

Sexual Signalling in Male Vervet Monkeys (*Chlorocebus pygerythrus*):  
Influences of Hormones, Behaviour, and Parasitism on Scrotal and Penile  
Skin Colouration

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## Abstract

Male secondary sexual characteristics may convey information about the signaller. The red penis and blue scrotum of male vervet monkeys make them a good species to examine variation in signal expression. We quantified colour in vervets at Lake Nabugabo, Uganda from standardized photos. We hypothesized that inter- and intra-individual variation in the expression of the genital colour of males is influenced by parasites, dominance rank, age, androgens and glucocorticoids. Our results indicate that blue scrotal colour between males is predicted by rank and parasites, and red penis colour is predicted by age (or sexual maturity) and parasites. Within males, our findings indicate that short-term variation in blue scrotal colour characteristics and contrast are negatively correlated with measures of parasitism and red penis hue becomes redder (less pink) as males increase in rank. These findings support the Hamilton-Zuk hypothesis and suggest that vervet genitals are a badge of status symbol.

## Dedication

This thesis is dedicated to my mother Laurie Snyder who taught me to be curious.

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# Introduction

## Animal Communication

At its base, animal communication involves a signal being transmitted from a sender to a signal receiver. This signal can influence competitive or cooperative interactions. The sender will only signal if there is a benefit to themselves, and a receiver can benefit from receiving a signal, although this may be involuntary on the part of the signaller (Marler, 1961). Such signalling can affect an individual's fitness by influencing an individual's reproductive success and survival. Signals can be enriched through context and increasing complexity to reduce uncertainty and influence behaviour (Marler, 1965; Seyfarth & Cheney, 2017).

Signals may supplement or substitute existing social knowledge. For example, in species where unfamiliar individuals regularly encounter one another, it may be important to quickly assess a possible competitor's strength or a possible mate's quality. So-called "badge of status" signals may supply useful information when social knowledge is not available, such as when new individuals immigrate into a social group (Bergman & Sheehan, 2013; Setchell & Wickings, 2005). Even where individuals are familiar with one another, co-residents may rely on signals to supplement existing but incomplete knowledge in response to changing within-group social factors, such as dominance relationships (Bergman & Sheehan, 2013).

Visual, olfactory, and auditory signals can relay information about a conspecific, such as physical strength, reproductive status, hormonal status, genetics, health, or relatedness (Holekamp & Strauss, 2016). Sexually dimorphic traits - such as body size, weaponry, ornamentation, and colouration - that vary between males and females of a given species may act in communication and be sexually selected (Clutton-Brock & Huchard, 2013). Information being signalled and received from sexually selected traits may be the result of either intersexual (i.e., mate choice) or intrasexual (i.e., male-male, female-female competition) selection or as a combination of both (Clutton-Brock & Huchard, 2013). Traits selected by intersexual selection most likely function to communicate that an individual is a quality mate. In this case, the signaller of such traits benefits by receiving increased access to resources (such as females), and the receiver (female) benefits by producing offspring with strong sexually selected traits, or "good genes" (Trivers & Campbell, 1972). Traits selected relating to intrasexual selection likely function to improve success in

competitive situations or avoid mismatched contests between males (Andersson, 1994; Andersson & Iwasa, 1996). Evolution of sexual signalling traits most likely function to improve an individual's access to a particular resource (e.g., access to females; Andersson & Iwasa, 1996).

Zahavi (1975) proposed the Handicap Principle, which states that only traits that are costly to produce would honestly represent signaller quality, where quality is a measure of genetic desirability (“good genes”) or competitive ability. In other words, only a high-quality individual can bear the cost of producing the signal, such that a low-quality individual could only produce a reduced signal, if at all (Grafen, 1987; Higham & Maestripieri, 2014). The Handicap Principle is often applied to both inter- and intra-sexual selection. For example, widowbird (*Eucleptes ardens*) tail length is used as a signal in female mate choice. Male widowbirds have long tails that represent a significant aerodynamic impediment that only high-quality individuals can support (Andersson & Simmons, 2006). Red deer (*Cervus elaphus*) males hold harems and are challenged for control of their harem by competing males. Male red deer employ a vocal roar signal as an advertisement of potential fighting ability to other males, a signal that is only effective if it relays honest information about the quality of the male, as this signal will inform whether a conspecific engages in a physical contest with the signalling male (Clutton-Brock & Albon, 1979; Grafen, 1990). A signaller will ‘cheat’ if there are no underlying costs associated with increasing the signal (Higham, 2016; Számándó, 2011). Therefore, honest signalling may be maintained by the production cost of the signal itself, or socially maintained by punishing misleading signallers, such as increasing aggressions directed towards the signaller (Lachmann et al., 2001).

In this thesis, I examine the expression of the male vervet monkey (*Chlorocebus pygerythrus*) genital colouration in the population at Lake Nabugabo, Uganda. Specifically, I aim to understand inter

and intraindividual colour variation of the distinctive blue scrotum and red penis of vervet monkey males, and how the colour is related to parasites, rank, age, androgens and glucocorticoids.

