the subjective classifications had the strongest correlation with Nix measures compared to objective color measures. This result suggests that older methods of color classification in primates that use the human eye remain entirely relevant for primate color analyses (particularly for catarrhine primates). However, humans will naturally have differences in color perceptions, and this trend could be subject to low experimental repeatability if adopted in long-term research with many different observers (Endler, 1990). In addition, although human subjective measures may be useful for studying the visual perception of a catarrhine primate with a similar visual system to humans (Bowmaker et al., 1991; Jacobs & Deegan 1999), visual systems vary widely across animals and include those with the ability to see spectra not visible to humans (e.g., UV). In studies on animals that have spectral sensitivities with less overlap with humans, ImageJ micaToolbox would be the preferred method as it allows conversion of images to the cone-catch sensitivity of the animal and has capabilities for UV-sensitive cameras (Troscianko & Stevens, 2015; van den Berg et al., 2020). In addition, the ImageJ micaToolbox offers a suite of tools for studying contrast and pattern which the Adobe method does not provide (van den Berg et al., 2020). In studies on wild populations where the animal is immobilized, the Nix measurement method may be the most useful, as is a lightweight tool for measuring color that has been proven effective for measuring pelage, feathers, or skin in museum specimens

(Potash et al., 2020). However, Nix measurements are designed for human visual systems which limits its use to animals with similar visual systems to humans.

We found that chest redness increased with activity ($p < 0.05$), heat application ($p < 0.05$), and skin surface temperature ($p < 0.05$), and chest luminance tended to decrease with skin surface temperature ($p = 0.09$, **Appendix A: Fig. S7B**), although the difference was not statistically significant, all supporting the more general hypothesis that chest redness in gelada males is associated with increased blood flow. The activity associated with male displays temporarily increased a male's chest redness (**Fig. 5**). Displays in geladas (and the activity and vocalizations that accompany them) are hypothesized to be used by leader males to ward off challenging bachelors — similar to chacma baboons (*Papio ursinus*), where adult males give a contest call with acoustic properties tied to rank that mediates male-male aggression by preventing fights between disparately ranked males (Kitchen et al., 2003). The change in chest color for post-display male geladas likely results from increased blood flow to the area due to elevated heart rate and blood pressure resulting from the increase in activity. In humans, exercise increases skin blood flow to dissipate heat generated by contracting muscles (Johnson, 1986; Kenney & Johnson, 1992), and in rhesus macaques, high skin blood flow increases skin redness (Rhodes et al., 1997). This suggests that redness in geladas might relate to overall activity levels and might explain some of the differences found across leader males. However, we do not know whether these brief episodes of activity might also increase longer term *baseline* redness for these males. We would like to know how long these transient increases in redness persist and whether differences in basal metabolic activity are associated with differences in baseline redness. In addition, the variation in redness among leader males could also reflect the oxygen content of their blood because red skin coloration can be produced by increased oxygenation

(Changizi et al., 2006). In a high-altitude environment like the Simien Mountains National Park where the oxygen is only about 66% that at sea level (Tinsley Johnson et al., 2018), this could be a salient factor limiting male fighting ability.

We also found that experimentally increasing the temperature of the chest on immobilized males temporarily increased chest redness (**Fig. 6**). We suggest that this trend is caused by the local effects of vasodilation, which has been shown to increase skeletal muscle blood flow in humans (Heinonen et al., 2011). This suggests that some of the differences in redness across males are due to differences in the local control of blood flow to the chest patch. Permanent developmental changes in this regulation could explain the observation of increases in redness in males that transition from bachelor to leader (Bergman et al., 2009). We propose that one-time ontogenetic regulatory changes associated with becoming a leader male result in a local, sustained increase in blood flow to the chest patch. This suggests that only males who have gone through this developmental switch regulate blood flow to their chest differently than other geladas. This potential developmental switch resembles signals triggered by social circumstances in other primates like the Bornean orangutan (*Pongo pygmaeus*), where cheek-pads (flanges) develop only for dominant males (Kuze et al., 2005), and mandrills (*Mandrillus sphinx*), where dominant males with access to mating opportunities exhibit a larger morphological variant (fatted; Wickings & Dixson, 1992).

Finally, we found that redder chests have a higher surface temperature (**Fig. 7**). This is likely a result of increased blood flow to the skin, and may be a signal production cost for the maintenance of redder chest patches. The ability of males to withstand a higher rate of heat loss may indicate a greater metabolic capacity, which is particularly salient for a species that regularly experiences below-freezing temperatures (Tinsley Johnson et al., 2018). In addition, the lower partial pressure of atmospheric oxygen may amplify the level of cold that animals experience in high-altitude environments (Chappell & Hammond, 2004; Cheviron et al., 2013; Hayes, 1989; Milledge et al., 2007). In golden snub-nosed monkeys (*Rhinopithecus roxella*), for example, a decrease of 5–10 degrees in winter months is associated with an energetic deficit, which suggests even small annual fluctuations in temperature can have a sizable impact on primate metabolic demands (Hou et al., 2020). To reduce heat loss, cold-adapted homeotherms often decrease energy expenditure by altering their behavior (Terrien et al., 2011). In geladas, we routinely see males and females sit with heads lowered over their chest patches on cold mornings, resulting in less exposed chest patches; they also huddle together with chest patches facing inward, which may also serve to reduce heat loss (**Appendix A: Fig. S8**). Huddling behavior for thermoregulation has been observed in other primates that live in extreme environments (titi monkeys, *Callicebus nigrifrons*: Gestich et al., 2014; ring-tailed lemurs, *Lemur catta*: Kelley et al., 2016; golden snub-nosed monkeys, *Rhinopithecus roxellana*: Hou et al., 2020) and has been demonstrated to effectively conserve energy expenditure (vervet monkeys, *Chlorocebus pygerythrus*: McFarland et al., 2015) and increase body temperature (Japanese macaques, *Macaca fuscata*: Hanya et al., 2007). Although we found that redder chests are associated with higher surface temperatures, we cannot yet determine whether this heat loss incurs a meaningful metabolic cost. Future research using non-invasive metabolic indices will help to determine how energy balance relates to chest redness.

Together, these findings move us closer to understanding the potential information content of red chests. Our findings suggest that redder chests can indicate males with sufficient energetic reserves to either maintain high rates of physical activity or to offset the metabolic costs of losing heat by directing blood flow specifically to the chest patch (or both). This information is particularly relevant to bachelor males because takeover challenges can be physically demanding battles of attrition that can last for days (Pappano & Beehner, 2014). Therefore, attending to chest redness may allow bachelor males to avoid challenging the males who are most likely to win such a contest. However, further research is needed to better understand both the energetic costs associated with redness and how other males respond to variation in redness.

It might seem extravagant that geladas — found only in a high-altitude, cold environment — are the only primate with a bare patch of highly vascularized skin on their chest. Signals are, however, not wasteful over-investments (Penn & Számadó, 2019). They evolve because selection favors efficient and optimal investment into signal expression, and some individuals can afford to invest much more in this expression and some much less. Individuals are expected to allocate their investment into their own signal according to their own ability and condition. For example, moths invest less in an anti-predatory signal (that happens to impair thermoregulation) in cold environments (Lindstedt et al., 2009). Particularly in extreme environments, animals that are doing well despite the harsh conditions will be the most able to invest in a signal that flouts the specific risks posed by the extreme environment. Thus, it is probably not a coincidence that geladas, living at both a temperature and oxygen extreme, appear to have a sexual signal associated with vasodilation, heat loss, and possibly even oxygen saturation. Gelada leader males that communicate their ability to overcome these constraints and bachelor males that attend to this information will both have an advantage. Therefore, signal form might match a species' environment not only in terms of efficacy of transmission (Patricelli & Hebets, 2016) but also in the type of information it can potentially convey. For geladas, the mix of red-colored blood signaling and an extreme cold environment may have provided an elegant demonstration of the natural links between signal form and function.

2.6 Acknowledgements

We are grateful to the Ethiopian Wildlife Conservation Authority for granting us permission to conduct this research. We also want to thank the many staff and wardens (Berhanu Gebre, Maru Biadglegn, Abebaw Azanaw Haile) of the Simien Mountains National Park, our Ethiopian staff (Esheti Jejaw, Ambaye Fanta, Setey Girmay, Yeshi Dessie, Dereje Bewket, Atirsaw Aduga, Tariku W/Aregay, Shifarew Asrat), our research assistants in the field (Clay Wilton, Julie Jarvey, Levi Morris, Tara Regan, Caitlin Hawley, Peter Clark, Evan Sloan, Megan Gomery, Laura Morrissey, Emily Downey, Eddy Wild, Liz Babbitt, Maddie Melton, Anna Cryer). We thank Jeanne Altmann from the Amboseli Baboon Project for loaning us the Raytek MX6 PhotoTemp Infrared Thermometer.

2.7 Author contributions

Conceptualization, data curation, formal analysis, investigation, visualization: PMD, RFP, JCB, TJB; data collection: PMD, RFP, SS, ISC, KLC, AL, FA, NSM, JCB, TJB; writing—original draft: PMD, RFP, JCB, TJB; writing—review and editing: all the authors; supervision: JCB, TJB. All the authors reviewed the results and approved the final version of the manuscript.

2.8 Funding

This work was supported by the National Science Foundation (BCS-2041542, BCS-0715179, BCS-1732231, BCS-1723237, BCS-2010309, BCS-1723228, IOS-1255974, IOS-

1854359), the Leakey Foundation (AWD015438), the Leakey Foundation Baldwin Award (AWD012312), the National Geographic Society (NGS-8100– 06, NGS-8989–11, NGS-1242, and NGS-50409R-18), the Fulbright Scholars Program, Nacey Maggioncalda Foundation, Sigma Xi, the University of Michigan, Arizona State University, and Stony Brook University.

Chapter 3 : Vascularization Underlies Differences in Sexually Selected Skin Coloration in a Wild Primate[3]

3.1 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,

---

[3] Under review at *Molecular Ecology*. https://doi.org/10.22541/au.167359516.63326414/v1

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.

3.2 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. 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).

Sexually selected signals in males are often mediated by testosterone, a steroid hormone involved in regulation of reproductive function in male vertebrates (Ketterson & Nolan Jr, 1999; Plant & Zeleznik, 2014) that is associated with reproductive benefits (Enstrom et al., 1997) and physiological costs (Muehlenbein, 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 sexual 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.

3.3 Materials and methods

3.3.1 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.

3.3.2 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; P. M. DeLacey et al., 2022). 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 (**Appendix B: Fig. S9**), 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 under 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 as more female photos were taken with the Sony camera. 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.

3.3.3 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.

3.3.4 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 (RQN) 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, as its richer annotation yielded a higher number of mapped reads compared to the *Theropithecus gelada* genome (Chiou et al., 2022).

3.3.5 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).

3.3.6 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., 2019). 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.

3.3.7 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 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.

3.4 Results

3.4.1 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). However, males had a wider range of chest redness within individuals compared to females (β=0.63, P=0.007, **Fig. 8**). While under anesthesia, male geladas did not have redder chests than females (β=0.04, P=0.40, **Fig. 9).**

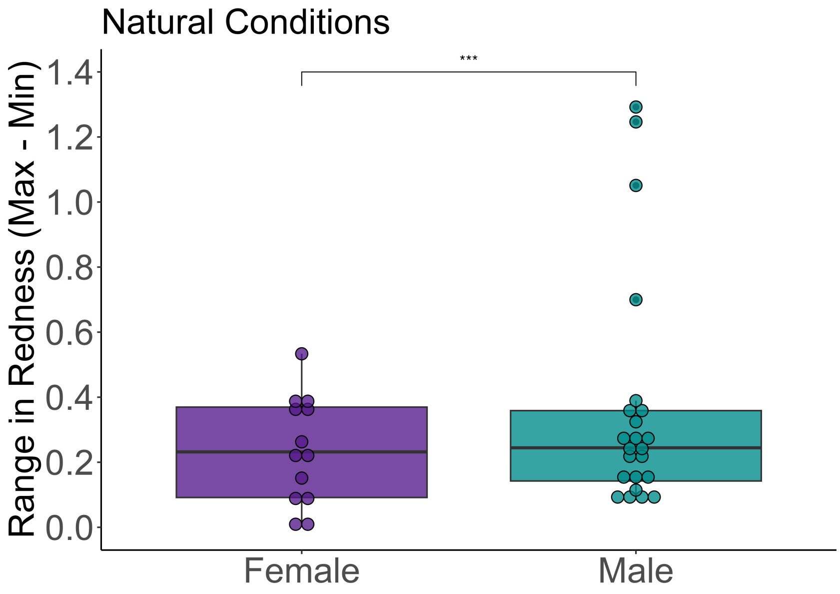


Figure 8. Adult males had a wider range in chest redness within individuals compared to females under natural conditions.

Figure 9. 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.

3.4.2 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, **Appendix B: Table S7, Fig. S10**). 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, **Appendix B: Fig. S11**; 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 (**Appendix B: Fig. S12**).

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%), in a model controlling for the effects of RQN, 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. 10).** The sample size for subadult females (*n*=2) was too small to draw conclusions about sex-bias gene expression trends in this age category.

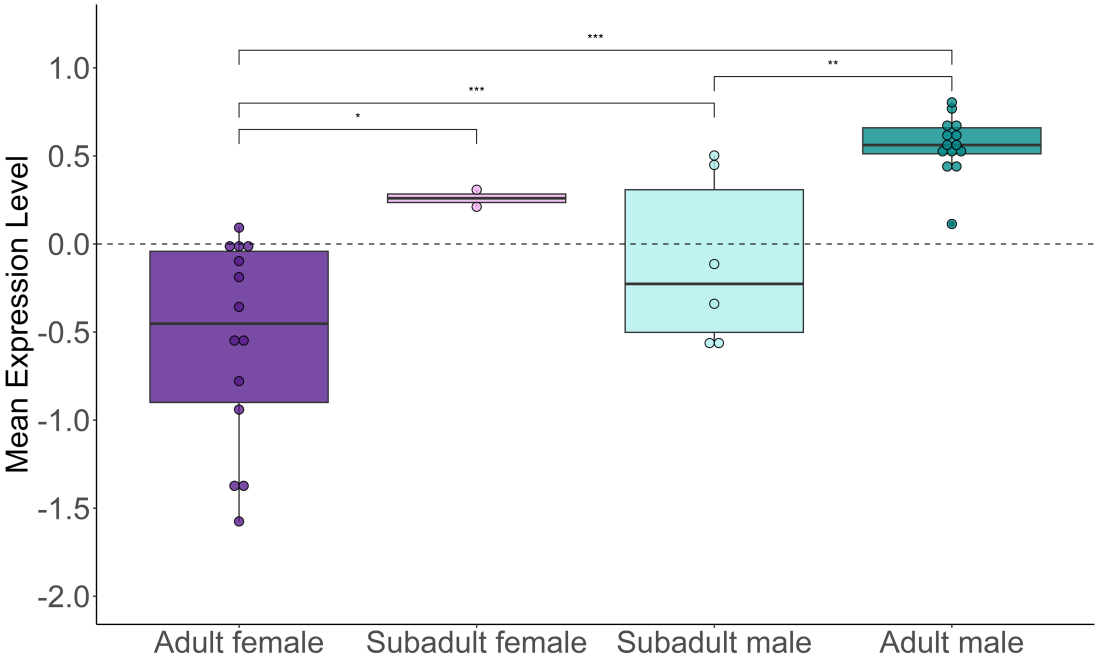


Figure 10. Subadult males exhibited intermediate expression of sex-biased genes between adult males and adult females. Sex-biased gene expression for each individual across all age categories.

3.4.3 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. 11**). 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}; **Appendix B: Fig. S13**). 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 (**Appendix B: Fig. S14).**

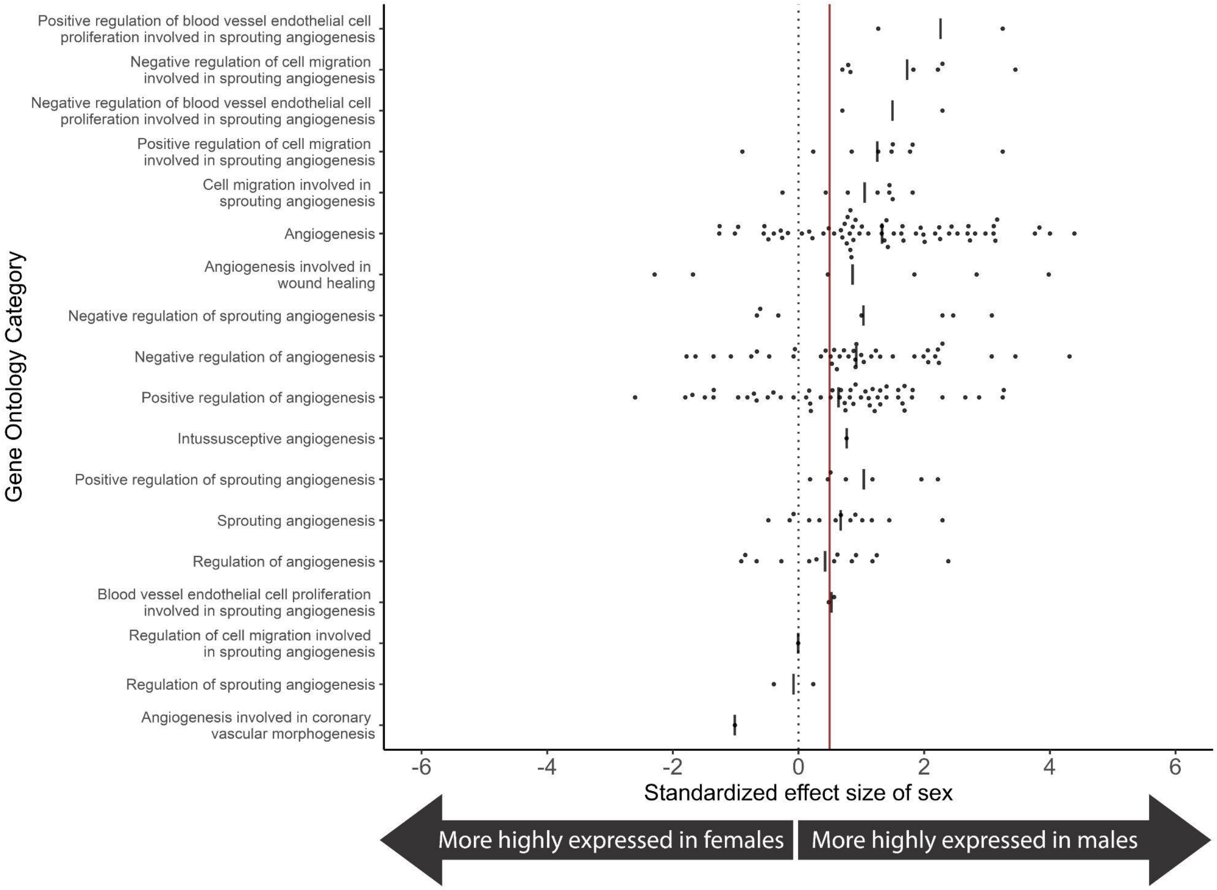


Figure 11. Genes more highly expressed in males were enriched for biological processes associated with angiogenesis. Points represent individual genes within each subcategory of the angiogenesis gene ontology category, and the solid vertical lines reflect the average standardized effect of sex for genes of each subcategory. The solid red line represents the median standardized effect of sex for all genes not in angiogenesis-associated categories.