## Primate Signalling

The males of some primate species exhibit conspicuous sexually dimorphic skin coloration that function as a signal to conspecifics, such as the red nose and blue cheeks of mandrills (*Mandrillus sphinx*; Renoult et al., 2011), red chest patches of geladas (*Theropithecus gelada*;

Bergman et al., 2009)), red faces of rhesus macaques (*Macaca mulatta*; Dubuc et al., 2014), and blue scrota, red penis and red perianal area of vervet monkeys (*Chlorocebus pygerythrus*; Henzi, 1982). However, not all individuals express skin colouration equally or consistently across their lifetime (Bradley & Mundy, 2008). For example, qualitative observations of male vervet monkeys suggest scrota ranging from deep blue to a blue-green color (Henzi, 1985; Turner et al., 2019, Figure 1). As the most colourful mammalian order (Bradley & Mundy, 2008; Setchell 2015), primates are good study subjects for the study of sexual signals in gregarious living organisms.

Current research on colour signalling in primates continues to support the hypothesis that sexual selection is a driver of interindividual colour variation (Setchell & Wickings, 2005). Colour signals appear to play a role in both intersexual and intrasexual selection. For example, in rhesus macaques, red facial skin colour is likely related to intersexual selection, because males with redder faces received more sexual solicitations from females (Dubuc et al., 2014b). Red nose colour in male mandrills may also play a role in intrasexual selection, because it is rank dependent (Setchell & Wickings, 2005). In captive vervets, males of similar scrotal colouration displayed more agonistic behaviours towards one another than dissimilar males (Gerald, 2001). In vervets, scrotal colour may also play a role in intersexual selection since females perceived scrotal colour differences between males, however they did not adjust their behaviour based on the colouration (Rojas et al., 2018). Therefore, vervet genital coloration has the potential to be involved in both intrasexual competition and in intersexual selection.

Signalling can be complex and span multiple modalities (Hebets & Papaj, 2005). Multicomponent signals stimulate the receiver in the same sensory modality (e.g., visual) but elicit multiple responses, while multimodal signals stimulate different sensory modalities (e.g., visual and chemical) of the receiver (Rojas et al., 2018). Multicomponent signalling involved in sexual selection occurs in many bird species (Grether et al., 2004), such as bird-of-paradise calls (*Paradisaea raggiana*; Frith, 1981), and red junglefowl (*Gallus gallus*) comb feather colour and length (Zuk et al., 1992). In a study on the colourful faces of mandrills, Renoult *et al.* (2011) found that high-ranking males had more saturated red and blue tones than low-ranking males, with the two colours working jointly as a unimodal multicomponent signal may have evolved to improve signal detection by maximizing hue disparity. Multicomponent signals, such as vervet monkey male genitalia, can increase the repertoire of meaning for signals that are evolutionarily

constrained (Marler, 1965; Seyfarth & Cheney, 2017). Placement of the signal may also function to improve signal saliency (Dubuc et al., 2016). An example of multicomponent visual signalling occurs in rhesus macaques, as they are able to discern and respond to conspecifics facial expressions and coloration (Bethell et al., 2012). Since rhesus macaque red facial skin is a sexual signal, signal saliency and meaning may be increased or supplemented by facial expression. Likewise, because the vervet male red and blue signal is located on the genitals with the red and blue located next to one another, signal meaning and saliency may be increased by colour contrast, by displaying an erect penis, or by display of the red-white-and-blue (RWB) display, which involves a male lifting their tail to reveal the red perianal area beside a white patch of hair and blue scrotum (Struhsaker, 1967).

### The Anatomy and Physiology of Colour Signals

Most, but not all mammalian taxa, have colour vision. While most eutherian mammals have dichromatic vision, trichromacy evolved independently in both catarrhines (Afro-Asian monkeys and apes) and platyrrhines (Central and South American primates) (Sumner & Mollon, 2003), enabling them to see red, green, and blue (Bradley & Mundy 2008; SurrIDGE et al., 2003). There is a long and rich history of colour vision research in mammals that has found two distinct kinds of cells in the retina. Colour vision is associated with cone cells, while black-and-white and low-light vision are associated with rod cells. For trichromatic primates, specialized cone cells located in the central fovea of the eye contain photoreceptors that are sensitive to various wavelengths of light; they are short wave (SW; “blue”), medium wave (MW; “green”), and long wave (LW; “red”) cones, also known as the tristimulus values (Sumner & Mollon, 2003). Colour vision is likely a process of two non-mutually exclusive mechanisms. The Young-Helmholtz theory of trichromacy suggests that red, green, and blue receptor cells respond to specific light wavelengths (Helmholtz, 1896). The colour-opponency models suggest that cone receptors are neurally linked with opposing continuous colours (red-green, blue-yellow, and the achromatic black-white) and activation of one receptor in the pair inhibits the other receptor in the pair (Vorobyev & Osorio, 1998).

Trichromacy likely evolved as a response to selective pressure to increase foraging efficiency in diurnal frugivorous primates (Sumner & Mollon, 2003) and was secondarily co-opted

for sexual signalling (Hiramatsu et al., 2017; Skalnikova et al., 2020). This could explain why there is a wide range of colourful visual signals present in primates, much more so than any other mammal (Setchell, 2015). Vervet monkeys already possess a sensory bias for the colour red from foraging for red fruit (Fernandez & Morris, 2007), and likely the red genital signal evolved to take advantage of this sensory bias, with the blue scrotal colouration possibly serving to enhance the red display (Arnqvist, 2006). This is consistent with the findings of Renoult et al. (2011), who suggested that the red colouration of the mandrill nose was a highly conserved evolutionary trait, while the contrasting blue cheek colouration likely evolved secondarily to increase the conspicuousness of the red nose signal.