3.5 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. 8-9)**. Further, subadults displayed an intermediate gene-expression pattern from adult males and adult females (**Fig. 10**). 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. 11**). 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. 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 (A. F. 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.

3.5.1 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.

3.5.2 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.

3.6 Acknowledgements

We are grateful to the Ethiopian Wildlife Conservation Authority for granting us permission to conduct this research. We also want to thank the many staff and wardens (Berhanu Gebre, Maru Biadglegn, Abebaw Azanaw Haile) of the Simien Mountains National Park, our Ethiopian staff (Esheti Jejaw, Ambaye Fanta, Setey Girmay, Yeshi Dessie, Dereje Bewket, Atirsaw Aduga, Tariku W/Aregay, Shifarew Asrat), our research assistants in the field (Clay Wilton, Julie Jarvey, Levi Morris, Tara Regan, Caitlin Hawley, Peter Clark, Evan Sloan, Megan Gomery, Laura Morrissey, Emily Downey, Eddy Wild, Liz Babbitt, Maddie Melton, Anna Cryer).

This work was supported by the National Science Foundation (BCS-2041542, BCS-0715179, BCS-1732231, BCS-1723237, BCS-2010309, BCS-1723228, IOS-1255974, IOS-1854359), the Leakey Foundation (AWD015438), the Leakey Foundation Baldwin Award (AWD012312), the National Geographic Society (NGS-8100–06, NGS-8989–11, NGS-1242, 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.

Chapter 4 : The Relationship Between Chest Redness, Testosterone, and Tenure Length in Male Geladas (*Theropithecus gelada*)

4.1 Abstract

Males in many primate species exhibit striking secondary sexual traits that enhance reproductive fitness, but reproductive outcomes are often difficult to study in long-lived species. Here, we use long-term data collected from wild male geladas (*Theropithecus gelada*) to assess a putative mechanism and a proxy of reproductive fitness associated with a sexually selected signal, the red chest patch. We used digital photographs (n=623 photos from n=38 males) and fecal androgen metabolites (fAMs; n=658 samples from n=48 males) to evaluate causes and consequences of the variation in the red chest patch in wild male geladas by examining the factors that predict chests redness (1) across males of all statuses (including bachelor, leader, and follower males), (2) across dominant leader males only (an between-subjects design), and (3) within dominant leader males (a within-subjects design). For the last two categories, we then examined one potential reproductive fitness consequence of red chests, tenure as leader male. Higher cumulative rainfall, which is tightly correlated with green grass availability, predicted higher chest redness and fAMs for all individuals, likely indicating males can produce redder chests when their energy levels are higher. We detected a trend towards a relationship between chest redness and fAMs both across males and across leaders. Across leaders, males with redder chests trend towards longer tenures while leaders with lower fAMs trend towards shorter tenures. Contrary to expectations, leaders with longer tenures had redder chests at the beginning of their

tenure compared to the end of their tenure perhaps suggesting that males increase redness when fighting whether it is the beginning or end of their tenure. Together, these results tentatively suggest circulating testosterone works in tandem with blood flow mechanisms to increase chest redness and that redder chests extend tenure length, providing evidence for improved reproductive fitness outcomes for leader males with redder chests.

4.2 Introduction

Adult males of many primate species exhibit striking secondary sexual traits such as brightly colored sexual skin, capes, beards, mustaches, and other facial adornments (Dixson et al., 2005). For a trait to be considered sexually selected, whether used to attract mates or intimidate rivals, it must exhibit: (1) sexual dimorphism, (2) variation within the same sex, (3) conspecific discrimination of signal variations, (4) behavioral choices in the recipient that are consistent with reproduction, and (5) differential fitness outcomes where high quality signals result in greater reproductive success (Snowdon, 2004). The first four criteria are possible to investigate in observational field studies, but reproductive outcomes are often difficult to study in long-lived species like primates. Many studies have used a proxy of reproductive success such as dominance rank or mating success within a season as a stand in for lifetime reproductive success to compensate for primate's long lifespans (Dubuc, Allen, et al., 2014; Grueter, Zhu, et al., 2015; Setchell & Dixson, 2001). These metrics are useful for getting a snapshot of a male's reproductive success in the short-term, but does not capture the full picture of a male's long-term reproductive fitness outcomes.

In addition to establishing a trait as a sexually selected signal, Tinbergen's four complementary levels of analysis provide another framework of understanding how and why a behavior or trait evolved (Tinbergen, 1963). Classically, Tinbergen's questions are organized into the mechanism (immediate causes for a behavior), development (ontogenetic changes that cause a behavior), evolutionary history (trajectory of a behavior across many generations), and function (fitness consequences of a behavior) levels of analysis (Bateson & Laland, 2013). Recent reframing organizes these levels into a single temporal timescale divided into causes (encompassing mechanism, development, and evolutionary history) which precede a single instance of behavior and consequences (encompassing function) that come after this behavior (Bergman & Beehner, 2022). Here we use the causes and consequences framework to investigate a putatively sexually selected trait, the red chest patch, in male geladas (*Theropithecus gelada*).

Geladas exhibit a unique hour-glassed patch of skin on their chest and neck that differs between males and females as females have a smaller within individual range in redness compared to males (DeLacey et al., *under* review) and a sexual swelling of cutaneous vesicles surrounding the chest region where vesicle turgidity varies across the ovarian cycle (Roberts et al., 2017). Chest redness varies within males where "leader" males, the dominant male of "reproductive units" (one dominant adult male, one or more subordinate adult males, up to a dozen adult females, and related offspring), have the reddest chests compared to "follower" males, subordinate males with 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 mediate male challenges allowing males to assess others prior to engaging in conflict (Benítez, 2016). 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, suggesting bachelors, who must challenge and replace a leader male to gain reproductive opportunities (Pappano & Beehner, 2014), use chest color in decisions of which male to target (Benítez, 2016).

We have previously identified that redder chests are associated with increased physical activity (Bergman et al., 2009; DeLacey et al., 2022) and higher chest skin surface temperature (DeLacey et al., 2022). Geladas also exhibit sex differences in gene expression where higher expression in males was associated with angiogenesis, blood pressure, and blood vessel maintenance (DeLacey et al., *under review*). Together, these mechanistic results suggest that males may have more variable chest redness due to increased blood flow and blood vessel branching in the chest skin. Contrary to our expectations, genes encoding proteins that interact with androgen or estrogen were not more highly expressed in males, but other aspects of hormonal regulation may be more informative than sex differences in gene expression. Specifically, gonadal testosterone has been implicated in mediating male reproductive trade-offs in vertebrates by increasing reproductive effort while simultaneously inhibiting aspects of survival (Heyland et al., 2005; Ketterson & Nolan Jr, 1999; Zera & Harshman, 2001). Nevertheless, the differential fitness outcomes associated with a redder chest patch within leaders have not yet been investigated. Here we investigate fecal androgen metabolites (fAMs) as hormonal mechanistic cause along with a functional consequence using tenure length, or the length of time a male remains leader of a reproductive unit, as a proxy of reproductive success.

Here we use a combination of long-term photogrammetric, hormonal, demographic, and climatic data collected from a population of wild geladas living in the Simien Mountains National Park, Ethiopia to assess factors that predict red chests across males of all statuses, across leaders, and within leaders. We tested the hypotheses that (1) climatic variables and circulating testosterone levels influence chest redness and (2) a redder chest patch extends tenure length for leaders by warding off would-be rivals. We predicted that for all groups, (1a) higher rainfall and ambient temperatures would increase both chest redness and fAMs and (1b) higher fAMs would predict redder chests. Across males, (2a) leaders would have the reddest chests compared to all status categories and (2b) both bachelors and leaders would have high fAMs compared to other status categories. Across leaders, (2c) males with more adult females in their unit and longer tenures would have the reddest chests and highest fAMs. Within leaders, (2d) males would have redder chests at the beginning of their tenure compared to the end of their tenure.

4.3 Methods

4.3.1 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.

4.3.2 Photo collection, measurement, and analyses

To assess male chest redness in geladas, we used objective color measurement methods for digital photographs taken while conducting daily activities like resting, grazing, or grooming. 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). Chest redness was measured from 623 digital chest photographs of adult males (n=38) collected between 2006 and 2013.

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 by four undergraduate research assistants 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).

4.3.3 Fecal sample collection, extraction, and storage

Fecal sample collection, extraction, and storage methods followed protocols previously validated for geladas (Beehner et al., 2009; Beehner & McCann, 2008a; Pappano et al., 2010). We collected fecal samples within minutes of defecation from known individuals. Specifically, approximately 0.5 g of wet feces were added to 3.0 ml of a methanol/acetone solution (4:1, v:v) and homogenized for 1 min using a battery-powered vortexer. Samples remained in the organic solution for 3-4 h, and once fecal matter settled to the bottom of the tube, we filtered 2.5 ml of the fecal homogenate through a 0.2 μm PTFE filter and washed the filter with an additional 0.7 ml of methanol/acetone solution. We then added 7 ml of distilled water and mixed the solution. We prepped a reverse-phase C18 solid-phase extraction cartridge (Sep-Pak Plus, Waters Corporation, Milford, MA) according to the manufacturer’s instructions (with 2 ml methanol followed by 5 ml distilled water) and loaded the aqueous homogenate onto the Sep-Pak cartridge. We then washed the cartridge with 2 ml of a sodium azide solution (0.1%) and sealed the cartridge in Whirl-pak bags with a desiccant (~2g silica beads). After drying for 3 days at room temperature, cartridges were frozen at -20°C. The dry weight of the extracted fecal sample was measured a few weeks later using a portable scale (to ±0.001 g). Cartridges were then shipped to the University of Michigan at room temperature and again stored at -20°C until hormone metabolites were eluted from the cartridge.

4.3.4 Fecal androgen metabolite assay

In the Beehner Endocrine Laboratory, steroids were eluted from cartridges with 2.5 ml of 100% methanol and stored at -20°C until the time of the assay. We measured fecal androgen metabolites (fAMs) using an epiandrosterone (5-androstan-3-ol-17-one) microtiter plate enzyme-immunoassay (EIA), which is a C17 group-specific enzyme immunoassay that detects 17 oxo-androgens with 100% cross-reactivity (Möhle et al., 2002; Palme, 1993). This EIA has been previously validated for use in geladas (Sen et al., 2022), and has successfully measured male androgen status from fecal samples in rhesus macaques (Higham, Heistermann, et al., 2013) and barbary macaques (Rincon et al., 2017). In short, 50 μg of samples (diluted at 1:20) and standards (range: 3.9-250.0 pg/well) were added to each plate in duplicate, followed by the addition of 50 μl biotin-labeled hormone and 50 μl antibody to each well. Plates were incubated overnight at 4°C, washed three times with the PBS wash solution (containing 0.002% Tween 20), followed by the addition of 150 μl streptavidin horseradish peroxidase to each well and incubated in the dark on a shaker for 1 h. Plates were then washed and 100 μl of TMB substrate solution was added to each well and incubated in the dark for 30-40 min. The reaction was stopped by adding 50 µl of sulfuric acid to each well. The absorbance was measured using an automatic plate reader at a wavelength of 450nm.

4.3.5 Weather variables

We included rainfall and temperature variables in both our chest redness and androgen analyses as previous research has demonstrated a positive correlation between rainfall/temperature and chest redness (Benítez, 2016; DeLacey et al., 2019) and testosterone levels for leader males (Pappano & Beehner, 2014). Rainfall (mm) was recorded using a rain gauge located at the Sankaber camp on a near daily basis, and daily minimum/maximum temperature (°C) were recorded in a shaded location at the field station. We calculated cumulative rainfall for each sample collected by summing rainfall measures from the previous 90 days as this measure is positively correlated with green grass availability in this area (Jarvey et al., 2018).

4.3.6 Age, status, group size, and tenure length

For each male, we calculated age by finding the difference between the estimated date of birth from the Simien Mountains Gelada Research Project demography and the date the sample was collected. Similarly, male status (leader, former leader, follower, or bachelor) was entered as the status on the day of the sample collection according to the demographic data. We excluded young follower males (i.e., followers who have not previously been leader) from across male analyses due to a small sample size (n=3) and the transient nature of follower males, some of whom do not often stay with a unit for more than 6 months and instead shift between units and all male groups.

For analyses within leader males, we defined unit size as the total number of adult females in each reproductive unit as outlined by previous research in geladas (Feder et al., 2022; Tinsley Johnson et al., 2021) on the day of sample collection. We only included leader males in the within and across leader male analyses if they had a known takeover date (observed ousting a male to become leader) and a known overthrow date (observed being ousted by another male and losing leader status). We calculated tenure length in years by calculating the difference between these two dates. Similarly, days since takeover was calculated as the number of days that had passed since the takeover date on the day of the sample collection, and days to overthrow was calculated as the number of days leading up to the overthrow on the day of sample collection.

4.3.7 Data analyses

All statistical analyses were performed in R version 4.2.1 (R Core Team, 2022). Linear mixed models were run in the R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017), and model comparisons were run in bbmle (Bolker & Team, 2010). Plots were constructed with ggplot2 (Wickham et al., 2016). For all analyses including model selection, only the best model that had the lowest AICc in model comparisons is included in the results.

Across males of all statuses

We assessed redness from 623 chest photos from 38 individual males (n=14 bachelors, n=20 former leaders, n=31 leaders), fAMs from 658 fecal samples from 48 individual males (n=12 bachelors, n=17 former leaders, n=38 leaders), and the relationship between redness and fAMs from 239 paired samples collected within 60 days of one another from 22 individual males (n=7 bachelors, n=10 former leaders, n=18 leaders). We included camera model as a random effect when redness was the outcome variable and individual as a random effect for all models. For each outcome variable, we used weighted AIC to compare models with combinations of the following fixed effects: status, age, log cumulative rainfall (mm) from the past 90 days, daily minimum temperature (°C), and daily maximum temperature (°C).

Across leaders

For our analyses across leaders, we restricted our dataset to only include leaders with tenures 6 months or longer and known takeover (when they transitioned from bachelor to leader) and overthrow dates (when they lost their leader position to another male). We assessed redness for 247 chest photos collected from 20 leader males, fAMs for 235 fecal samples from 20 leader males (note: not the same set of 20), and 97 matched redness and fAM samples collected within 60 days of one another from 10 leaders. We included camera model as a random effect when redness was the outcome variable and individual as a random effect for all models. For each outcome variable, we used weighted AIC to compare models with combinations of the following fixed effects: log cumulative rainfall (mm) from the past 90 days, daily minimum temperature (°C), daily maximum temperature (°C), group size, age, days since takeover, days to overthrow, and tenure length.

Within leader males

We further restricted our data set within leaders to include only males with tenure lengths 8 months or greater and chest redness samples collected during both the first four months and last four months of tenure. For the 10 leader males that fit the criteria, we calculated mean chest redness during the first four months of tenure and the mean chest redness during the last four months of tenure. We then calculated a z-score ((sample mean-population mean)/population standard deviation)) for the first and last four months of tenure using that individual's average and standard deviation of redness across their entire tenure as the population mean and standard deviation. We then used paired t-tests to assess whether there was a difference between chest redness z-scores in the first four months compared to the last four months of tenure for males with short tenure lengths (8 months-2 years; n=4 males), medium tenure length (2-4 years; n=3 males), and long tenure lengths (4-6 years; n=3 years).

4.4 Results

4.4.1 Across males of all statuses

Similar to previous studies on this same topic (Benítez, 2016; P. DeLacey et al., 2019), we also found that males with redder chests experienced more cumulative rainfall from the past 90 days (β=0.04, P=1.15x10^{-6}) and higher maximum temperatures (β=0.03, P=1.02x10^{-8}). Leader males had redder chests compared to former leaders (β=0.12, P=0.01) but not bachelors (β=0.07, P=-0.36; **Fig 11**).

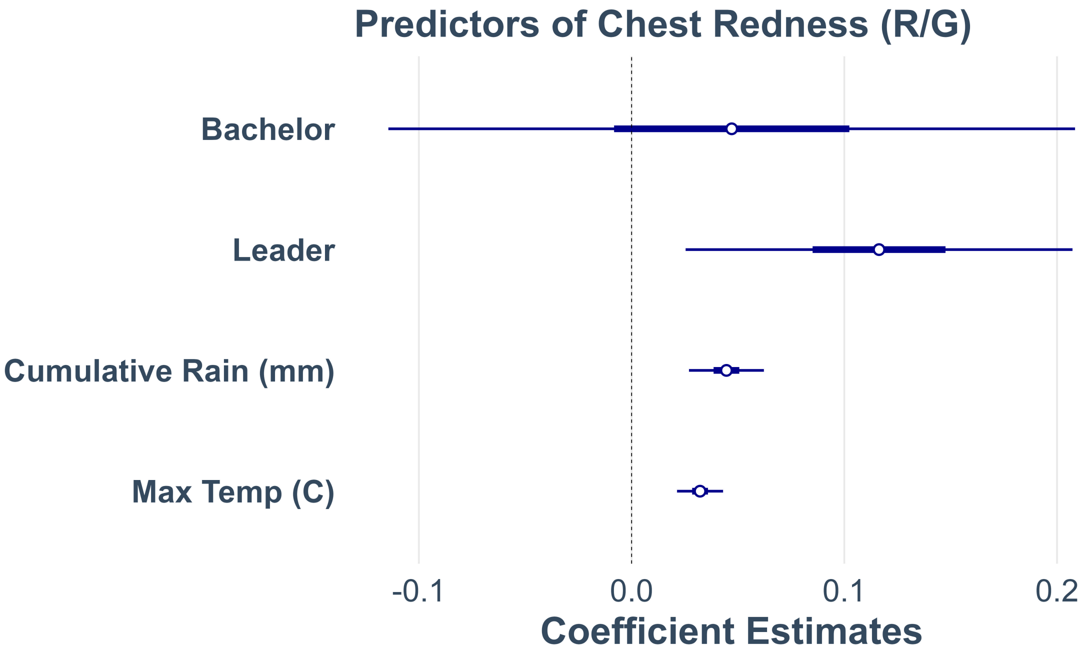


Figure 12. Across males, redder chests were associated with higher cumulative rainfall from the past 90 days and higher maximum temperatures. Leader males had redder chests compared to former leaders but not bachelors. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

Higher fAMs were associated with higher cumulative rainfall from the past 90 days (β=0.04, P=9.17x10^{-5}) and with being a younger male (β=-0.04, P=0.03). Leader males had higher fAMs than former leaders (β=0.14, P=0.02) but not bachelors (β=0.1, P=0.24, **Fig. 13**)

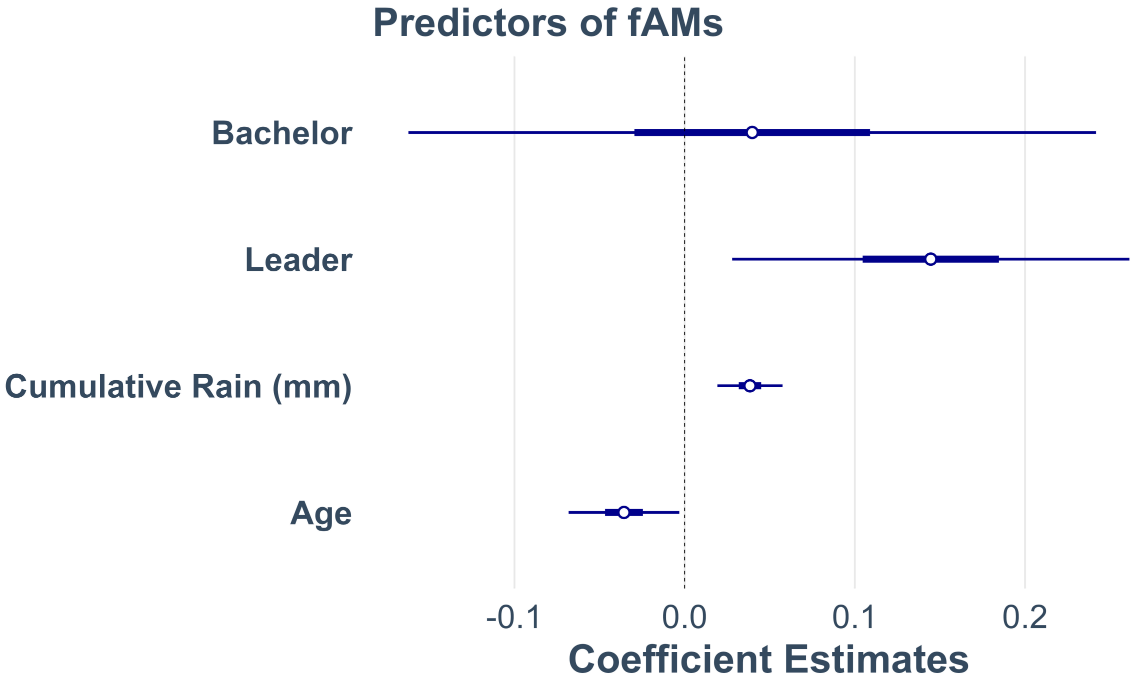


Figure 13. Across males, higher fAMs were associated with higher cumulative rainfall from the past 90 days and younger males. Leader males had higher fAMs compared to follower males but not bachelors. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

Across males, for chest photos and fecal samples collected within 60 days of one another, we found a trend towards a positive correlation between chest redness and fAMs when including age and status in the model (β=0.11, P=0.10, **Fig. 14**).