The central qualities for quantifying colour are hue, saturation (the colour aspects) and luminance (the achromatic aspect). Hue is a measure of the colour (such as red, blue, green, etc.), while saturation measures the purity of the colour in relation to the grey achromatic axis, or how much grey is in the colour. Luminance relates to specific photoreceptor sensitivity and contrast with neighboring patches and is related to how much white light is reflected; more luminant colours will appear whiter (Renoult et al., 2017). There are interspecific differences in wavelength sensitivity of photopigments, such that species often have a unique spectral sensitivity, which can influence colour perception of hue, luminance, and saturation. For example, relative to humans, macaques and chimpanzees have higher SW and lower LW sensitivity (Stevens et al., 2009). Correspondingly, rhesus macaque males are able to discern minute changes in red female face colour indicative of reproductive status, potentially representing a fine-tuning of the photoreceptors for the specific signal function in this species (Hiramatsu et al., 2017). Spectral sensitivities of vervet monkeys appear to be very similar to those of humans (G. Jacobs, pers. comm.; Jacobs and Deegan 1999).

Blue and white skin in animals is generally produced by collagen fibers, and sometimes additionally influenced by melanin pigmentation (Prum, 2004). Variation of blue skin colour may be due to thickness of the dermal collagen layer or due to variation in the layer of melanocytes underlying the collagen array, both of which may be subject to hormonal regulation (Diaz et al., 1986; Prum & Torres, 2004). While the non-iridescent property of blue skin may be due to the melanocyte layer, mandrills have structurally produced non-reflective blue skin on their faces even

in the absence of a melanocyte layer; indicating that in mandrills, the dermal collagen layer is sufficient to produce the blue colouration (Bagnara et al., 2006). In contrast, a melanocyte layer is present in male vervet scrotal tissue, indicating that colour intensification may be due in part to the melanocyte layer underneath (Bagnara et al., 2006; Prum & Torres, 2004). Shawkey & Hill (2006) examined the role of the melanocyte layer in colour purity by comparing the feathers of amelanotic versus regularly pigmented Steller's Jay (*Cyanocitta stelleri*) feathers. Bird feathers that lacked melanin and had an intact spongy (structural) layer appeared white to human observers. Using spectrometry, the amelanotic feathers were found to have more saturated wavelengths across all visible wavelengths (hence the white appearance of the feathers), however there was a peak in the blue/green spectra. The normal feathers had reflectance peaks in the blue/UV range, and exhibited a more saturated colour (Shawkey & Hill, 2006). This suggests that the purpose of the melanocyte layer may be to absorb incoherently scattered light, increasing the purity of the colour (Prum & Torres, 2004). Alternatively, as structural colours are often iridescent, wavelength can vary based on refracting angle and polarization of the incident light, thus emit different wavelengths based on the structural aspect of the collagen array (Dumanli & Savin, 2016). Thus, it is possible that the mechanisms that guide colour hue, luminance, and saturation variation may result from an interactive effect of pigment and structural colour production.

Red skin colouration used for signalling in animals is often produced either by a high carotenoid diet or high capillary density at the skin surface with oxygenated hemoglobin (Marty et al., 2009). In primates, red skin colouration is produced by the latter mechanism, and as a result can exhibit short-term changes (Freeman-Gallant et al., 2009). In male rhesus macaques, the red facial skin colouration varies with blood flux in the epidermis, which is subject to hormonal regulation by androgens (Vandenbergh, 1965; Rhodes et al., 1997).

### Autecology of Colour Signalling

Life history trade-offs based on functional traits are driven by the allocation of limited internal resources (Stearns, 1992). Environmental and individual factors may influence resource availability, and allocation of internal resources may vary throughout an individual's life history stages. Traits that are functionally linked in a trade-off are predicted to be negatively correlated (Zera & Harshman, 2001). The "Y" model of resource allocation proposes that the allocation of

internal resources likely act to increase current and future reproductive success of individuals within the constraints of survival or somatic growth (van Noordwijk & de Jong, 1986). Individuals that pay high current reproductive costs likely will experience survival costs or decreased future reproductive potential (Stearns, 1989; McLean et al., 2019). Analyses of resource allocation can be examined at the level of genotype, phenotype, or at the level of endocrine and physiological mechanisms that connect the genotype to the phenotype (Stearns, 1989). While this model is often applied to female birds in relation to clutch size, it is theoretically possible that the current and future potential reproductive effort of signalling by males will also be constrained by costs to survival. For example, Androgens (AR) modulate a trade-off between energy investment in reproduction or immune function within individuals, thereby modulating trait expression (Prall & Muehleisen 2014). Within males, signal honesty is maintained by internal costs associated with elevating their signal (Higham, 2016). If there are no underlying costs to producing the signal, the signaller will produce an elevated signal (Számándó, 2011). Therefore, individual variation of these underlying costs associated with producing an elevated signal may lead to individual variation in signalling.

### The Endocrinology of Colour Signals: Androgens and Glucocorticoids

Androgens (AR) are a group of sex steroids that play a critical role in spermatogenesis, influence sexual behaviour, stimulate the development and maintenance of secondary sexual characteristics, and facilitate reproductive aggression in males (Dixson, 2012). In primates, testosterone is the major circulating AR and is produced in small amounts in the adrenal cortex of males and females, and in the Leydig cells of in the testicles of males (Nieschlag & Behre, 1990). Consistently maintaining high levels of ARs may incur costs, such as increased risk of injury due to higher levels of aggression, potential immune suppression, and physiological costs associated with production of the hormone (Wingfield et al., 1990; Beehner et al., 2006).