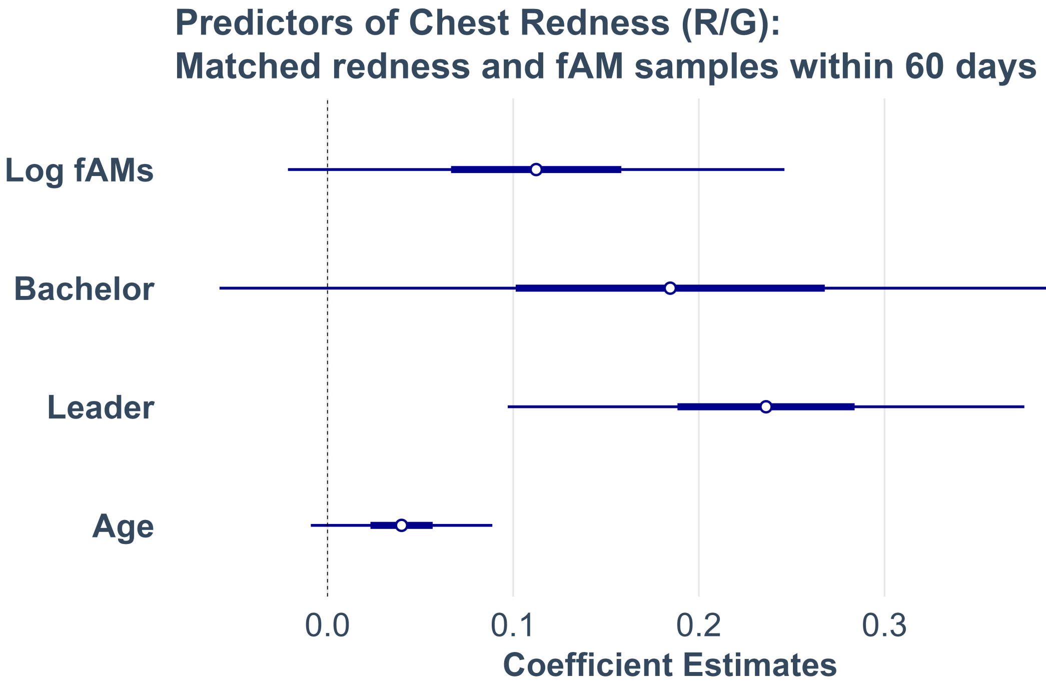


Figure 14. For paired chest photo and fAM samples collected from the same individual within 60 days of one another, there was a positive but non-significant relationship between chest redness and fAMs. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

4.4.2 Across leaders

Across leader males, redder chests were associated with higher maximum temperatures (β= 0.04, P=1.72x10^{-5}), higher cumulative rainfall from the past 90 days (β=0.05, P=4.09x10^{-4}), when leaders were closer to their overthrow date (β=-0.08, P=0.001), and trended towards longer tenures (β=0.05, P=0.22, **Fig. 15**).

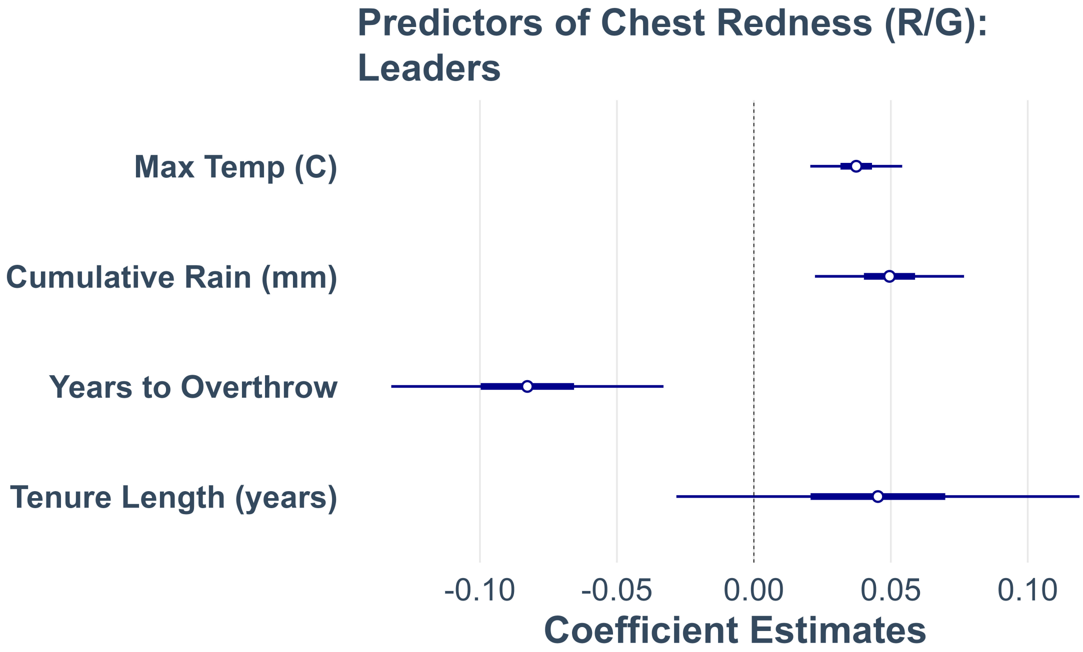


Figure 15. Across leaders, redder chests were associated with (i) higher maximum temperatures, (ii) higher cumulative rainfall from the past 90 days, (iii) a time when leaders were closer to being overthrown, and (iv) longer tenures (although this last one was a trend that did not reach significance). The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

Across leader males, higher fAMs were associated with higher cumulative rainfall in the past 90 days (β=0.04, P=0.02), being further away from being overthrown (β=0.1 , P=0.005), and shorter tenure lengths (β=-0.01, P=0.0004, **Fig. 16**). For chest photos and fecal samples collected within 60 days of one another, we found a trend towards elevated chest redness and increased levels of fAMs when including age, status, and tenure length in the model (β=0.12, P=0.19, **Fig. 17**).

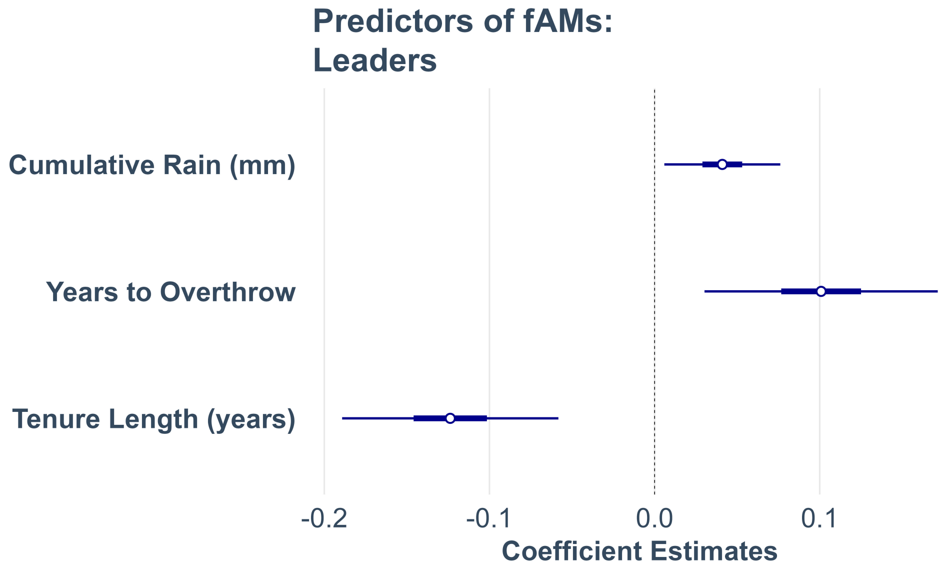


Figure 16. Across leaders, higher fAMs were associated with (i) higher cumulative rainfall from the past 90 days, (ii) a timer period further from being overthrown, and (iii) shorter tenure lengths. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

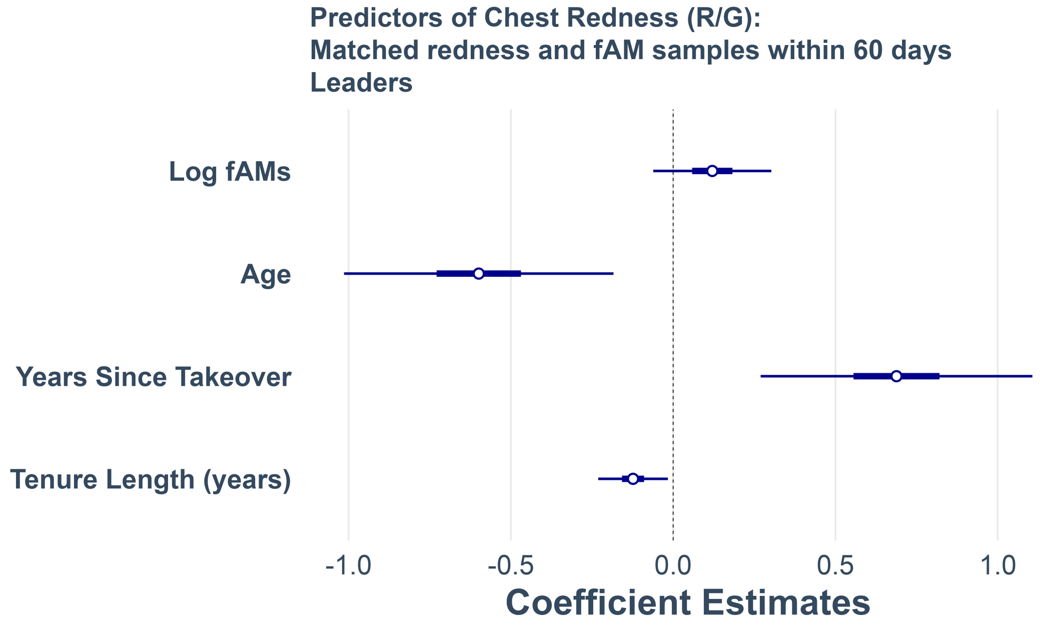


Figure 17. Across leaders, for paired chest photos and fecal samples collected from the same individual within 60 days of one another, there was a positive but non-significant relationship between chest redness and fAMs. The thinner line represents the 95% confidence interval while the thicker line represents the 50% confidence interval.

Within leaders

Within leaders compared to their own average chest redness across tenure, males with shorter tenures trended towards having redder chests in their first 4 months of tenure compared to their last 4 months of tenure (paired t-test, t=2.92, *P*=0.06). Conversely, males with longer tenures trended towards having redder chests in their last 4 months of tenure compared to their first 4 months of tenure (paired t-test: t=-3.68, *P*=0.07). Chest redness did not differ between the first and last 4 months for medium-length tenure males (t=-0.21, *P*=0.85; **Fig. 18**).

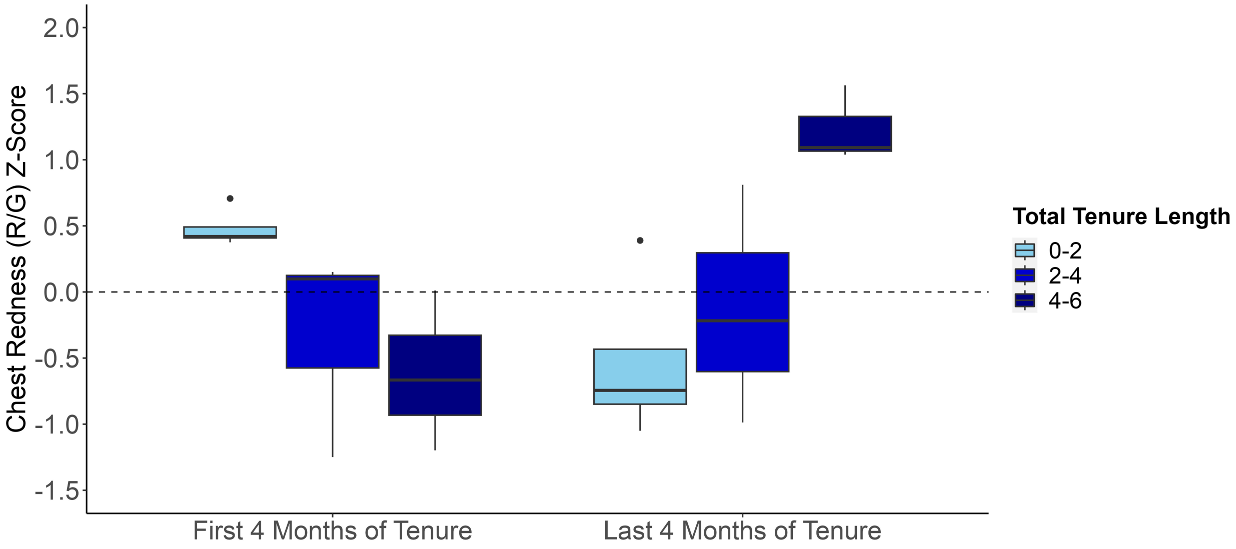


Figure 18. Within leaders, males with shorter tenures trended towards higher redness during their first 4 months than their last 4 months compared to their own overall average. Males with longer tenures trended towards lower redness during their first 4 months than their last 4 months compared to their own overall average.

4.5 Discussion

We used long-term data collected from wild geladas to assess a putative mechanism and proxy of reproductive fitness associated with a sexually selected signal. Higher cumulative rainfall predicted higher chest redness and fAMs for all individuals. Leaders had redder chests and higher fAMs than former leaders but not bachelors (**Fig. 12, 13)**. We found a trend towards a relationship between chest redness and fAMs both across males and across leaders (**Figs. 14, 17)**. Across leaders, males with redder chests trend towards longer tenures (**Fig. 15)** and males with lower fAMs trend towards shorter tenures (**Fig. 16)**, but group size was not correlated with chest redness or fAMs. Contrary to expectations, within leaders, males with longer tenures had redder chests at the end of their tenure compared to the beginning of their tenure perhaps suggesting that males increase redness when fighting whether it is the beginning or end of their tenure (**Fig. 18)**. Together, these results tentatively suggest circulating testosterone works in tandem with blood flow mechanisms to increase chest redness and that redder chests extend tenure length.

Rainfall and temperature variables influenced both chest redness and fAMs. Chest redness is higher with higher cumulative rainfall (mm) from the past 90 days and higher maximum daily temperatures (°C) both across males and within leaders (**Figs. 12, 15**). Although our first investigation into the chest patch did not detect an effect of weather variables on chest redness (Bergman et al., 2009), a later study found that males exhibited redder chests after displaying in the wet season compared to dry season months (Benítez, 2016). This analysis encompasses the longest period to date and is likely the best representation of the impact of weather variables on chest redness. Cumulative rainfall from the past 90 days is tightly linked to the availability of green grass in the Simien Mountains which may indicate all males are able to produce redder chests when their energy levels are higher (Jarvey et al., 2018). Males may have redder chests on days with higher maximum temperatures because they are less likely to be under thermoregulatory stress on these days, as we know redder chests are hotter (**Chapter 2: Fig. 7**) and may result in heat loss through this area of highly vascularized exposed skin. Further, fAMs are higher with higher cumulative rainfall from the past 90 days both within and across males (**Figs. 13, 16)** which aligns with past research in geladas and baboons (Gesquiere et al., 2011; Pappano & Beehner, 2014).

Across males of all statuses, we found that leader males had redder chests and higher fAMs compared to former leaders, but not compared to bachelors (**Figs. 12, 13).** For chest redness, this partially aligns with previous research where we found leaders had the reddest chests compared to all other status categories (Bergman et al., 2009). The bachelor status category was composed of mostly early prime-aged males (9.1 +/- 1.4 years; Beehner et al., 2009; Bergman et al., 2009) but can include young males who recently joined the group or mid prime-aged males close to overthrowing a leader (Pappano, 2013). The heterogenous composition of bachelor units likely influenced the difference in results where this analysis contains more mid prime-aged males who are already antagonizing leaders than the previous study. For fAMs, our result aligns with previous findings as bachelors had higher fAMs compared to leaders for part of the year (Pappano & Beehner, 2014). Further, we found that across males, younger males had lower fAMs, which is consistent with previous research in geladas and is expected as young adult males transition to prime-age (Sen et al., 2022).

When investigating the relationship between chest redness and fAMs, we found a trend towards a relationship between redder chests and higher fAMs both across males and across leaders (**Fig. 14, 17**). This tentatively suggests that circulating testosterone levels contribute to redder chests as we predicted. However, several limitations must be taken into consideration when interpreting this result. We matched chest redness photos with fecal samples collected within 60 days of one another to optimize our sample size from a long-term data set. However, it would be ideal to target data collection to minimize the time between chest photo and testosterone sample. Although blood samples would allow the most accurate point samples of testosterone, anesthetizing animals is invasive and thus only conducted annually in this population on a small subset of individuals. Urinary testosterone, which has been validated for use in other primates (Möhle et al., 2002), collected on the day of the chest photo would allow for a more direct assessment of the relationship between testosterone and redness. Further, testosterone may only indirectly influence chest redness through the aromatization to estrogen in the surface skin similar to facial redness in rhesus macaques (Rhodes et al., 1997). If given permission to conduct an experiment on a captive population of geladas (e.g., Bronx Zoo or San Diego Zoo geladas), comparing chest redness in adult male geladas before and after an injection of fadrozole (an aromatase inhibitor that prevents testosterone from converting to estradiol), would help determine whether estradiol also plays an important role in regulating chest redness.

We investigated a proxy of reproductive success, tenure length, in association with chest redness and fAMs across leader geladas. We found that leaders with redder chests trended towards a longer tenure length (**Fig. 15)**, and leaders with lower fAMs trended towards a shorter tenure length (**Fig. 16)**. These results align with our predictions that a redder chest may increase tenure length by warding off would-be rival males, and thus indirectly contributes to reproductive success by allowing leaders a longer period to increase their total number of offspring sired during their reproductive tenure. Contrary to predictions, larger group sizes, which provide more reproductive opportunity as there are more adult females in the unit, did not predict higher chest redness or fAMs. However, the number of cycling females may be more important than total group size and will be included in future analyses.

The weak relationship between chest redness and tenure length could be a result of leaders employing “multimodal signaling”, or signals made up of several components occurring in several sensory modalities (Candolin, 2003), as males also display with the loud call, a vocalization display which is often accompanied by running, throwing rocks, climbing trees, or shaking branches (Benítez et al., 2016). Bachelors may evaluate the chest redness along with the loud call as multimodal signals sometimes indicate different male qualities (e.g., chest patch signals current condition while the loud call signals genetic quality) or indicate condition over multiple time scales (e.g., chest patch signals short-term condition while loud call represents long-term growth or juvenile development; Møller et al., 1998; Moller & Pomiankowski, 1993; Scheuber et al., 2003). Perhaps bachelors weigh both signals when deciding which leader to target for overthrow, muddying the relationship between chest redness and tenure length.

Within leaders, we found that males with shorter tenures (8 months-2 years) had redder chests in their first 4 months compared to their last 4 months of tenure whereas males with longer tenures (4-6 years) instead had redder chests in the last 4 months of their tenure (**Fig. 18**). Males with very red chests coming into their leader position may “fizzle out” quickly and employ a “live-fast, die-young” strategy where they compress their reproductive success into a short period of time (Robinson et al., 2006). However, the rise in redness towards the end of tenure for males with longer tenures could be a result of sampling being skewed to leaders responding to challenges from bachelors as we know males are redder after a vocal display compared to their own baseline (DeLacey et al., 2022). These trends are preliminary due to our small sample sizes. More consistent sampling throughout each male’s tenure will help gain a more accurate view of how chest redness changes over time. Further, although fine-grained data is not yet available, it would be interesting it target future research towards determining whether having a redder chest relative to the other leaders in the band is more important in extending tenure rather than maintaining a certain threshold level of chest redness.

Here, we provide the first initial evidence that high levels of fAMs may serve to increase chest redness in geladas alongside increased blood flow in this patch of highly vascularized exposed skin. We found a trend between redder chests and longer tenure lengths in leaders, providing the first link between chest redness and a reproductive fitness proxy. Going forward, we hope to target sampling to better understand how strongly fAMs predict chest redness and how chest redness varies over tenure by sampling on a smaller timescale.

Chapter 5 : Conclusion

5.1 Overview

In this dissertation, I examine the causes and consequences of the red chest patch, a putatively sexually selected signal, in male geladas through a series of comprehensive studies incorporating multiple lines of inquiry. The framework that I have set up with this research can be expanded to study signaling in other wild primates. I first compared multiple objective color measurement methods to determine the consistency and accuracy of each method in measuring gelada chest skin redness. These results led us to use the Adobe method for all subsequent color measurement analyses. I then measured four potential mechanisms that could mediate variation in chest redness – behavioral, thermoregulatory, gene expression pathway, and hormonal mechanisms. I presented photogrammetric, demographic, behavioral, climatic, transcriptomic, and hormonal data to support these findings. I found that redder chests were associated with increased physical activity and higher chest skin surface temperature, and that genes more highly expressed in males were associated with blood vessel development and maintenance. Together, these results provide 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. I then used a combination of paired chest photos and fecal samples to determine whether circulating androgen levels in males influences chest redness and found a weak positive relationship between chest redness and fAMs. Finally, I investigated whether chest redness was associated with tenure length, a proxy of reproductive success, and found leaders with redder chests trend towards longer tenures. Below, I discuss my main conclusions and future directions for this research.

5.1.1 What are the causal mechanisms associated with redder chest patches?

In Chapters 2 and 3, we demonstrated that chest redness in male geladas is linked to blood flow in the skin. Within leader males, chest redness increases after vigorous physical activity (male displays including a ritualized loud call followed by running, throwing rocks, climbing trees, or shaking branches) compared to their own baseline. Redder chests have a higher surface temperature across all male social statuses and experimentally raising the temperature of the chest through external heat application temporarily increased redness in both anesthetized males and females. Together, these physical correlates suggest that chest redness in geladas is mediated by increased blood flow with a concomitant increase in surface temperature. A similar mechanism is also present in humans where skin blood flow increases to dissipate heat generated by muscle contractions during exercise in humans (Kenney & Johnson, 1992) and rhesus macaques where skin blood flow increases sexual skin redness (Rhodes et al., 1997). The blood flow mechanism for increasing redness in this exposed patch of highly vascularized skin may indicate the chest patch as a condition-dependent signal as only males with a greater metabolic capacity and the ability to withstand a higher rate of heat loss in this cold, high-altitude environment are able to produce the signal.

Interestingly, while both males and females demonstrated a substantial overlap in chest redness under both natural and anesthetized conditions, males exhibited a wider within-individual range in chest redness under natural conditions. Having established sex differences in chest redness under natural conditions, we next examined underlying gene expression levels to identify specific molecular mechanisms leading to these differences. We measured gene expression levels in chest skin biopsies collected from a subset of male and female geladas using well established RNA sequencing methods. We detected sex differences in gene expression where higher expression in males was associated with angiogenesis, blood pressure, and blood vessel maintenance providing molecular evidence (in addition to the above physical correlates) that blood flow and vascularization may underlie the greater male range in chest redness. This larger network of blood vessels may allow males to rapidly increase chest redness after displays to ward off challenging bachelor males. Among males, leaders spend less time resting, more time engaging in low-intensity aggression, and produce more loud calls per vocal display bout compared to bachelors (Benitez, 2016; Perlman, 2021). This frequent activity may build up vascular networks which allows an instance of increased blood flow to prompt a larger increase in chest redness.

Genes encoding proteins that interact with androgen and estrogen were not more highly expressed in males. However, this could indicate that both males and females use the same androgen and estrogen regulation pathways in the chest skin which prevented us from detecting a sex difference in gene expression. Circulating hormones such as testosterone play a role in sexual skin redness in other primates (Dixson et al., 2005; Rhodes et al., 1997; Setchell & Dixson, 2001) and high doses of circulating testosterone are known to dilate vascular networks and increase blood flow (Molinari et al., 2002; Webb et al., 1999) which led us to further examine whether androgens are associated with chest redness in male geladas. In Chapter 4, using a large data set of redness measurements from digital photographs and fAMs measured from non-invasively collected samples, we detected a non-significant trend towards a positive correlation between chest redness and fAMs in males of all status categories and across leaders. These results tentatively suggest circulating androgens may work in tandem with blood flow mechanisms to increase chest redness. The limitations associated with this result are discussed in section 5.2 below.

5.1.2 What are the functional consequences associated with redder chest patches?

To be considered a sexually selected trait, variation in the chest patch must result in differential fitness outcomes where a redder chest results in greater reproductive success (Snowdon, 2004). In Chapter 4, we used tenure length, or the length of time a male remains leader of a reproductive unit, as a proxy of reproductive success and found that across leaders, males with redder chests trend towards longer tenures, but group size did not influence chest redness. These results suggest that a redder chest can increase tenure length by warding off bachelors, and thus indirectly contributes to reproductive success by allowing leaders a longer time to sire more offspring. Interestingly, our analysis within leaders also revealed a pattern that was contrary to what we expected – leaders with longer tenures had redder chests at the end of their tenure compared to the beginning. This could be attributable to the nature of challenge itself where leaders rise in redness when being challenged by bachelors towards the end of their tenure before their ultimate overthrow. Further, it could be related to the quality of male, where higher quality males with longer tenures are still able to rise in redness in response to challenges even at the end of their tenure. Although the small sample sizes currently make it difficult to draw conclusions about longitudinal chest redness trends within leaders in this population, unraveling how chest redness impacts male reproductive success will be an exciting next step for this research.

5.2 Limitations of study and future directions

When a young graduate student embarks on their mission to collect their ideal dissertation data, most soon learn that the "ideal" dissertation data collection regime never unfolds as planned. I am certainly not an exception here. Like many of my peers, my years as a graduate student were impacted by the COVID-19 pandemic. After spending eight months working from home alongside my five roommates during the height of the pandemic, my lab mate Sofia Carrera and I traveled to Ethiopia in October 2020 with an abundance of COVID-19 precautions (including quarantine periods in Addis Ababa, several unpleasant nasal swabs, and daily at-home testing with a kit provided by Noah Snyder-Mackler). On November 3, 2020, the day of the U.S. Presidential election between incumbent President Donald Trump and Joe Biden, a civil war began in Ethiopia in the Tigray region, directly north of our field site. Needless to say, Sofia and I quickly evacuated the field site to Addis and soon after returned to the United States.

Upon my return home, I was left without my "ideal" fine-grained dissertation data collected from a targeted subset of leader males. However, I was fortunate to work with an established project that had a wealth of long-term data to use to continue answering my research questions. Although demographic, behavioral, and hormonal data has been collected (mostly) continuously at the Sankaber field site since 2006, chest photos were collected less consistently between 2006 and 2010. The major hurdle in my data interpretation comes back to using coarse-grained chest photo data. In Chapter 4, when examining the relationship between chest redness and fAMs, we matched samples collected within 60 days from the same individual. We did detect a trend towards a positive correlation between chest redness and fAMs, but closing the gap in time between sample collection would provide more insight into the relationship between chest redness and fAMs. Further, as with any long-lived study subject, it is difficult to accrue longitudinal data to address questions about lifetime reproductive success. We did detect a trend towards a positive correlation between chest redness and tenure length from n=20 leaders but adding more leader males to this data set will help determine whether the relationship holds.

As gelada chest patch research continues, I recommend combining long-term data with targeted short-term sampling to examine how chest redness varies across tenure and to determine whether hormones also influence chest redness. Specifically, I propose (1) for the SMGRP to continuously collect monthly chest photos of all small band leader males at rest and within 10 minutes of a loud call to be added to the long term database, and (2) a short-term project where two sets, one at rest and one within 10 minutes of a loud call, of a paired chest photo and a fecal sample are collected from small band leader males within 24 hours on a weekly or biweekly basis over 12 months. The long-term data would increase both the sample size and data consistency to assess how chest redness varies within leaders across tenure. When takeovers arise, it would allow comparison of leader male redness on the band-level to see whether bachelors target paler leaders. The targeted short-term data would allow us to further probe the relationship between chest redness and fAMs to see if the positive correlation holds when analyzing data on a smaller time scale.

Our findings in Chapter 2 and 3 provide substantial evidence that chest redness in geladas is driven by increased blood flow near the surface of the skin, specifically through increased blood vessel branching in the chest skin. Increasing blood flow to this patch of highly vascularized skin may expose males to heat loss as geladas frequently experience below-freezing temperatures (Tinsley Johnson et al., 2018). In another cercopithecoid that must endure cold temperatures, the golden snub-nosed monkey (*Rhinopithecus roxella*), a decrease of 5-10 degrees in winter months is associated with an energetic deficit, which demonstrates even a small decrease in temperature can have a large impact on primate metabolic demands (Hou et al., 2020). The ability of gelada males to withstand a higher rate of heat loss may indicate a greater metabolic capacity, creating a signal production consequence for this putatively condition-dependent signal. However, we must first assess whether heat loss impacts energy metabolism in geladas before drawing these conclusions. Future research could measure triiodothyronine (T3), a hormone involved in thermoregulation and energy balance (Urlacher et al., 2022; van den Berg et al., 2020) already validated in geladas (Perlman, unpublished data), levels from fecal samples from the targeted short-term data set to determine whether males with redder chests experience increased metabolic demands. If possible, it would be useful to also measure fecal glucocorticoids (fGCs) alongside T3 as it is helpful to combine two endocrine axes when examining energetic challenges and the metabolic strategies used to cope with them (Touitou et al., 2021), and there is evidence that geladas show elevated GCs during periods of cold stress (Beehner & McCann, 2008b; Tinsley Johnson et al., 2018).

In addition to chest redness, future analyses could assess variation in the color contrast between the chest patch and the surrounding fur as we have observed males vary from brown to silver-gray fur surrounding the chest patch. Although time did not allow for this analysis during my time as a graduate student, recent developments in the ImageJ micaToolbox allows objective contrast measurement using this free and open-source software (van den Berg et al., 2020). Using our newly organized long-term photo database (organized by a team of undergraduate students), we could select a subset of well-sampled males to measure the contrast between chest patch and redness to see if this aspect of coloration is also important to the observer. I predict that the silver-gray fur would create a more striking contrast with a red chest patch, perhaps emphasizing the chest patch signal to rival bachelor males.

5.3 Concluding remarks

In this dissertation, I focused on the causes and consequences of a putatively sexually selected signal in male geladas. This thesis includes the first study of mechanisms causing the variation in chest redness observed across males (Chapter 2-3) and of a reproductive success metric associated with the signal (Chapter 4). All chapters advance our understanding of a unique sexually selected signal of male competitive ability in a non-human primate. Future research will focus on the potential signal production costs associated with producing the signal and fluctuations over tenure within leaders. Understanding the causes and consequences of the male gelada chest patch will improve our understanding of how signals can evolve in a complex multilevel society to communicate condition to conspecifics.

Appendix A

Supplementary Material Accompanying Chapter 2

Supplementary Table 1. The Red values for the ColorChecker chart and Nix for each of the 24 ColorChecker chart squares.

Square number	Square name	Chart Red	Nix Red
1	dark skin	115	115
2	light skin	194	193
3	blue sky	98	91
4	foliage	87	89
5	blue flower	133	124
6	bluish green	103	90
7	orange	214	221
8	purplish blue	80	67.33
9	moderate red	193	193
10	purple	94	86
11	yellow green	157	153

12	orange yellow	224	226.67
13	blue	56	46.33
14	green	70	60
15	red	175	173
16	yellow	231	233
17	magenta	187	186
18	cyan	8	0
19	white	243	239
20	neutral 8	200	196
21	neutral 6.5	160	157
22	neutral 5	122	118
23	neutral 3.5	85	83
24	black	52	50

Supplementary Table 2. The Green values for the ColorChecker chart and Nix for each of the 24 ColorChecker chart squares.

Square number	Square name	Chart Green	Nix Green
1	dark skin	82	84

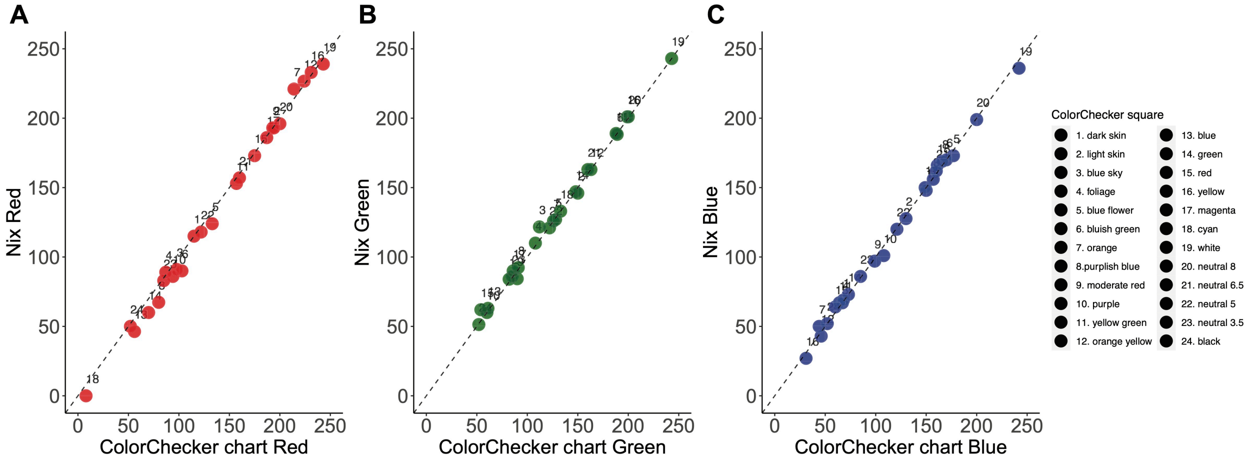
2	light skin	150	146
3	blue sky	112	121.67
4	foliage	108	110
5	blue flower	128	127
6	bluish green	189	188.33
7	orange	126	126
8	purplish blue	91	92.33
9	moderate red	90	84.33
10	purple	60	60
11	yellow green	188	189
12	orange yellow	163	163
13	blue	61	63
14	green	148	147
15	red	54	62
16	yellow	199	201
17	magenta	86	90.00
18	cyan	133	133

19	white	243	243
20	neutral 8	200	201
21	neutral 6.5	160	163
22	neutral 5	122	121
23	neutral 3.5	85	86
24	black	52	51.33

Supplementary Table 3. The Blue values for the ColorChecker chart and Nix for each of the ColorChecker chart squares.

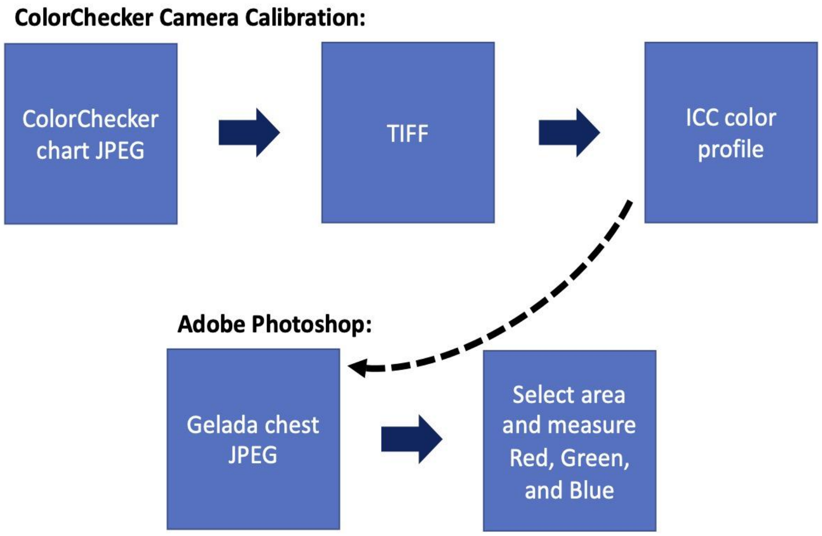
Square number	Square name	Chart Blue	Nix Blue
1	dark skin	68	69
2	light skin	130	127.67
3	blue sky	157	156
4	foliage	67	67
5	blue flower	177	173
6	bluish green	170	170
7	orange	44	50
8	purplish blue	166	169

9	moderate red	99	97
10	purple	108	101
11	yellow green	64	67
12	orange yellow	46	43
13	blue	150	148
14	green	73	73
15	red	60	64
16	yellow	31	27
17	magenta	149	150
18	cyan	161	166
19	white	242	236
20	neutral 8	200	199
21	neutral 6.5	160	162
22	neutral 5	121	120
23	neutral 3.5	85	86
24	black	52	52

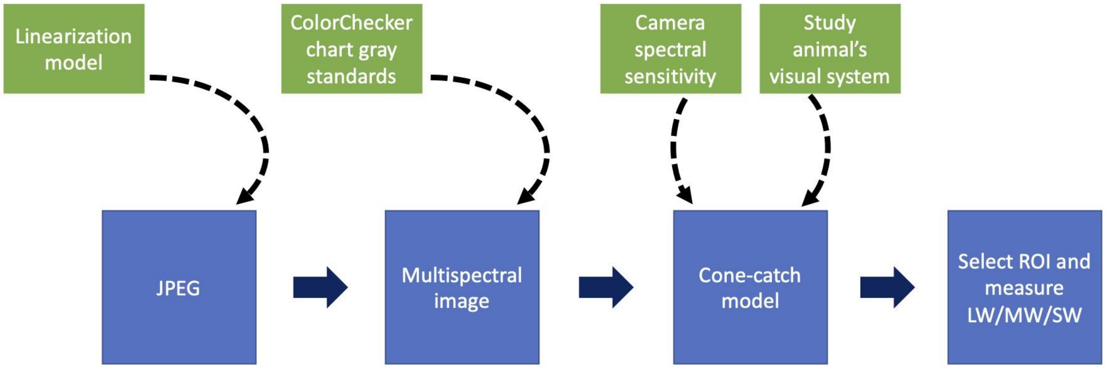


Supplementary Figure 1. The relationship between Nix and the ColorChecker chart for A) Red values, B) Green values, and C) Blue values of the 24 ColorChecker chart squares.