The Challenge Hypothesis was developed to explain variation in AR levels in male birds (Wingfield et al., 1990), and has since been expanded to other vertebrates, including primates (Wingfield, 2017). The original iteration of the Challenge Hypothesis is based on the principle that there are three levels of AR responsiveness that reflect the costs and benefits of androgens in different social contexts; a non-breeding baseline, a breeding baseline that allows for basic sexual

functions brought on by environmental cues, and a physiological maximum level in response to male reproductive aggression (Wingfield et al., 1990, Hirschenhauser & Oliveira, 2004). The Challenge Hypothesis proposes that species with monogamous mating systems characterized by male parental care and low intrasexual aggression will experience fewer AR surges and fluctuations than polygynous or multi-male multi-female system, which are characterized by less male parental care and more intrasexual competition (Goymann, 2009). Among individuals, AR profiles can vary drastically even within the same life stage (Goymann et al., 2019). Increases in testosterone due to breeding seasonal variation and male-male competition are also likely influenced by additional factors, such as exposure to receptive females, non-social environmental cues and physiological maximum thresholds of AR responsiveness, dominance status, and age (Goymann, 2009; Muller, 2017; Goymann et al., 2019).

Glucocorticoids (GC) are a group of hormones that play a role in the stress response. GCs facilitate appropriate physiological and behavioural responses to stressors in an effort to maximize individual fitness (Sapolsky et al., 2000). Release of GCs is mediated by the Hypothalamic-Pituitary-Adrenal (HPA) axis (reviewed in Higham, 2016), and the predominant circulating GC in primates is cortisol (Abbott et al., 2003). When a stressor is perceived, the hypothalamus releases corticotropin releasing factor, which triggers the release of adrenocorticotrophic hormone that binds to the adrenal glands, in turn stimulating the release of GCs (Becker et al., 2002). Individual GC levels both between and within males can be influenced by a variety of social and ecological factors that vary by unique circumstance (Sapolsky, 2005). Changes in GC levels have been linked to a variety of social and ecological variables (Higham, 2016), such as social status (Abbott et al., 2003), directed and received aggression (Surbeck et al., 2012), ecological season (Gesquiere et al., 2008; Beehner & McCann 2008; Schoof et al., 2016), mating season (Ostner et al., 2008; Girard-Buttoz et al., 2009), and presence of ovulatory females (Schoof et al., 2014; 2016). Both dominant and subordinate individuals can exhibit elevated GC levels. These GC elevations are linked to physical and psychosocial stressors, and can vary depending on social conditions such as hierarchy stability and available coping strategies (i.e., access to grooming partners), or ecological conditions such predation pressure, nutritional stress, and pathogen exposure (Buchanan, 2000). While short-term GC secretion can be beneficial because they lead to appropriate responses, long-term secretion can lead to physiological and behavioural impediments, such as immune, reproductive,

and growth suppression (Cavigelli & Caruso 2015; Sapolsky, 2002). Understanding GC fluctuations can be useful for comprehending how dominance status is acquired and maintained, as rank-related costs are linked with the costs and benefits of GC production (Goymann & Wingfield, 2004). In addition, immunosuppressive effects of GCs may generate a trade-off between immune functioning and sexual signal development, which could further reinforce the production of honest signals (Buchanan, 2000).

### *Parasites, Hormones and Honest Signalling*

The Hamilton-Zuk Hypothesis, an extension of the Handicap Principle, proposes that one possible quality advertised by signals is that of parasite resistance (Hamilton & Zuk, 1982; Nunn & Altizer 2006). Choosing a mate with few parasites and advertising ability to resist parasites may be especially important for species and populations that are vulnerable to parasites, and since disease resistance is heritable, some sexually-selected traits are likely to correlate with parasite resistance (Hamilton & Zuk, 1982). If individuals are choosing mates that are less parasitized, this may be because they are benefitting from less chance of having parasites transmitted during mating, or because they will be more likely to pass on genes for parasite resistance to their offspring (Able, 1996). Thus, pathogen virulence and transmission mode could influence exposure to parasites which may influence signal production depending on how susceptible the individual is (Folstad & Karter, 1992). Good health may be evident in fur (Regan, 1998), plumage (Zuk et al., 1990), skin (Ayres, 1996; Mayor et al., 2015), brightness of colour (Isbell, 1995), and agonistic displays (Lea et al., 2018). Research to date suggest an inconsistent relationship between health and primate skin colouration is equivocal. Blue vervet monkey scrotal colouration likely fades with poor nutrition (Isbell, 1995) and bald uakari red facial skin colouration fades with increasing parasitic load (Ayres, 1996). In contrast, there is no correlation between red nose colour and parasitic or hematological measures in mandrills (Setchell et al., 2009).

Mounting an immune response (e.g., leukocyte production) can be energetically costly. Variation in immune response may exist to maximize potential fitness (Viney et al., 2005); tolerating some low level of infection may be more energetically efficient than mounting an immune response to fight the infection (Hanssen et al., 2004). That said, previous research has

demonstrated that vervet monkeys that had higher levels of gastrointestinal parasites decreased duration of resting events post-deworming, indicating that parasites may represent an energetic burden (Chapman et al., 2016). Additionally, poor nutritional status may cause increased infection susceptibility, as energetic resources are re-allocated away from immune function (Beldomenico & Begon, 2010), as documented in multiple primate species (Agostini et al., 2017).

The Immunocompetence Handicap Hypothesis (ICHH), an extension of the Hamilton-Zuk hypothesis, aims to explain parasitic burden and testosterone as regulatory mechanisms of secondary sexual characteristics (Folstad & Karter, 1992). Testosterone (T) is hypothesized to suppress the immune response while at the same time elevating production of secondary sexual characteristics (Folstad & Karter, 1992, Braude et al., 1999). According to the ICHH, only healthy males can withstand the immunosuppressive effects of elevated T levels, which are paramount to the development of secondary sexual characteristics; therefore, only healthy males can afford to express or amplify signals (Folstad & Karter, 1992). However, others have hypothesised that immune response is not suppressed in response to increased AR levels, but rather redistributed to areas that may require more immediate attention, such as to the skin and other tissues for wound healing, in the event of a physical altercation (Braude et al., 1999).

The stress-mediated immunocompetence handicap hypothesis is another iteration of the ICCH and proposed that in that there is a potential trade-off between signal production and long-term physiological stress and immune response (Buchanan 2000). This trade-off may be involved in maintaining honest signals, as elevated glucocorticoid (GC) levels may denote immune impediment. In barn owls (*Tyto alba*), glucocorticoids mediated production of melanin-based traits (Roulin et al., 2008). However, in mandrills, GCs were not related to signal expression of the red nose despite individuals with higher GC levels being more parasitized (Setchell et al., 2010).

### Function of the Colour Signal in Primates

A sexual signal may reflect an individual's age, mate quality, health status, or competitive ability. For example, in birds, common yellowthroat males display a yellow bib and black mask (a sexual signal), and older males had larger bibs and masks and were also more successful at securing

mates (Freeman-Gallant et al., 2009). Age may also influence parasite burden; often older individuals are more parasitized (Poulin & Morand, 2000), which may influence signal production. In male geladas, chest patch redness is associated with age, suggesting that it is a signal of sexual maturity; that said, chest patch colour and age were closely tied to dominance status (Bergman et al., 2009). Thus, sexual maturity and dominance rank may be connected, and as such both conveyed in the sexual signal.

Dominance status may be reflected in a sexual signal. Dominance relationships are near ubiquitous in group-living species. Dominance is an expression of the relative competitive ability of an individual; as such, it is generally mediated by a pattern of repeated dyadic, agonistic interactions in which the dominant (“winner”) individual gains priority-of-access to resources over the subordinate (“loser”) individual. Dominance status refers to an individual’s position within a dyad, while dominance rank refers to an individual’s position within a hierarchy (Drews, 1993). Many primates live in relatively stable social groups with sex-specific dominance hierarchies (Dixson, 2012). Hierarchies can benefit both male and female high-ranking individuals by increasing fitness. In female primates, high rank is generally associated with high fecundity and infant survival rates, potentially due to physical condition, lower stress, or the ability to exercise mate choice (Majolo et al., 2012). In male primates, high rank is frequently but not ubiquitously associated with more copulations and higher fecundity, potentially due to increased competitive success, mate guarding, or female preference for dominant individuals (Majolo et al., 2012). Hierarchies may also be beneficial for lower-ranking individuals during periods of social stability, as the hierarchy system may reduce overt conflict, the risk of injury and death, and stress levels (Holekamp & Strauss, 2016). Numerous individual and social factors influence an individual’s dominance rank, such as age, hormones, individual competitive ability, kinship, length of tenure, or number of competitors (Drews, 1993, Holekamp & Strauss, 2016).

In many social primate species, males ascertain rank through aggressive encounters with other males (Clutton-Brock & Huchard, 2013). Sexual signals that signal competitive ability of an individual may relate to rank, as more competitive individuals are more likely to engage in aggressive encounters with other highly competitive individuals (Gerald, 2001). Using qualitative observations, Isbell (1995) found no correlation between rank and scrotal blueness from visual

observations in wild vervets, however the red portion of the signal was not accounted for, nor was photography used as a tool to quantify variation.

Hormones may influence rank. Since ARs are involved in the production and maintenance of secondary sexual characteristics and has been linked with levels of aggression (Dixson, 2012), there is a possible link between dominance, ARs and colour signal production. For example, in male mandrills, the highest-ranking males had highest circulating testosterone level as well as the most intense colour signal (Wickings & Dixson, 1992). In mandrill and macaques red skin colouration is influenced by testosterone, however blue skin colour in mandrill is not related to testosterone (Setchell & Dixson, 2001b; Vandenberg, 1965). Dominance rank and fecal AR levels of male mandrills were significantly correlated, and red colour and fecal AR levels were significantly correlated, however there was not a correlation between red colouration and rank (controlling for age related colour effects; Setchell et al., 2008). GC's may also have an impact on individual rank, although it is unclear and situation-dependent whether high- or low-ranking monkeys are more likely to have higher levels of stress (Sapolsky, 2005; Setchell et al., 2010). Conversely, GCs may influence dominance rank, as higher levels of GCs represent a cost, so an individual with higher GCs may be less likely to engage in agonistic behaviours to achieve higher rank (Setchell et al., 2010).