Supplementary Figure 2. Measuring chest patch skin surface temperature. One of the authors (TJB) using a Raytek MX6 PhotoTemp Infrared Thermometer ("temperature gun") to measure the surface temperature of an adult male's chest patch. All measurements were taken from 1-2 meters away.



Supplementary Figure 3. Adobe measurement process



Supplementary Figure 4. ImageJ micaToolbox measurement process.

Method precision linear regression models

To ensure consistency in the Adobe and ImageJ micaToolbox methods across lighting conditions, we measured redness and luminance in the "light skin" and "moderate red" squares of the ColorChecker chart across four light conditions: (1) backlit, (2) cloud, (3) shade, and (4) full sun (N=10 photo sets per condition, 40 photos total; see Methods). We ran four linear regression models of redness by light condition (light skin and moderate red square for Adobe and ImageJ micaToolbox) and two linear regression models of luminance by light condition (light skin and moderate red square for ImageJ micaToolbox). The results are summarized in the "Method precision" subheading of the Results, but we report the full results below.

Supplementary Table 4. Results of linear regression model for redness across light condition for Adobe Photoshop.

Light skin square

Variable	Predictor	Estimate	SE	t	*p* value
Redness: Red to Green Ratio (Red/Green)	**Intercept (Cloud)**	**1.199**	**0.005**	**250.557**	**<0.001**
	Backlit	**0.052**	**0.007**	**7.620**	**<0.001**
	Shade	0.007	0.007	1.023	0.314
	Sun	0.004	0.007	0.579	0.567

Moderate red square

Variable	Predictor	Estimate	SE	t	*p* value
Redness: Red to Green Ratio (Red/Green)	Intercept (Cloud)	2.034	0.023	86.985	<0.001
	Backlit	-0.016	0.033	-0.483	0.633
	Shade	-0.027	0.032	-0.825	0.416
	Sun	-0.044	0.032	-1.368	0.182

Supplementary Table 5. Results of linear regression model for redness across light condition for ImageJ.

Light skin square

Variable	Predictor	Estimate	SE	t	*p* value
Redness: R-G Opponency (LW-MW)/(LW+MW)	**Intercept (Cloud)**	**0.098**	**0.001**	**94.164**	**<0.001**
	Backlit	**-0.008**	**0.001**	**-5.767**	**<0.001**
	Shade	-0.003	0.001	-1.769	0.087
	Sun	-0.002	0.001	-1.351	0.187

Moderate red square

Variable	Predictor	Estimate	SE	t	*p* value
Redness: R-G Opponency (LW-MW)/(LW+MW)	**Intercept (Cloud)**	**0.177**	**0.003**	**63.153**	**<0.001**
	Backlit	0.001	0.004	0.243	0.810
	Shade	**-0.009**	**0.004**	**-2.285**	**0.030**
	Sun	**-0.010**	**0.004**	**-2.487**	**0.019**

Supplementary Table 6. Results of linear regression model for luminance across light condition for ImageJ.

Light skin square

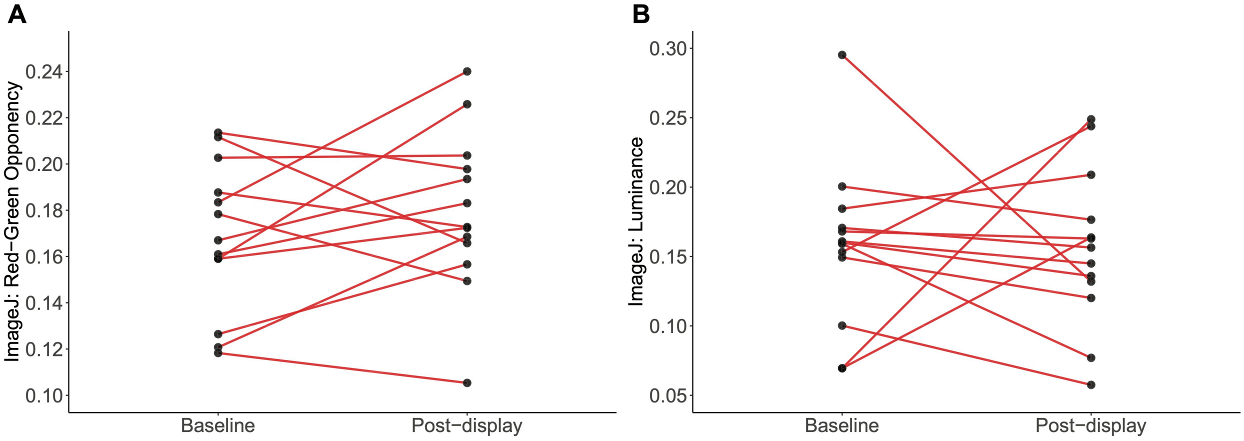
Variable	Predictor	Estimate	SE	t	*p* value
Luminance: (LW+MW)/2	**Intercept (Cloud)**	**0.295**	**0.004**	**78.195**	**<0.001**
	Backlit	0.008	0.005	1.530	0.137
	Shade	-0.009	0.005	-1.654	0.108
	Sun	0.001	0.005	0.146	0.885

Moderate red square

Variable	Predictor	Estimate	SE	t	*p* value
Luminance: (LW+MW)/2	**Intercept (Cloud)**	**0.140**	**0.004**	**38.391**	**<0.001**
	Backlit	0.007	0.005	1.276	0.212
	Shade	0.004	0.005	0.790	0.436
	Sun	0.004	0.005	0.712	0.482

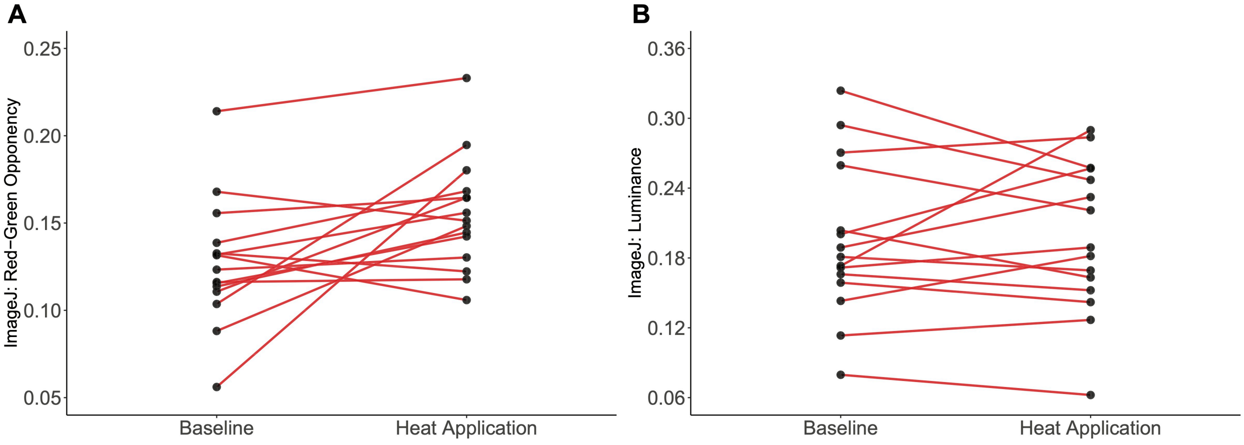
ImageJ micaToolbox results

Although our method validation suggested Adobe Photoshop best suited chest redness measurement in field conditions, we present ImageJ micaToolbox redness (Red-Green Opponency) and luminance results below (these results are equivalent to **Figs. 5-6** in the main text but for ImageJ micaToolbox instead of Adobe Photoshop). The poor performance of ImageJ micaToolbox could be attributed to the method only using two gray standards from the ColorChecker chart rather than all 24 squares or the use of JPEGs as the software has only recently been updated for use with JPEG images and functions best with RAW file formats. Contrary to results demonstrated by Adobe, the ImageJ results did not demonstrate redder chest patches for males immediately after a display (paired t-test: t=1.18, df=12, *p*=0.26; **Fig. S5A**) or lower luminance chest patches (paired t-test: t=-0.05, df=12, *p*=0.97; **Fig. S5B**) for the same males when compared to their own "baseline" photos ("**Activity and redness")**.



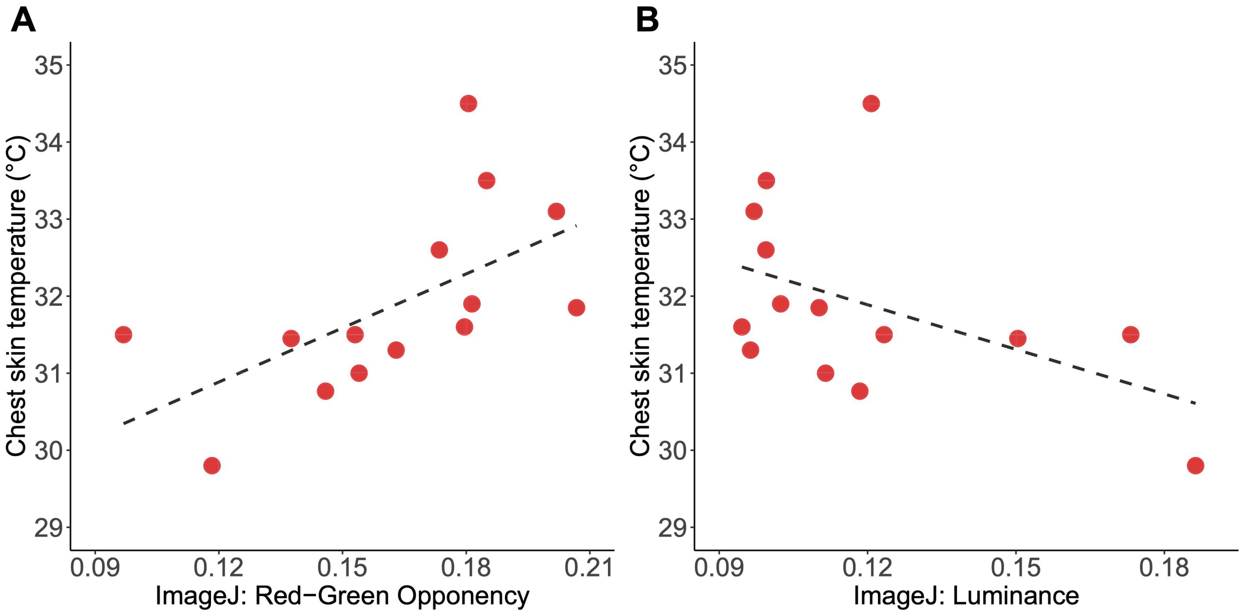
Supplementary Figure 5. Within individual leader males following a display ("post-display") as compared to "baseline" conditions measured for: **A**) Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox.

ImageJ results aligned with the Adobe result that chest redness (Red-Green Opponency) increased after direct application of a heat pack to the chest for 1 min compared to baseline conditions (paired t-test: t=2.71, df=14, *p*=0.02; **"Heat manipulation and redness"; Fig. S6A**). However, for ImageJ results, chest luminance did not change between baseline and heat application ((paired t-test: t=0.26, df=14, *p*=0.8; **Fig. S6B**).

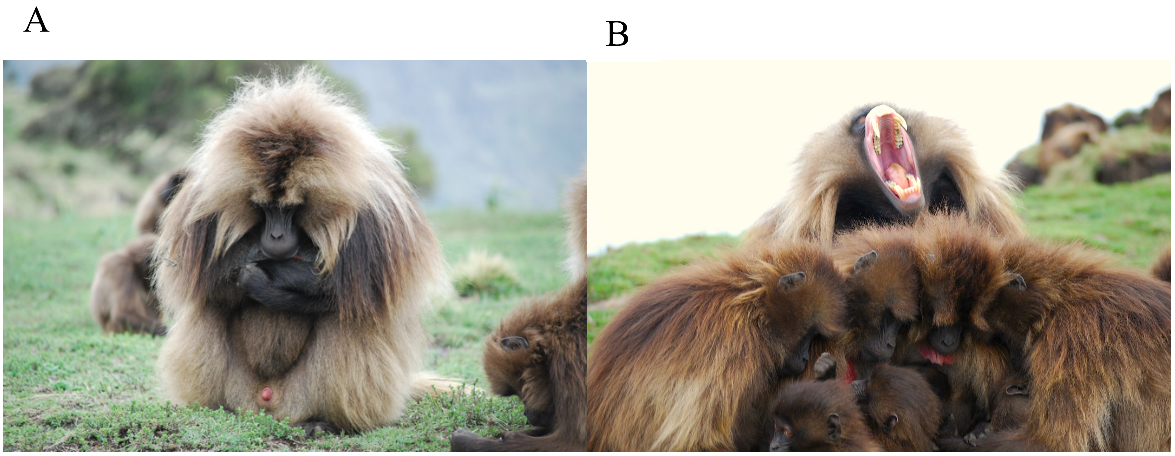


Supplementary Figure 6. Within individual adult males, direct application of a heat pack to the chest as compared to measurements taken immediately prior for: **A)** Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox.

ImageJ results once again aligned with the Adobe result that chest skin surface temperature increased with chest redness (Red-Green Opponency) (b=23.39, SE=8.94, t=2.62, *p*=0.03; "**Redness and skin temperature"; Fig. S7A**) and trended towards decreasing with luminance in adult males (b=-19.3, SE=10.4, t=-1.86, *p*=0.09; **Fig. S7B**).



Supplementary Figure 7. Chest patch surface temperature (°C) measured with a Raytek MX6 PhotoTemp Infrared Thermometer compated with **A)** Chest redness (Red-Green Opponency (LW-MW)/(LW+MW)) and **B)** Chest luminance ((LW+MW)/2) measured in ImageJ micaToolbox.

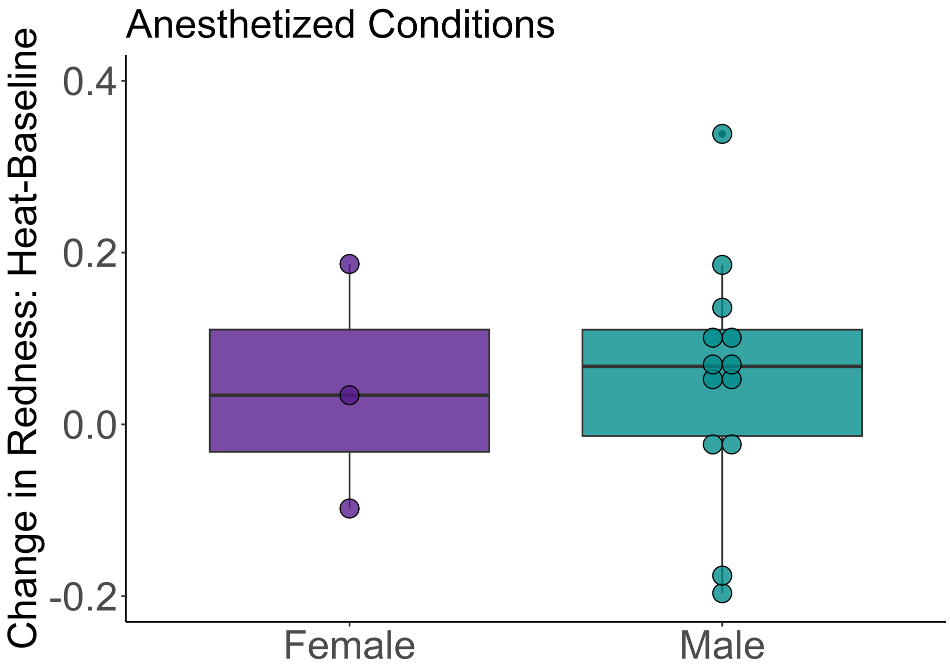


Supplementary Figure 8. **A)** An adult male gelada sitting with his head lowered over his chest patch on a cold morning. **B)** A reproductive unit of geladas huddling together with their chest patches turned inwards (photographs by Julie Jarvey).

Appendix B

Supplementary Material Accompanying Chapter 3

In addition to the 38 photos taken at the start of anesthetization (hereafter, "baseline"; **Fig. 8**), we took 16 photos from a subset of adult males (*n*=13) and females (*n*=3) after a heat pack was applied to one side of the chest for 1 minute (hereafter "heat application"). Although we were unable to make statistically meaningful comparisons due to a smaller sample size of photos from anesthetized adult females (*n*=3), females seem to follow a similar pattern to males for change in redness between baseline and application of a heat pack directly to the chest skin (**Fig. S9**).

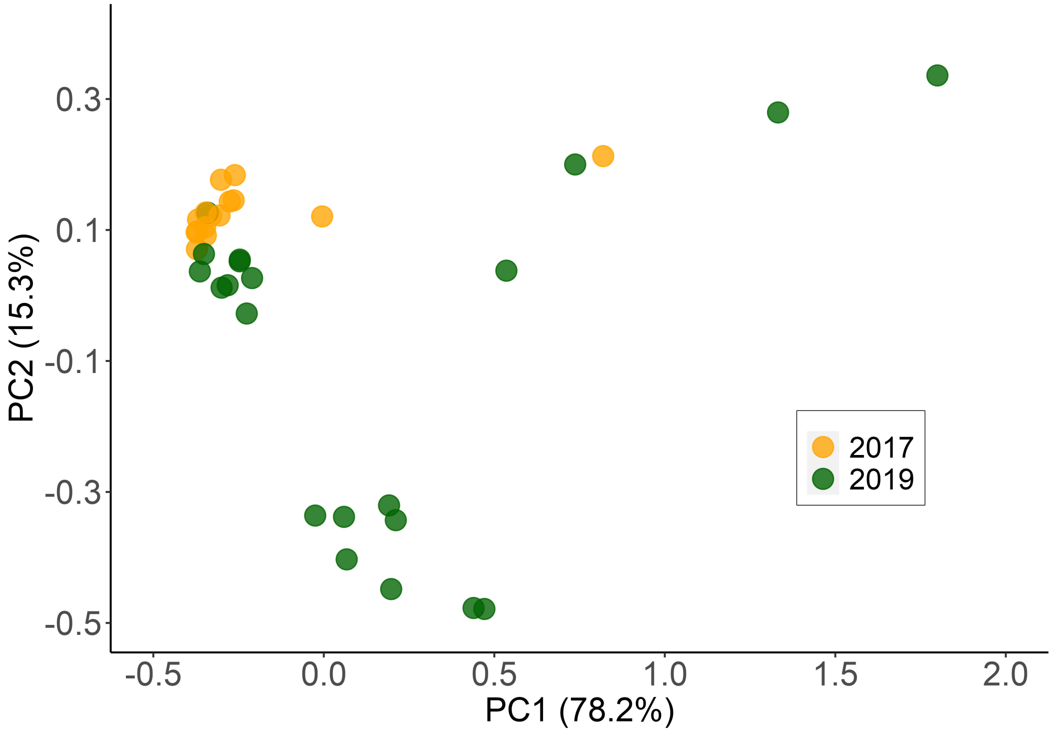


Supplementary Figure 9. Adult males and females show a similar pattern in change in redness between baseline and application of a heat pack directly to the skin.