Health may also be reflected in the signal. In stickleback fish (*Gasterosteus aculeatus*) and guppies (*poecilia reticulata*) redder males were healthier and less parasitized (Milinski & Bakker, 1990; Nicoletto, 1993). Health reflected in a sexual signal has been observed in primates as well. In the bald uakari, red facial skin is a good indicator of individual health status (Mayor et al., 2015), and in vervets blue scrotal skin fades with poor nutrition (Isbell, 1995). One possible function of sexual selection is for females to choose mates with “good genes.” Some such genes may be those involved with the major histocompatibility complex (Penn & Potts, 1999). MHC genes are involved in immune function (Klein, 1986), and may influence the choosier sex to choose a mate that complements their own MHC allele diversity, choosing healthier mates with higher immune (or MHC) function based on sexual signal expression advertising individual health (Milinski, 2006).

## Study Site and Species

Vervet monkeys (*Chlorocebus pygerythrus*) live in multi-male multi-female troops year-round, with sex-specific linear hierarchies, and males exhibit a distinctive blue scrotum, red penis and red perianal area (Isbell, 1995). Males have been observed to lift their tail revealing the red perianal area, a white patch of hair, and blue scrotum, known as the red-white-and-blue display (Struhsaker, 1967). Female vervets exhibit concealed ovulation, in which signs of ovulation are not externally discernable to human observers (Andelman, 1987). Wild vervets have been observed to breed seasonally, however the vervets at the Lake Nabugabo Research Station in Uganda are capable of breeding year-round, possibly due to accessible and consistent anthropogenic food sources in the area (Schoof et al., 2015). Based on paternity analyses of a South African vervet population, male dominance and mating success were not correlated, and both high and low-ranking males likely both have some access to females (Minkner et al., 2018). In addition to producing a highly conspicuous genital signal, vervet monkeys are an excellent species in which to study color signalling and its relationship to dominance status because they have easily quantifiable sex-specific hierarchies (Cheney & Seyfarth, 1990). Female vervets are capable of resisting mating attempts from males (Struhsaker, 1967), therefore sexual signalling by males may also be driven by female choice. Male vervets frequently disperse to different groups, so group members may use the signal to supplement information about the incoming male, either competitive ability or in mate choice by females (Bergman & Sheehan, 2013; Setchell & Wickings, 2005). Subadult males were included in this study because they are capable of fertilizing and competing for females and exhibit colour development, even though they are frequently low ranking due to social immaturity and not having reached full adult body size (Cheney et al., 1988; Cowlshaw & Dunbar, 1991).

The vervet male's colourful genitals have been of fascination to scientists for years. Isbell et al., (1995) found that scrotal colour was predicted by individual health, as such perhaps parasitism may influence the production of the blue scrotal colour. Henzi (1982) found that subadult males have a paler blue scrotum than adult males, and Cramer (2013) found that as adult males get older the blue scrotal colour lightens, indicating that the scrotum may be a signal of age. There is also evidence that rank and vervet scrotal colour are related. In an experimental study where male scrota were painted different shades of blue, Gerald and colleagues (2001) found that

colour-matched males were more likely to aggress one another, suggesting the signal is a signal of rank. More recently, Young et al. (2020) reported that genital colour in a South African population of vervets was not predicted by rank, age, tenure length, injuries and GCs. However, males that were more dominant were more likely to present the red white and blue display, which also provides evidence that the signal may be related to dominance (Young et al., 2020). Henzi (1982) found that subordinate males were more likely than dominant males to retract their scrotum into their inguinal canal, hiding the scrotum, possibly as a means to avoid aggression from high ranking males and thereby suggesting that scrotal colour is tied to competitive ability or rank. While most studies focus on the blue scrotal colour, studies on the red penis aspect have been overlooked.

The objectives of my research are to: 1) examine the variation of the colour signal (hue, luminance, saturation and contrast) of both the red penis and blue scrotum in relation to androgens, glucocorticoids, age, dominance rank, and parasitism between individual males and 2) examine the variation of the colour signal (hue, luminance, saturation and contrast) of the red penis and blue scrotum in relation to the trade-offs of androgens, glucocorticoids, and dominance rank within individuals (Table 1 and 2, respectively).



Figure 1: Photo of two males from the same group (KS) taken during the same time of year (February 2019) at Lake Nabugabo, Uganda. There is clear colour variation of the blue scrotum and red penis. Photos by Karin Snyder.

**Table 1:** Hypothesis 1: Inter-individual variation in the expression of genital colour signal (blue scrotal and red penile) is influenced by individual (age), social (dominance rank), and physiological (AR, GC, parasitism) factors

<u>Prediction</u>	<u>Predictor Variable</u>	<u>Response Variable</u>
<p><b>Parasites</b> Males that are more parasitized will vary in colour from those that have less parasites.</p>	<p>Parasite prevalence Parasite richness</p>	
<p><b>Rank</b> If colour signals male competitive ability, reflected in their rank, then males of varying ranks will have different colours.</p>	<p>Ordinal rank</p>	<p>Blue hue, luminance, and saturation</p>
<p><b>Age</b> If colour signals age, then older males will vary in colour to younger individuals.</p>	<p>Minimum age</p>	<p>Red hue, luminance, and saturation</p>
<p><b>Androgens (AR)</b> If AR influences signal expression, then higher AR levels will produce a varied colour signal.</p>	<p>Androgens (ng/g)</p>	<p>Blue/red contrast</p>
<p><b>Glucocorticoids (GC)</b> If GCs influence signal expression, then we could expect colour variation between of varying GC levels.</p>	<p>Glucocorticoids (ng/g)</p>	

**Table 2:** Hypothesis 2: Intra-individual variation of colour over both the short- (3 months) and long-term (3 years) is based on the allocation and trade-offs of internal resources.