Sample collection year (**Table S7**) is associated with the first and second principal components of gene expression which explained 78.2% and 15.5% of the variance, respectively (PC1: year β=0.39, *P*=0.02; PC2: year β=-0.22, *P*=0.003, **Fig. S10**). This variance could be a result of storage time, a batch effect of the RNAlater™ in which samples were stored, or temperature differences during shipment to the United States. This finding led us to include sample collection year in subsequent linear models of sex on the first and second principal component of gene expression.

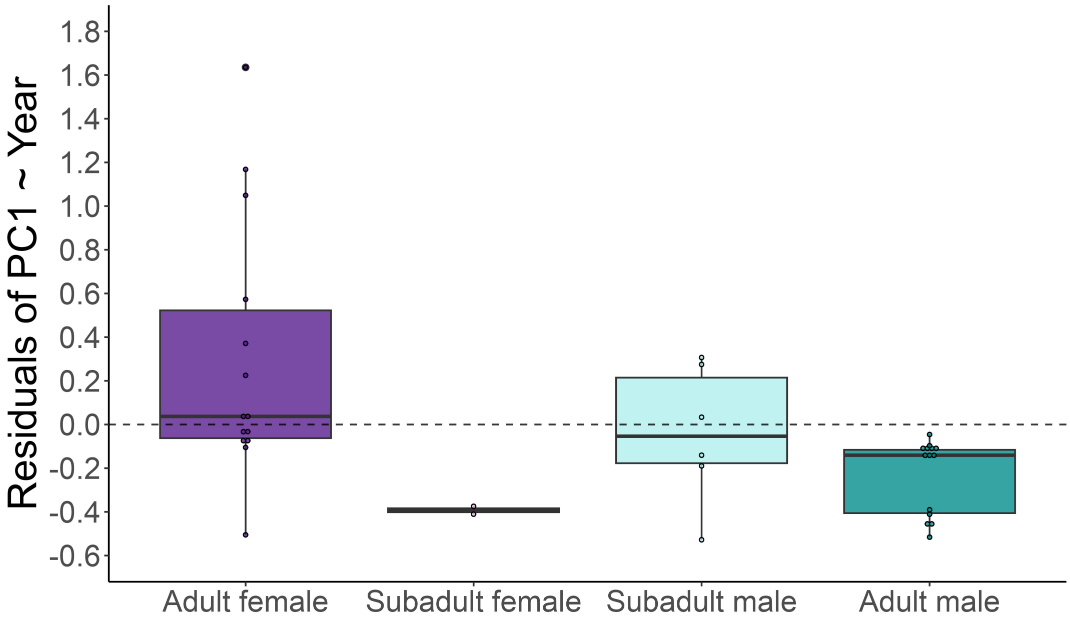
Supplementary Table 7. Sample collection by year, sex, and age category for the 36 samples used in analyses.

Year	Subadult female	Adult female	Subadult male	Adult male	Total
2017	2	6	1	8	17
2019	0	8	5	6	19

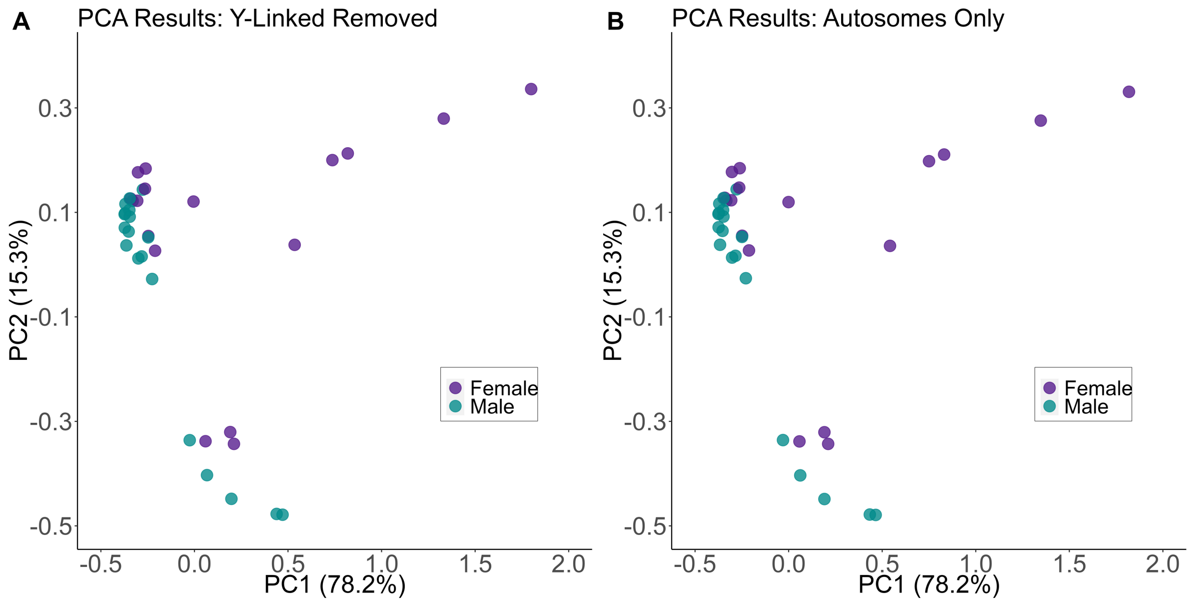


Supplementary Figure 10. Sample collection year was significantly associated with the first and second principal component of gene expression.

When controlling for sample collection year, males and females differed along the first principal component of gene expression, which explained 78.2% of the overall variance in gene expression (**Fig. S11**). Year explained 12.2% of the variance and sex explained 13.1% of the variance in the first principal component of gene expression. We removed Y-linked genes from all analyses as they would be biased towards males. We did include X-linked genes in downstream analyses, but here we tested whether results of the sex difference in gene expression are influenced by X-linked genes. We found that the results of the principal component analysis did not change when we removed genes located on the X chromosome (**Fig. S12**).

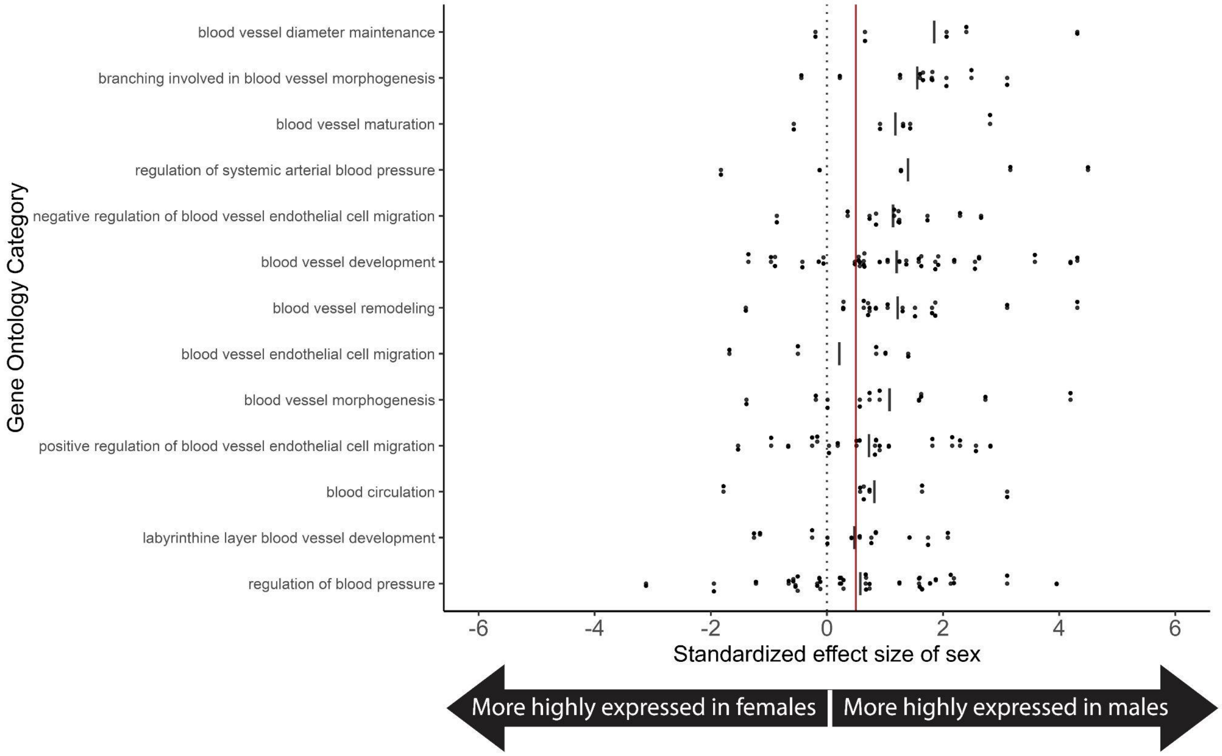


Supplementary Figure 11. The first principal component of gene expression by age category while controlling for year of sample collection.



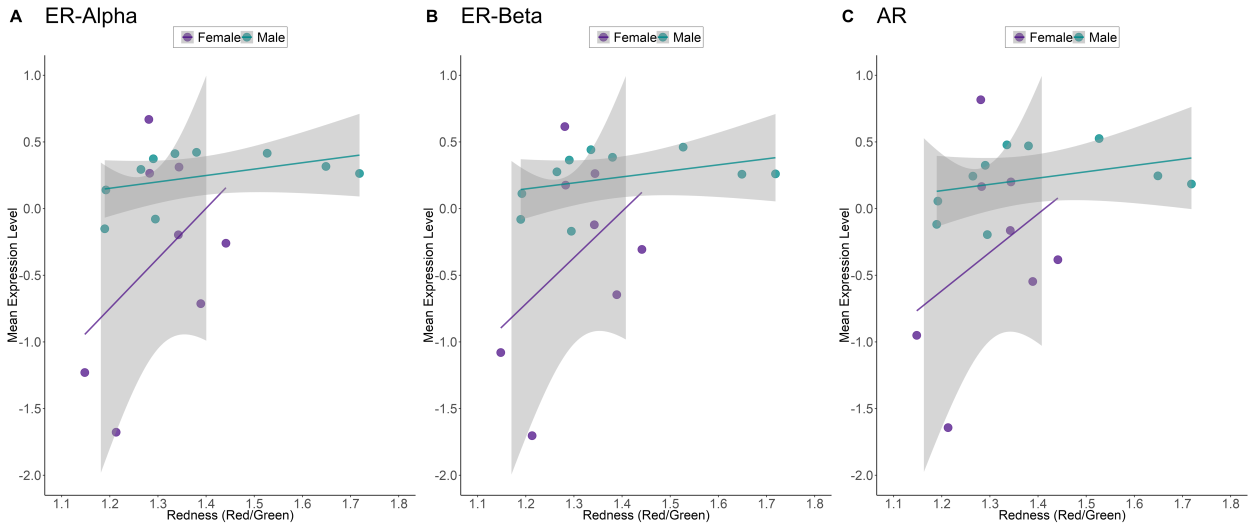
Supplementary Figure 12. The results of the principal component analysis did not change between **A)** when we removed genes located on the Y chromosome and **B)** when we analyzed only those found on autosomes (both X-linked and Y-linked genes removed).

To test our prediction that males would exhibit increased expression of genes associated with blood flow, we searched for relevant terms (including the phrase "blood") and removed those not involved in blood pressure regulation and blood vessel maintenance (i.e., "establishment of blood-retinal barrier"). We compared the standardized effect of sex for these 149 blood pressure regulation and blood vessel maintenance genes to the standardized effect of sex for all other 10,066 detectably expressed genes with a Kolmogorov-Smirnov's test. Genes more highly expressed in males were enriched for biological processes associated with blood pressure regulation and blood vessel maintenance (K-S Test: D=0.19, P=2.59x10^{-5}). Enriched genes in the blood pressure regulation group included functions such as regulation of systemic arterial blood pressure while genes in the blood vessel maintenance group included regulation of blood vessel diameter, branching, remodeling, and development (**Fig. S13**).



Supplementary Figure 13. Genes more highly expressed in males were enriched for biological processes associated with blood pressure regulation and blood vessel maintenance. Beeswarm dots represent individual genes within the top 13 most represented subcategories of the blood pressure and blood vessel maintenance gene ontology category. 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 blood pressure regulation of blood vessel maintenance associated categories.

Although we did not find evidence for increased expression of genes associated with estrogen or androgen regulation in males, we further investigated whether the average standardized expression of these genes correlated with chest redness at baseline while under anesthesia within females or within males. For the subset of individuals for which we had matched skin biopsies and chest redness data at baseline while under anesthesia (n=10 males, n=8 females), we Z-transformed expression values for each set of genes associated with PPI networks for ERα, ERβ, and AR across these 18 individuals and averaged Z-transformed expression levels of these genes per individual to obtain a composite ERα, ERβ, and AR gene expression score for each individual. We then ran three linear regression models within females and three linear regression models within males with expression score as the outcome variable and chest redness as the predictor variable. We did not find a correlation between average standardized sex-bias gene expression level of ERα, ERβ, or AR and chest redness within females (ERα: β=3.75, P=0.28; ERβ: β=3.47, P=0.29; AR: β=2.89, P=0.38) or within males (ERα: β=0.48, P=0.21; ERβ: β=0.45, P=0.26; AR: β=0.47, P=0.31; **Fig. S14**). The small sample sizes and the impact of anesthesia on chest redness may have influenced these results.



Supplementary Figure 14. Expression of genes associated with ERα, ERβ, or AR did not correlate with chest redness at baseline while under anesthesia within males or within females.

Bibliography

Akdemir, D., & Godfrey, O. U. (2015). *EMMREML: Fitting Mixed Models with Known Covariance Structures*.

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

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*.

Andersson, M. (1994). *Sexual selection*. Princeton University Press.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.*, *67*, 1–48.

Bateson, P., & Laland, K. N. (2013). Tinbergen's four questions: an appreciation and an update. *Trends in Ecology & Evolution*, *28*(12), 712–718. https://doi.org/https://doi.org/10.1016/j.tree.2013.09.013

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.

Beehner, J. C., Gesquiere, L., Seyfarth, R. M., Cheney, D. L., Alberts, S. C., & Altmann, J. (2009). Testosterone related to age and life-history stages in male baboons and geladas. *Horm. Behav.*, *56*(4), 472–480.

Beehner, J. C., & McCann, C. (2008a). Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). *Physiology and Behavior*, *95*(3), 508–514. https://doi.org/10.1016/j.physbeh.2008.07.022

Beehner, J. C., & McCann, C. (2008b). Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). *Physiol. Behav.*, *95*(3), 508–514.

Bell, R. B., Bradley, B. J., & Kamilar, J. M. (2021). The Evolutionary Ecology of Primate Hair Coloration: A Phylogenetic Approach. *Journal of Mammalian Evolution*, *28*(3), 911–927. https://doi.org/10.1007/s10914-021-09547-8

Benitez, M. E. (2016). *Sizing up strangers: sexual selection and vocal signals in gelada males* (J. C. Beehner, Ed.). University of Michigan.

Benítez, M. E., 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*, 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. *Proc. Biol. Sci.*, *277*(1696), 3045–3053.

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). *Biol. J. Linn. Soc. Lond.*, *94*(2), 231–240.

Bergman, T. J., & Beehner, J. C. (2022). Leveling with Tinbergen: Four levels simplified to causes and consequences. *Evolutionary Anthropology: Issues, News, and Reviews*, *31*(1), 12–19.

Bergman, T. J., Beehner, J. C., & Cheney Dorothy L and Seyfarth, R. M. (2003). Hierarchical classification by rank and kinship in baboons. *Science*, *302*(5648), 1234–1236.

Bergman, T. J., Ho, L., & Beehner, J. C. (2009). Chest color and social status in male geladas (Theropithecus gelada). *Int. J. Primatol.*, *30*(6), 791–806.

Bergman, T. J., & Sheehan, M. J. (2013). Social knowledge and signals in primates. *American Journal of Primatology*, *75*(7), 683–694. https://doi.org/10.1002/ajp.22103

Bolker, B., & Team, R. D. C. (2010). bbmle: Tools for general maximum likelihood estimation. *R Package Version 0.9*, *5*.

Bowmaker, J. K., Astell, S., Hunt, D. M., & Mollon, J. D. (1991). Photosensitive and photostable pigments in the retinae of Old World monkeys. *J. Exp. Biol.*, *156*, 1–19.

Bradbury, J., & Vehrencamp, S. (2011). *Principles of animal communication* (Second). Sinauer Associates.

Bradley, B. J., & Mundy, N. I. (2008). The primate palette: The evolution of primate coloration. *Evol. Anthropol.*, *17*(2), 97–111.

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.*, *34*(5), 525–527.

Brown, K. R., & Jurisica, I. (2005). Online predicted human interaction database. *Bioinformatics*, *21*(9), 2076–2082.

Caine, N. G., & Mundy, N. I. (2000). Demonstration of a foraging advantage for trichromatic marmosets (Callithrix geoffroyi) dependent on food colour. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1442), 439–444.

Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews*, *78*, 575–595.

Caro, T. (2005). The Adaptive Significance of Coloration in Mammals. *BioScience*, *55*(2), 125–136. https://doi.org/10.1641/0006-3568(2005)055[0125:TASOCI]2.0.CO;2

Caro, T., Brockelsby, K., Ferrari, A., Koneru, M., Ono, K., Touche, E., & Stankowich, T. (2021). The evolution of primate coloration revisited. *Behav. Ecol.*, *32*(4), 555–567.

Carvalho, L. S., Pessoa, D. M. A., Mountford, J. K., Davies, W. I. L., & Hunt, D. M. (2017). The genetic and evolutionary drives behind primate color vision. *Frontiers in Ecology and Evolution*, *5*, 34.

Changizi, M. A., Zhang, Q., & Shimojo, S. (2006). Bare skin, blood and the evolution of primate colour vision. *Biol. Lett.*, *2*(2), 217–221.

Chappell, M. A., & Hammond, K. A. (2004). Maximal aerobic performance of deer mice in combined cold and exercise challenges. *J. Comp. Physiol. B*, *174*(1), 41–48.

Cheviron, Z. A., Bachman, G. C., & Storz, J. F. (2013). Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice. *J. Exp. Biol.*, *216*(Pt 7), 1160–1166.

Cicchetti, D. v. (1994). Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol. Assess.*, *6*(4), 284–290.

Cooper, V. J., & Hosey, G. R. (2003). Sexual Dichromatism and Female Preference in Eulemur fulvus Subspecies. *International Journal of Primatology*, *24*(6), 1177–1188. https://doi.org/10.1023/B:IJOP.0000005986.21477.ad

Cuthill, I. C., Stevens, M., Sheppard, J., Maddocks, T., Párraga, C. A., & Troscianko, T. S. (2005). Disruptive coloration and background pattern matching. *Nature*, *434*(7029), 72–74. https://doi.org/10.1038/nature03312

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*(7578), 367–370.

Dale, J., Lank, D. B., & Reeve, H. K. (2001). Signaling individual identity versus quality: a model and case studies with ruffs, queleas, and house finches. *Am. Nat.*, *158*(1), 75–86.

Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. Murray.

Darwin, C. (1872a). Chapter 5: Special Expressions of Animals. In *The expression of the emotions in man and animals* (pp. 115–145). D. Appleton & Company.

Darwin, C. (1872b). Chapter 13: Self-Attention, Shame, Shyness, Modesty; Blushing. In *The expression of the emotions in man and animals* (pp. 309–346). D. Appleton & Company.

Darwin, C. (1876). Sexual Selection in Relation to Monkeys. *Nature*, *15*, 18–19.

Davies, N. B., & Halliday, T. R. (1978). Deep croaks and fighting assessment in toads Bufo bufo. *Nature*, *274*(5672), 683–685.

DeLacey, P., Bergman, T., & Beehner, J. (2019). Temperature alters chest redness in male geladas. *American Journal of Physical Anthropology*, 55.

DeLacey, P. M., Perlman, R. F., Sen, S., Schneider-Crease, I., Chiou, K. L., Lemma, A., Ayele, F., Higham, J. P., Lu Amy and Snyder-Mackler, N., Beehner, J. C., & Bergman, T. J. (2022). Assessing male gelada chest patches: color measurement and physiological mechanisms. *Mamm. Biol.*

Deschner, T., Heistermann, M., Hodges, K., & Boesch, C. (2004). Female sexual swelling size, timing of ovulation, and male behavior in wild West African chimpanzees. *Hormones and Behavior*, *46*(2), 204–215. https://doi.org/https://doi.org/10.1016/j.yhbeh.2004.03.013

Dixson, A., Dixson, B., & Anderson, M. (2005). Sexual selection and the evolution of visually conspicuous sexually dimorphic traits in male monkeys, apes, and human beings. *Annu. Rev. Sex Res.*, *16*, 1–19.

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.

Dixson, A. F. (2012). *Primate Sexuality: Comparative Studies of the Prosimians, Monkeys, Apes, and Human Beings: Vol. 2nd Edition*. Oxford University Press.

Dominy, N. J., & Lucas, P. W. (2001). Ecological importance of trichromatic vision to primates. *Nature*, *410*(6826), 363–366. https://doi.org/10.1038/35066567

Douglas, P. H., Hohmann, G., Murtagh, R., Thiessen-Bock, R., & Deschner, T. (2016). Mixed messages: wild female bonobos show high variability in the timing of ovulation in relation to sexual swelling patterns. *BMC Evolutionary Biology*, *16*(1), 140. https://doi.org/10.1186/s12862-016-0691-3

Dubuc, C., Allen, W. L., Maestripieri, D., & Higham, J. P. (2014). Is male rhesus macaque red color ornamentation attractive to females? *Behav. Ecol. Sociobiol.*, *68*(7), 1215–1224.

Dubuc, C., Brent, L. J. N., Accamando, A. K., Gerald, M. S., MacLarnon, A., Semple, S., Heistermann, M., & Engelhardt, A. (2009). Sexual skin color contains information about the timing of the fertile phase in free-ranging macaca mulatta. *International Journal of Primatology*, *30*(6), 777–789. https://doi.org/10.1007/s10764-009-9369-7

Dubuc, C., Winters, S., Allen, W. L., Brent, L. J. N., Cascio, J., Maestripieri, D., Ruiz-Lambides, A. v, Widdig, A., & Higham, J. P. (2014). Sexually selected skin colour is heritable and related to fecundity in a non-human primate. *Proc. Biol. Sci.*, *281*(1794), 20141602.

Durinck, S., Spellman, P. T., Birney, E., & Huber, W. (2009). Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat. Protoc.*, *4*(8), 1184–1191.

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.

Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc. Lond.*, *41*(4), 315–352.

Enstrom, D. A., Ketterson, E. D., & Nolan VAL, J. R. (1997). Testosterone and mate choice in the dark-eyed junco. *Anim. Behav.*, *54*(5), 1135–1146.

Exnerová, A., Landová E, Štys, P., Fuchs, R., Prokopová, M., & Cehláriková, P. (2003). Reactions of passerine birds to aposematic and non-aposematic firebugs (Pyrrhocoris apterus; Heteroptera). *Biological Journal of the Linnean Society*, *78*(4), 517–525. https://doi.org/10.1046/j.0024-4066.2002.00161.x

Fashing, P. J., Nguyen, N., Venkataraman, V. v, & Kerby, J. T. (2014). Gelada feeding ecology in an intact ecosystem at Guassa, Ethiopia: Variability over time and implications for theropith and hominin dietary evolution. *American Journal of Physical Anthropology*, *155*(1), 1–16. https://doi.org/https://doi.org/10.1002/ajpa.22559

Feder, J. A., Beehner, J. C., Baniel, A., Bergman, T. J., Snyder-Mackler, N., & Lu, A. (2022). Social drivers of maturation age in female geladas. *Behav. Ecol.*, *33*(3), 654–664.

Fedigan, L. M., Melin, A. D., Addicott, J. F., & Kawamura, S. (2014). The heterozygote superiority hypothesis for polymorphic color vision is not supported by long-term fitness data from wild neotropical monkeys. *PLoS One*, *9*(1), e84872.

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

Gamer, M., Lemon, J., Fellows, I., & Singh, P. (2019). *irr: Various coef- ficients of interrater reliability agreement* (R package version 0.84.1.).

Gerald, M. S. (2001). Primate colour predicts social status and aggressive outcome. *Anim. Behav.*, *61*(3), 559–566.

Gesquiere, L. R., Onyango, P. O., Alberts, S. C., & Altmann, J. (2011). Endocrinology of year-round reproduction in a highly seasonal habitat: Environmental variability in testosterone and glucocorticoids in baboon males. *American Journal of Physical Anthropology*, *144*(2), 169–176. https://doi.org/https://doi.org/10.1002/ajpa.21374

Gesquiere, L. R., Wango, E. O., & Alberts Susan C and Altmann, J. (2007). Mechanisms of sexual selection: sexual swellings and estrogen concentrations as fertility indicators and cues for male consort decisions in wild baboons. *Horm. Behav.*, *51*(1), 114–125.

Gestich, C. C., Caselli, C. B., & Setz, E. Z. F. (2014). Behavioural Thermoregulation in a Small Neotropical Primate. *Ethology*, *120*(4), 331–339. https://doi.org/https://doi.org/10.1111/eth.12203

Govardovskii, V. I., Fyhrquist, N., Reuter, T., & Kuzmin D G and Donner, K. (2000). In search of the visual pigment template. *Vis. Neurosci.*, *17*(4), 509–528.

Grafen, A. (1990). Biological signals as handicaps. *J. Theor. Biol.*, *144*(4), 517–546.

Greenberg, D., Snyder, K. P., Filazzola, A., Mastromonaco, G. F., & Schoof, V. A. M. (2022). Hormonal correlates of male dominance rank, age, and genital colouration in vervet monkey (Chlorocebus pygerythrus). *Gen. Comp. Endocrinol.*, *316*, 113948.

Grueter, C. C., Isler, K., & Dixson, B. J. (2015). Are badges of status adaptive in large complex primate groups? *Evol. Hum. Behav.*, *36*(5), 398–406.

Grueter, C. C., Zhu, P., Allen, W. L., Higham, J. P., Ren, B., & Li, M. (2015). Sexually selected lip colour indicates male group-holding status in the mating season in a multi-level primate society. *R Soc Open Sci*, *2*(12), 150490.

Hallgren, K. A. (2012). Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutor. Quant. Methods Psychol.*, *8*(1), 23–34.

Hanya, G., Kiyono, M., & Hayaishi, S. (2007). Behavioral thermoregulation of wild Japanese macaques: comparisons between two subpopulations. *American Journal of Primatology*, *69*(7), 802–815. https://doi.org/https://doi.org/10.1002/ajp.20397

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. *Proc. Natl. Acad. Sci. U. S. A.*, *112*(14), 4393–4398.

Hau, M., Wikelski, M., Soma, K. K., & Wingfield, J. C. (2000). Testosterone and year-round territorial aggression in a tropical bird. *Gen. Comp. Endocrinol.*, *117*(1), 20–33.

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

Hayes, J. P. (1989). Field and Maximal Metabolic Rates of Deer Mice (Peromyscus maniculatus) at Low and High Altitudes. *Physiol. Zool.*, *62*(3), 732–744.

Heinonen, I., Brothers, R. M., Kemppainen Jukka and Knuuti, J., Kalliokoski, K. K., & Crandall, C. G. (2011). Local heating, but not indirect whole body heating, increases human skeletal muscle blood flow. *J. Appl. Physiol.*, *111*(3), 818–824.

Hendershott, R., Hu, G., Groves, C., & Behie, A. (2019). Natal-to-juvenile pelage change in free-living François' (Trachypithecus francoisi) and Cat Ba langurs (T. poliocephalus). *Vietnamese Journal of Primatology*, *3*, 41–54.

Heyland, A., Hodin, J., & Reitzel, A. M. (2005). Hormone signaling in evolution and development: a non-model system approach. *Bioessays*, *27*(1), 64–75.

Higham, J. P. (2006). *The Reproductive Ecology of Female Olive Baboons (Papio Hamadryas Anubis), at Gashaka-Gumti National Park, Nigeria*.

Higham, J. P., Heistermann, M., & Maestripieri, D. (2013). The endocrinology of male rhesus macaque social and reproductive status: a test of the challenge and social stress hypotheses. *Behav. Ecol. Sociobiol.*, *67*(1), 19–30.

Higham, J. P., Heistermann, M., Saggau, C., Agil, M., Perwitasari-Farajallah, D., & Engelhardt, A. (2012). Sexual signalling in female crested macaques and the evolution of primate fertility signals. *BMC Evolutionary Biology*, *12*(1), 89. https://doi.org/10.1186/1471-2148-12-89

Higham, J. P., Hughes, K. D., Brent, L. J. N., Dubuc, C., Engelhardt, A., Heistermann Michael and Maestriperi, D., Santos, L. R., & Stevens, M. (2011). Familiarity affects the assessment of female facial signals of fertility by free-ranging male rhesus macaques. *Proc. Biol. Sci.*, *278*(1723), 3452–3458.

Higham, J. P., Pfefferle, D., Heistermann, M., Maestripieri, D., & Stevens, M. (2013). Signaling in multiple modalities in male rhesus macaques: sex skin coloration and barks in relation to androgen levels, social status, and mating behavior. *Behav. Ecol. Sociobiol.*, *67*(9), 1457–1469.

Hill, W. C. O. (1955). A note on integumental colours with special reference to the genus Mandrillus. *Säugetierkd. Mitt*, *3*, 145–151.

Höjesjö, J., Johnsson, J. I., Petersson, E., & Järvi, T. (1998). The importance of being familiar: individual recognition and social behavior in sea trout (Salmo trutta). *Behav. Ecol.*, *9*(5), 445–451.

Holzer, B., Jacot, A., & Brinkhof, M. W. G. (2003). Condition-dependent signaling affects male sexual attractiveness in field crickets, Gryllus campestris. *Behav. Ecol.*, *14*(3), 353–359.

Hou, R., Chapman, C. A., Jay, O., Guo, S., Li, B., & Raubenheimer, D. (2020). Cold and hungry: combined effects of low temperature and resource scarcity on an edge-of-range temperate primate, the golden snub-nose monkey. *Ecography*, *43*(11), 1672–1682. https://doi.org/https://doi.org/10.1111/ecog.05295

Houde, A. (2019). *Sex, color, and mate choice in guppies*. Princeton University Press.

Hunt, D. M., Dulai, K. S., Cowing, J. A., Julliot, C., Mollon, J. D., Bowmaker, J. K., Li, W.-H., & Hewett-Emmett, D. (1998). Molecular evolution of trichromacy in primates. *Vision Research*, *38*(21), 3299–3306. https://doi.org/https://doi.org/10.1016/S0042-6989(97)00443-4

Hunter, C. P. (2001). *Ecological determinants of gelada ranging patterns (Theropithecus gelada)*. University of Liverpool.

Hytten, F. (1985). Blood volume changes in normal pregnancy. *Clin. Haematol.*, *14*(3), 601–612.

Jablonski, N. G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of the National Academy of Sciences*, *107*(supplement_2), 8962–8968. https://doi.org/10.1073/pnas.0914628107

Jacobs, G. H. (2007). New World Monkeys and Color. *International Journal of Primatology*, *28*(4), 729–759. https://doi.org/10.1007/s10764-007-9168-y

Jacobs, G. H. (2008). Primate color vision: A comparative perspective. *Visual Neuroscience*, *25*(5–6), 619–633. https://doi.org/DOI: 10.1017/S0952523808080760

Jacobs, G. H., & Deegan 2nd, J. F. (1999). Uniformity of colour vision in Old World monkeys. *Proc. Biol. Sci.*, *266*(1432), 2023–2028.

Jacobs, G. H., Neitz, M., Deegan, J. F., & Neitz, J. (1996). Trichromatic colour vision in New World monkeys. *Nature*, *382*(6587), 156–158. https://doi.org/10.1038/382156a0

Jarvey, J. C., Low, B. S., Pappano, D. J., Bergman, T. J., & Beehner, J. C. (2018). Graminivory and fallback foods: annual diet profile of geladas (Theropithecus gelada) living in the Simien Mountains National Park, Ethiopia. *Int. J. Primatol.*, *39*(1), 105–126.

Johnson, J. M. (1986). Nonthermoregulatory control of human skin blood flow. *J. Appl. Physiol.*, *61*(5), 1613–1622.

Kamilar, J. M., & Bradley, B. J. (2011). Interspecific variation in primate coat colour supports Gloger's rule. *Journal of Biogeography*, *38*(12), 2270–2277. https://doi.org/https://doi.org/10.1111/j.1365-2699.2011.02587.x

Karavanich, C., & Atema, J. (1998). Individual recognition and memory in lobster dominance. *Anim. Behav.*, *56*(6), 1553–1560.

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. *Anim. Behav.*, *81*(4), 795–800.

Kassambara, A. (2020). *Package `ggpubr'* (R package version 0.4.0.). mran.microsoft.com. https://cran.r-project.org/web/packages/ggpubr/index.html

Kelley, E. A., Jablonski, N. G., Chaplin, G., Sussman, R. W., & Kamilar, J. M. (2016). Behavioral thermoregulation in Lemur catta: The significance of sunning and huddling behaviors. *American Journal of Primatology*, *78*(7), 745–754. https://doi.org/https://doi.org/10.1002/ajp.22538

Kenney, W. L., & Johnson, J. M. (1992). Control of skin blood flow during exercise. *Med. Sci. Sports Exerc.*, *24*(3), 303–312.

Ketterson, E. D., & Nolan Jr, V. (1999). Adaptation, Exaptation, and Constraint: A Hormonal Perspective. *Am. Nat.*, *154*(S1), S4–S25.

Keyser, A. J., & Hill, G. E. (2000). Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behav. Ecol.*, *11*(2), 202–209.

Khramtsova, E. A., Davis, L. K., & Stranger, B. E. (2019). The role of sex in the genomics of human complex traits. *Nat. Rev. Genet.*, *20*(3), 173–190.

Kingdon, J. S. (1980). The role of visual signals and face patterns in African forest monkeys (guenons) of the genus Cercopithecus. *The Transactions of the Zoological Society of London*, *35*(4), 425–475. https://doi.org/https://doi.org/10.1111/j.1096-3642.1980.tb00062.x

Kitchen, D. M., Seyfarth, R. M., Fischer, J., & Cheney, D. L. (2003). Loud calls as indicators of dominance in male baboons (Papio cynocephalus ursinus). *Behav. Ecol. Sociobiol.*, *53*(6), 374–384.

Kunzetsova, A., Brockhoff, P., & Christensen, R. (2017). lmerTest package: Tests in linear mixed effect models. *J. Stat. Softw.*, *82*, 1–26.

Kuze, N., Malim, T. P., & Kohshima, S. (2005). Developmental changes in the facial morphology of the Borneo orangutan (Pongo pygmaeus): possible signals in visual communication. *Am. J. Primatol.*, *65*(4), 353–376.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, Articles*, *82*(13), 1–26.

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

Leonhardt, S. D., Tung, J., Camden, J. B., Leal, M., & Drea, C. M. (2009). Seeing red: behavioral evidence of trichromatic color vision in strepsirrhine primates. *Behavioral Ecology*, *20*(1), 1–12.

Lev-Yadun, S. (2001). Aposematic (warning) Coloration Associated with Thorns in Higher Plants. *Journal of Theoretical Biology*, *210*(3), 385–388. https://doi.org/https://doi.org/10.1006/jtbi.2001.2315

Lindstedt, C., Lindström, L., & Mappes, J. (2009). THERMOREGULATION CONSTRAINS EFFECTIVE WARNING SIGNAL EXPRESSION. *Evolution*, *63*(2), 469–478. https://doi.org/https://doi.org/10.1111/j.1558-5646.2008.00561.x

Lopes-Ramos, C. M., Chen, C.-Y., Kuijjer Marieke L and Paulson, J. N., Sonawane, A. R., Fagny Maud and Platig, J., Glass, K., Quackenbush, J., & DeMeo, D. L. (2020). Sex Differences in Gene Expression and Regulatory Networks across 29 Human Tissues. *Cell Rep.*, *31*(12), 107795.

Lucas, P. W., Darvell, B. W., Lee, P. K. D., Yuen, T. D. B., & Choong, M. F. (1998). Colour Cues for Leaf Food Selection by Long-Tailed Macaques (Macaca fascicularis) with a New Suggestion for the Evolution of Trichromatic Colour Vision. *Folia Primatologica*, *69*(3), 139–154. https://doi.org/https://doi.org/10.1159/000021576

Lucas, P. W., Dominy, N. J., Riba-Hernandez, P., Stoner, K. E., Yamashita, N., Calderön, E. L.-, Petersen-Pereira, W., Rojas-DurÁN, Y., Salas-Pena, R., Solis-Madrigal, S., Osorio, D., & Darvell, B. W. (2003). EVOLUTION AND FUNCTION OF ROUTINE TRICHROMATIC VISION IN PRIMATES. *Evolution*, *57*(11), 2636–2643. https://doi.org/https://doi.org/10.1111/j.0014-3820.2003.tb01506.x

Mank, J. E., Nam, K., Brunström, B., & Ellegren, H. (2010). Ontogenetic complexity of sexual dimorphism and sex-specific selection. *Mol. Biol. Evol.*, *27*(7), 1570–1578.

Marty, J. S., Higham, J. P., Gadsby, E. L., & Ross, C. (2009). Dominance, Coloration, and Social and Sexual Behavior in Male Drills Mandrillus leucophaeus. *Int. J. Primatol.*, *30*(6), 807.

Maynard Smith, J. (1982). *Evolution and the theory of game*. Cambridge University Press.

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

Maynard Smith, J., & Harper, D. G. C. (1995). Animal Signals: Models and Terminology. *J. Theor. Biol.*, *177*(3), 305–311.

Maynard Smith, J., & Parker, G. A. (1976). The logic of asymmetric contests. *Anim. Behav.*, *24*(1), 159–175.

McFarland, R., Fuller, A., Hetem, R. S., Mitchell, D., Maloney, S. K., Henzi, S. P., & Barrett, L. (2015). Social integration confers thermal benefits in a gregarious primate. *Journal of Animal Ecology*, *84*(3), 871–878. https://doi.org/https://doi.org/10.1111/1365-2656.12329

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychol. Methods*, *1*(1), 30–46.