<u>Prediction</u>	<u>Predictor Variable</u>	<u>Response Variable</u>
<b>Rank</b> If colour signals competitive ability, individual dominance rank changes should be associated with changes in the color characteristics.	Change in Ordinal Rank	
<b>Age</b> If color signals age, color characteristics should change over time.	Minimum Age	Blue hue, luminance, and saturation
<b>Androgens (AR)</b> If AR regulates signal expression, individual AR variation will be reflected by related changes in color characteristics.	Androgens (ng/g)	Red hue, luminance, and saturation  Blue/red contrast
<b>Glucocorticoids (GC)</b> If GC regulates signal production, individual GC variation will be reflected by changes in colour characteristics	Glucocorticoids (ng/g)	

## Methods

### Study Subjects and Location

These data were collected by the field assistants and myself at the Nabugabo Research Site at Lake Nabugabo, Uganda under the supervision of Dr. Valérie A.M. Schoof. Data were collected on all adult and subadult males (>4 years) over two field seasons. During the first field season, May - June 2016, two neighboring groups of habituated vervet monkeys were observed: M group (n = 10 males) and HC group (n = 8 males). During the second field season, January - June 2019, three neighboring groups of habituated vervet monkeys were observed: M group (n = 9 males), KS group (n = 8 males) and HC group (n = 2 males). The habitat is characterized by wetlands, grasslands, patches of swamp forest, farmers' fields, degraded forests, and a few buildings (Chapman et al., 2016). The wet season occurs from March to mid-May, and November to December and the dry season from December through late February and mid-May through October (Chapman et al., 2016).

### Behavioural Data Collection and Dominance Calculations

Between 2012 and July 2019, *ad libitum* data collection was carried out to record data on all occurrences of male-male agonism, which is necessary to describe individual and group-level dominance hierarchy characteristics. We carried out 15- minute focal follows between January and June 2019, and 15-minute interval group scans conducted by the field assistants between 2012 to June 2019, which we used to supplement our *ad libitum* data. We included all observations of male-male submissive (e.g. submissive vocalizations, avoidance, cowering, fear grimace) and aggressive (i.e., bite, hit chase, lunge) interactions recorded since 2012 in order to construct a reliable hierarchy (Neumann et al., 2011; Young et al., 2017). For a full ethogram, see Appendix A. Because of the sequential and interconnected nature of behaviours during agonistic interactions, we defined bouts of agonism between a given male-male dyad as behaviours that occurred within 15 minutes of one another. The winner and loser from each bout were then recorded. In the case of no clear winner, this bout was recorded as a draw and removed from the analyses. In order to quantify male dominance rank for each individual, we calculated Elo scores for each individual, using the “EloRating” package in R (Neumann & Kulik, 2004, R Development Core Team, 2011).

Males were started with an Elo rating of 1000 for the first data point or when they entered a new hierarchy, and a “burn-in period” of at least 5 previous interactions for each male was necessary to determine the males’ actual rank. We used the optimized k function to determine the k-value (i.e., the amount that each agonistic encounter influences the Elo score by) that most suited our data for each group. Optimized k values were obtained by testing possible k-values between 5 and 200 using a resolution of 5000 (Newton-Fisher, 2017). By graphically examining where the loglikelihood values began to steadily decline for each group, we determined that  $k=76.75$  was best suited to use for all groups, as that loglikelihood for each group was the closest to the best possible loglikelihood while staying within an acceptable range. Finally, Elo scores were transformed to ordinal rank (numbered from most dominant to least dominant), which is the best measure for density-dependent competition in baboons (Levy et al., 2020), and we will apply here to vervets.

### Minimum Age Determination

While we had date of birth for some males in the study, it was not possible to know the age of newly immigrated males. To determine ages of such males, we calculated male minimum age (Young et al., 2020) by adding 60 months to the amount of time each male of unknown age had been in one of the study groups since transfer, based on male natal transfer age around 60 months (Cheney & Seyfarth, 1983).

### Colour Quantification and Analysis

Photographs were captured during two separate periods, in mid-January to mid-March 2019 (session 1), and May to June 2019 (session 2), to evaluate short-term seasonal and/or developmental changes in colouration. Photographs from May-June 2019 were compared to photographs of the same males taken in May-June 2016, photographed by Aneta Tasheva to examine long-term intra-individual variation.

Using the sequential method, we collected multiple digital images of each subject, followed immediately by an image of a color standard (X-rite ColorChecker Passport) under the same environmental conditions (Bergman & Beehner, 2008; Dubuc et al., 2014; Higham, 2006). All

images were taken from a distance of 2-10 meters in RAW format using a Canon EOS Rebel T4i DSLR camera with an 18-megapixel APS-C Hybrid CMOS sensor and an EF-S 55-250 mm f/4–5.6 IS lens (Dubuc et al., 2009; Higham 2013; Dubuc et al., 2014). White balance was set manually with a white standard and was recalibrated throughout the day as needed when lighting conditions changed. Aperture was set to the smallest setting to avoid spherical aberration and photos were slightly underexposed (based on visual examination of the histogram) to avoid clipping (Stevens et al., 2007). Linearization of the sensor and equalization of RGB values were assessed using gray card standards as per Stevens et al. (2007, 2009).