McNamara Jr, J. A., & Graber, L. W. (1975). Mandibular growth in the rhesus monkey (Macaca mulatta). *Am. J. Phys. Anthropol.*, *42*(1), 15–24.

Melin, A. D., Fedigan, L. M., Hiramatsu, C., Hiwatashi, T., Parr, N., & Kawamura, S. (2009). Fig Foraging by Dichromatic and Trichromatic Cebus capucinus in a Tropical Dry Forest. *International Journal of Primatology*, *30*(6), 753–775. https://doi.org/10.1007/s10764-009-9383-9

Melin, A. D., Mortiz, G. L., Fosbury, R. A. E., Kawamura, S., & Dominy, N. J. (2012). Why Aye-Ayes See Blue. *American Journal of Primatology*, *74*(3), 185–192. https://doi.org/https://doi.org/10.1002/ajp.21996

Milledge, J. S., West, J. B., & Schoene, R. B. (2007). *High Altitude Medicine and Physiology*. CRC Press.

Möhle, U., Heistermann, M., Palme, R., & Hodges, J. K. (2002). Characterization of urinary and fecal metabolites of testosterone and their measurement for assessing gonadal endocrine function in male nonhuman primates. *Gen. Comp. Endocrinol.*, *129*(3), 135–145.

Molinari, C., Battaglia, A., Grossini, E., Mary D A S G and Vassanelli, C., & Vacca, G. (2002). The effect of testosterone on regional blood flow in prepubertal anaesthetized pigs. *J. Physiol.*, *543*(Pt 1), 365–372.

Moller, A. P., & Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology*, *32*, 167–176.

Møller, A. P., Saino, N., Taramino, G., Ferrario, S., & Galeotti, P. (1998). Paternity and multiple signaling: effects of a secondary sexual character and song on paternity in the barn swallow. *The American Naturalist*, *151*(3), 236–242.

Mollon, J. D. (1989). "Tho'she kneel'd in that place where they grew…" The uses and origins of primate colour vision. *Journal of Experimental Biology*, *146*(1), 21–38.

Moreira, L. A. A., Duytschaever, G., Higham, J. P., & Melin, A. D. (2019). Platyrrhine color signals: New horizons to pursue. *Evol. Anthropol.*, *28*(5), 236–248.

Mourthe, I., Trindade, R. A., Aguiar, L. M., Trigo, T. C., Bicca-Marques, J. C., & Bonatto, S. L. (2019). Hybridization Between Neotropical Primates with Contrasting Sexual Dichromatism. *International Journal of Primatology*, *40*(1), 99–113. https://doi.org/10.1007/s10764-017-0011-9

Muehlenbein, M. P. (2006). Adaptive variation in testosterone levels in response to immune activation: empirical and theoretical perspectives. *Soc. Biol.*, *53*(1–2), 13–23.

Muehlenbein, M. P., & Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. *Am. J. Hum. Biol.*, *17*(5), 527–558.

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., … Volpi, S. (2020). The impact of sex on gene expression across human tissues. *Science*, *369*(6509), eaba3066. https://doi.org/10.1126/science.aba3066

Olsson, M. (1994). Rival recognition affects male contest behavior in sand lizards (Lacerta agilis). *Behav. Ecol. Sociobiol.*, *35*(4), 249–252.

Osorio, D., & Vorobyev, M. (2005). Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. Biol. Sci.*, *272*(1574), 1745–1752.

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Res.*, *48*(20), 2042–2051.

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.

Palme, R. (1993). Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. *Advances of Steroid Analysis*, 111–117.

Pappano, D. J. (2013). *The reproductive trajectories of bachelor geladas* [PhD thesis]. University of Michigan.

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. *R Soc Open Sci*, *1*(1), 140081.

Pappano, D. J., Roberts, E. K., & Beehner, J. C. (2010). Testing extraction and storage parameters for a fecal hormone method. *Am. J. Primatol.*, *72*(11), 934–941.

Parr, L. A., Winslow, J. T., Hopkins, W. D., & de Waal, F. B. M. (2000). Recognizing facial cues: Individual discrimination by chimpanzees (Pan troglodytes) and rhesus monkeys (Macaca mulatta). *J. Comp. Psychol.*, *114*(1), 47–60.

Pascalis, O., & Kelly, D. J. (2009). The Origins of Face Processing in Humans: Phylogeny and Ontogeny. *Perspect. Psychol. Sci.*, *4*(2), 200–209.

Patricelli, G. L., & Hebets, E. A. (2016). New dimensions in animal communication: the case for complexity. *Current Opinion in Behavioral Sciences*, *12*, 80–89.

Penn, D. J., & Számadó, S. (2019). The Handicap Principle: how an erroneous hypothesis became a scientific principle. *Biological Reviews of the Cambridge Philosophical Society*, *95*(1), 267–290. https://doi.org/10.1111/brv.12563

Penn, D. J., & Számadó, S. (2020). The Handicap Principle: how an erroneous hypothesis became a scientific principle. *Biol. Rev. Camb. Philos. Soc.*, *95*, 267–290.

Perlman, R. F. (2021). *The energetics of male reproductive strategies in geladas*. 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. *Mol. Biol. Evol.*, *31*(5), 1206–1219.

Pessoa, D. M. A., Maia, R., de Albuquerque Ajuz, R. C., de Moraes, P. Z. P. M. R., Spyrides, M. H. C., & Pessoa, V. F. (2014). The adaptive value of primate color vision for predator detection. *American Journal of Primatology*, *76*(8), 721–729. https://doi.org/https://doi.org/10.1002/ajp.22264

Petersdorf, M., Dubuc, C., Georgiev Alexander V and Winters, S., & Higham, J. P. (2017). Is male rhesus macaque facial coloration under intrasexual selection? *Behav. Ecol.*, *28*(6), 1472–1481.

Pflüger, L. S., Valuch, C., Gutleb Daria R and Ansorge, U., & Wallner, B. (2014). Colour and contrast of female faces: attraction of attention and its dependence on male hormone status in Macaca fuscata. *Anim. Behav.*, *94*, 61–71.

Plant, T. M., & Zeleznik, A. J. (2014). *Knobil and Neill's Physiology of Reproduction* (T. M. Plant & A. J. Zeleznik, Eds.). 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*, *64*(1), 97–107.

Poole, J. H. (1989). Announcing intent: the aggressive state of musth in African elephants. *Anim. Behav.*, *37*, 140–152.

Potash, A. D., Greene, D. U., Foursa, G. A., Mathis, V. L., Conner, L. M., & McCleery, R. A. (2020). A comparison of animal color measurements using a commercially available digital color sensor and photograph analysis. *Curr. Zool.*, *66*(6), 601–606.

Potti, J., & Canal, D. (2011). Heritability and genetic correlation between the sexes in a songbird sexual ornament. *Heredity*, *106*(6), 945–954.

Prum, R. O., & Torres, R. H. (2004). Structural colouration of mammalian skin: convergent evolution of coherently scattering dermal collagen arrays. *Journal of Experimental Biology*, *207*(12), 2157–2172. https://doi.org/10.1242/jeb.00989

Puff, C., & Nemomissa, S. (2005). *Plants of the Simen: a flora of the Simen Mountains and surroundings, northern Ethiopia* (Vol. 37). National Botanic Garden (Belgium).

R Core Team. (2022). *R: A language and environment for statistical computing* (4.2.1).

Reby, D., & McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Anim. Behav.*, *65*(3), 519–530.

Rettenmeyer, C. W. (1970). Insect Mimicry. *Annual Review of Entomology*, *15*(1), 43–74. https://doi.org/10.1146/annurev.en.15.010170.000355

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

Rich, T. J., & Hurst, J. L. (1999). The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. *Anim. Behav.*, *58*(5), 1027–1037.

Riechert, S. E. (1978). Games spiders play: Behavioral variability in territorial disputes. *Behav. Ecol. Sociobiol.*, *3*(2), 135–162.

Rigaill, L., Higham, J. P., Winters, S., & Garcia, C. (2019). The redder the better? Information content of red skin coloration in female Japanese macaques. *Behavioral Ecology and Sociobiology*, *73*(8), 103. https://doi.org/10.1007/s00265-019-2712-x

Rigaill, L., MacIntosh, A. J. J., Higham, J. P., Winters, S., Shimizu, K., Mouri, K., Furuichi, T., & Garcia, C. (2015). Multimodal Advertisement of Pregnancy in Free-Ranging Female Japanese Macaques (Macaca fuscata). *PLoS One*, *10*(8), e0135127.

Rincon, A. v, Maréchal, L., Semple Stuart and Majolo, B., & MacLarnon, A. (2017). Correlates of androgens in wild male Barbary macaques: Testing the challenge hypothesis. *Am. J. Primatol.*, *79*(10).

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 Res.*, *43*(7), e47.

Roberts, E. K., Lu, A., Bergman, T. J., & Beehner, J. C. (2017). Female reproductive parameters in wild geladas (theropithecus gelada). *Int. J. Primatol.*, *38*(1), 1–20.

Robinson, M. R., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M., & Kruuk, L. E. B. (2006). Live fast, die young: trade-offs between fitness components and sexually antagonistic selection on weaponry in Soay sheep. *Evolution*, *60*(10), 2168–2181. https://doi.org/https://doi.org/10.1111/j.0014-3820.2006.tb01854.x

Sayigh, L. S., Tyack, P. L., Wells, R. S., Solow, A. R., Scott, M. D., & Irvine, A. B. (1999). Individual recognition in wild bottlenose dolphins: a field test using playback experiments. *Anim. Behav.*, *57*(1), 41–50.

Scheuber, H., Jacot, A., & Brinkhof, M. W. G. (2003). Condition dependence of a multicomponent sexual signal in the field cricket Gryllus campestris. *Animal Behaviour*, *65*(4), 721–727.

Schmidt, K. L., & Cohn, J. F. (2001). Human facial expressions as adaptations: Evolutionary questions in facial expression research. In *American Journal of Physical Anthropology* (Vol. 116, Issue S33, pp. 3–24).

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods*, *9*(7), 671–675.

Sen, S., Carrera, S. C., Heistermann, M., Potter, C. B., Baniel, A., DeLacey Patricia M and Petrullo, L., Lu, A., & Beehner, J. C. (2022). Social correlates of androgen levels and dispersal age in juvenile male geladas. *Horm. Behav.*, *146*, 105264.

Setchell, J. M. (2005). Do female mandrills prefer brightly colored males? *Int. J. Primatol.*, *26*(4), 715–735.

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. *Horm. Behav.*, *39*(3), 177–184.

Setchell, J. M., & Wickings, E. J. (2005). Dominance, status signals and coloration in male mandrills (Mandrillus sphinx). *Ethology*, *50*, 25–50.

Setchell, J. M., Wickings, E. J., & Knapp, L. A. (2006). Signal content of red facial coloration in female mandrills (Mandrillus sphinx). *Proc. Biol. Sci.*, *273*(1599), 2395–2400.

Sheehan, M. J., & Tibbetts, E. A. (2011). Specialized face learning is associated with individual recognition in paper wasps. *Science*, *334*(6060), 1272–1275.

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M., & Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. *Journal of Experimental Biology*, *207*(14), 2471–2485. https://doi.org/10.1242/jeb.01047

Silk, J. B. (1999). Male bonnet macaques use information about third-party rank relationships to recruit allies. *Anim. Behav.*, *58*(1), 45–51.

Smith, A. C., Buchanan-Smith, H. M., Surridge, A. K., Osorio, D., & Mundy, N. I. (2003). The effect of colour vision status on the detection and selection of fruits by tamarins (Saguinus spp.). *Journal of Experimental Biology*, *206*(18), 3159–3165.

Snowdon, C. T. (2004). Sexual selection and communication. *Sexual Selection in Primates: New and Comparative Perspectives*, 57–70.

Snyder-Mackler, N., Alberts, S. C., & Bergman, T. J. (2012). Concessions of an alpha male? Cooperative defence and shared reproduction in multi-male primate groups. In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 279, Issue 1743, pp. 3788–3795).

Snyder-mackler, N., Beehner, J. C., & Bergman, J. (2012). Defining Higher Levels in the Multilevel Societies of Geladas (Theropithecus gelada). *International Journal of Primatology*, *33*, 1054–1068. https://doi.org/10.1007/s10764-012-9584-5

Snyder-Mackler, N., Beehner, J. C., & Bergman, T. J. (2012). Defining higher levels in the multilevel societies of geladas (Theropithecus gelada). *Int. J. Primatol.*, *33*(5), 1054–1068.

Stephen, I. D., Coetzee, V., Law Smith, M., & Perrett, D. I. (2009). Skin blood perfusion and oxygenation colour affect perceived human health. *PLoS One*, *4*(4), e5083.

Stevens, M., & Merilaita, S. (2011). *Animal camouflage: mechanisms and function*. Cambridge University Press.

Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C., & Troscianko, T. S. (2007). Using digital photography to study animal coloration. *Biol. J. Linn. Soc. Lond.*, *90*(2), 211–237.

Storey, J. D., Bass, A. J., Dabney, A., & Robinson, D. (2019). *qvalue: Q-value estimation for false discovery rate control*.

Surridge, A. K., Osorio, D., & Mundy, N. I. (2003). Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution*, *18*(4), 198–205. https://doi.org/https://doi.org/10.1016/S0169-5347(03)00012-0

Számadó, S. (2011). The cost of honesty and the fallacy of the handicap principle. *Anim. Behav.*, *81*(1), 3–10.

Talebi, M. G., Pope, T. R., Vogel, E. R., Neitz, M., & Dominy, N. J. (2006). Polymorphism of visual pigment genes in the muriqui (Primates, Atelidae). *Molecular Ecology*, *15*(2), 551–558. https://doi.org/https://doi.org/10.1111/j.1365-294X.2005.02822.x

Terrien, J., Perret, M., & Aujard, F. (2011). Behavioral thermoregulation in mammals: a review. *Front. Biosci.*, *16*, 1428–1444.

Thapar, V. (1986). *Tigers: Portrait of a Predator*. Collins.

Tibbetts, E. A., & Dale, J. (2007). Individual recognition: it is good to be different. *Trends Ecol. Evol.*, *22*(10), 529–537.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift Für Tierpsychologie*, *20*(4), 410–433.

Tinsley Johnson, E., Feder, J. A., Bergman, T. J., Lu, A., Snyder-Mackler, N., & Beehner, J. C. (2021). The Goldilocks effect: female geladas in mid-sized groups have higher fitness. *Proc. Biol. Sci.*, *288*(1952), 20210820.

Tinsley Johnson, E., Snyder-Mackler, N., Lu Amy and Bergman, T. J., & Beehner, J. C. (2018). Social and ecological drivers of reproductive seasonality in geladas. *Behav. Ecol.*, *29*(3), 574–588.

Tobias, J. A., Montgomerie, R., & Lyon, B. E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, *367*(1600), 2274–2293.

Touitou, S., Heistermann, M., Schülke, O., & Ostner, J. (2021). Triiodothyronine and cortisol levels in the face of energetic challenges from reproduction, thermoregulation and food intake in female macaques. *Hormones and Behavior*, *131*, 104968.

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

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox–a free software suite for objectively measuring reflectance, colour and pattern. *Methods Ecol. Evol.*, *6*(11), 1320–1331.

Tung, J., Alberts, S. C., & Wray, G. A. (2010). Evolutionary genetics in wild primates: combining genetic approaches with field studies of natural populations. *Trends Genet.*, *26*(8), 353–362.

Urlacher, S. S., Kim, E. Y., Luan, T., Young, L. J., & Adjetey, B. (2022). Minimally invasive biomarkers in human and non-human primate evolutionary biology: Tools for understanding variation and adaptation. *American Journal of Human Biology*, *34*(11), e23811.

Uy, J. A. C., Moyle, R. G., Filardi, C. E., & Cheviron, Z. A. (2009). Difference in Plumage Color Used in Species Recognition between Incipient Species Is Linked to a Single Amino Acid Substitution in the Melanocortin-1 Receptor. *The American Naturalist*, *174*(2), 244–254. https://doi.org/10.1086/600084

van den Berg, C. P., Troscianko, J., Endler John A and Marshall, N. J., & Cheney, K. L. (2020). Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods Ecol. Evol.*, *11*(2), 316–332.

Vandenbergh, J. G. (1965). Hormonal basis of the sex skin in male rhesus monkeys. *Gen. Comp. Endocrinol.*, *5*, 31–34.

Veilleux, C. C., Scarry, C. J., di Fiore, A., Kirk, E. C., Bolnick, D. A., & Lewis, R. J. (2016). Group benefit associated with polymorphic trichromacy in a Malagasy primate (Propithecus verreauxi). *Scientific Reports*, *6*(1), 1–10.

Waitt, C., Gerald, M. S., Little, A. C., & Kraiselburd, E. (2006). Selective attention toward female secondary sexual color in male rhesus macaques. *American Journal of Primatology*, *68*(7), 738–744. https://doi.org/https://doi.org/10.1002/ajp.20264

Waitt, C., Little, A. C., Wolfensohn, S., Honess, P., Brown, A. P., Buchanan-Smith, H. M., & Perrett, D. I. (2003). Evidence from rhesus macaques suggests that male coloration plays a role in female primate mate choice. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(suppl_2), S144–S146. https://doi.org/10.1098/rsbl.2003.0065

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? *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, *372*(1724).

Webb, C. M., McNeill, J. G., Hayward, C. S., & de Zeigler D and Collins, P. (1999). Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation*, *100*(16), 1690–1696.

Weir, L. K., Grant, J. W. A., & Hutchings, J. A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *Am. Nat.*, *177*(2), 167–176.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package `ggplot2’. *Create Elegant Data Visualisations Using the Grammar of Graphics. Version*, *2*(1), 1–189.

Wickings, E. J., & Dixson, A. F. (1992). Testicular function, secondary sexual development, and social status in male mandrills (Mandrillus sphinx). *Physiol. Behav.*, *52*(5), 909–916.

Winters, S., Allen, W. L., & Higham, J. P. (2020). The structure of species discrimination signals across a primate radiation. *ELife*, *9*, e47428. https://doi.org/10.7554/eLife.47428

Winters, S., Petersdorf, M., & Higham, J. P. (2019). Charles Darwin and Selection in Relation to Sex in the Colors of Monkeys. In G. Geher, D. S. Wilson, H. Head, & A. Gallup (Eds.), *Darwin's Roadmap to the Curriculum: Evolutionary Studies in Higher Education*. Oxford Scholarship Online.

Yalden, D. W., Largen, M. J., & Kock, D. (1977). Catalogue of the mammals of Ethiopia. 3. Primates. *Monitore Zoologico Italiano. Supplemento*, *9*, 1–52.

Yang, X., Schadt, E. E., Wang, S., Wang, H., Arnold, A. P., Ingram-Drake, L., & Drake Thomas A and Lusis, A. J. (2006). Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res.*, *16*(8), 995–1004.

Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *J. Theor. Biol.*, *67*(3), 603–605.

Zera, A. J., & Harshman, L. G. (2001). The Physiology of Life History Trade-Offs in Animals. *Annu. Rev. Ecol. Syst.*, *32*, 95–126.

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. *Mol. Ecol.*, *27*(24), 5049–5072.

Zouboulis, C. C., Chen, W.-C., Thornton, M. J., Qin, K., & Rosenfield, R. (2007). Sexual hormones in human skin. *Horm. Metab. Res.*, *39*(2), 85–95.

Zuckerman, S., & Parkes, A. S. (1939). Observations on the secondary sexual characters in monkeys. *Journal of Endocrinology*, 430–439.