A photo shoot is defined as a group of photos of the same individual at the same time and location with only one chart image. For each individual, the best photo shoot was selected for each session based on the criteria that the signal was not over- or underexposed, was not obstructed by anything in the foreground, and was in focus. From each photo shoot, a best photo was selected based on focus and visibility of the genital signal. All photographs were standardised and colour-quantified using Image J software (Schindelin et al., 2012), with the Multispectral Image Calibration and Analysis tool (MICA) plug-in (Troscianko & Stevens, 2015). We measured each associated colour chart photo (X-rite ColorChecker Passport) to create a cone mapping model using D65 irradiance spectrum with an  $R^2$  value no less than 0.98, indicating reliable conversion of camera measurements to cone-catch values (Troscianko & Stevens, 2015). Vervet spectral sensitivity measurements are not available in the published literature. Instead, we generated cone mapping models using human spectral sensitivity in Image J, as it is both similar to that of vervet monkeys and sufficient for detecting colour differences in images (Jacobs and Deegan, 1999). Regions of interest (i.e., scrotum and penis) were selected on each vervet photo and measured in accordance to the respective cone mapping model, generating LMS quantal catches (longwave, mediumwave, and shortwave) and luminance values (Troscianko & Stevens, 2015).

LMS values were transformed into CIE  $L^*a^*b^*$  colour space using the R script pavo (Maia et al., 2013; R Development Core Team, 2013) in which the L designates luminance,  $a^*$  designates the red/green channel and  $b^*$  designates the blue/yellow channel. We further converted to CIE LCh space which involves calculating hue angle derived from the formula  $H = \arctan(b^*/a^*)$  and saturation from the formula  $C = (a^{*2} + b^{*2})^{1/2}$  (Maia et al., 2013; Renoult et al., 2017). CIE  $L^*a^*b^*$

was developed for the human visual system, however it can be used with catarrhine primates, as the visual system is comparable (Renoult et al., 2011). From this model, hue and saturation are scaled so that one unit of each colour characteristic is equal to one just noticeable difference (JND), or the minimum amount a stimulus must increase for a change to be perceived (Fairchild, 2005). Contrast was measured based on distance in Euclidean space, calculated using pavo, such that red and blue values from the same individual that were further apart displayed a higher contrast by maximizing hue disparity (Fairchild, 2005; Renoult et al., 2011; Maia et al., 2013; Young et al., 2020).

### Fecal Sampling: Hormones

We aimed to collect fecal samples from all study males twice per month before noon, in order to avoid any natural circadian hormone variation (Behringer & Deschner, 2017). Samples were collected within 10 minutes of defecation and stored in cool pack while in the field. At the end of the field day, samples were split for future hormone analysis and parasite analysis, with the fecal sample for hormone analysis being transferred to a freezer at the Nabugabo Research Site. Fecal hormone samples underwent preliminary field processing, normally within one month of collection. Samples were thawed and a 50:50 ethanol:water mixture was added to 0.5 g of feces. After vortexing the sample for 10 minutes, we separated the hormone-containing supernatant from the fecal pellet by centrifuging for 20 minutes, and 2 mL of the supernatant was processed through a Maxi-Clean Prevail C-18 solid phase extraction (SPE) cartridge manufactured by S\*Pure Pte Ltd, followed by a 2 mL water wash. Cartridges were labelled, capped, and stored in a cool, dry place until transported to the Glendon Primate Behavioural Endocrinology Lab at York University, Toronto. Following a 1mL 5% methanol wash, samples were eluted from the cartridges using 2mL 100% methanol with an Alltech vacuum manifold.

Extracted hormone metabolites were then taken to the Toronto Zoo's Reproductive Sciences Lab for analyses. Both fecal glucocorticoid metabolites (fGCM) and fecal androgen metabolites (fARM) were quantified using enzyme immunoassay (EIA) protocols modified from Terwissen et al. (2014) and Majchrzak et al. (2015). To remove the methanol, the hormone-methanol solution was dried down and reconstituted in EIA buffer. For fGCM, the sample dilution

range was 4-fold diluted to 3-fold concentrated. For fARM, the sample dilution range was 2-fold to 4-fold diluted. Microtiter plates were coated with 50 uL of hormone specific antibody diluted in coating buffer and incubated overnight. Testosterone antibody (R156/R157) and cortisol antibody (R4866) were previously developed by C. Munro (UC Davis). Plates were subsequently washed with 0.15 M NaCl solution containing 0.05% Tween 20, and reconstituted hormone extracts (50 uL) along with 50 uL horseradish peroxidase diluted in EIA buffer were plated. After a two-hour incubation at room temperature, plates were washed and 100 uL substrate solution (i.e. colour reaction solution ABTS) was added. Absorbance was measured at 405 nm using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA, USA). All samples and standards were run in duplicate, and a mean fGCM and mean fARM metabolite level for each session (2016, 2019 session 1 and 2019 session 2) were calculated and reported as nanograms per gram (ng/g). All samples were stored for potential future use at ultra-low temperatures (-86°C) to reduce likelihood of sample loss by evaporation.

Biological validation of fecal GC levels have been previously conducted using an Adrenocorticotrophic hormone (ACTH) challenge in captive vervet monkeys (Young et al., 2017). To test for parallelism, serial dilutions in EIA buffer of the vervet hormone extract were compared against a 9-point standard curve (GC standard: Sigma H0135, 78-20,000 pg/mL; AR standard: Steraloids Inc., A6950, 48-12,5000 pg/mL). All samples and standards were run in duplicate. Linear regression indicates a high degree of parallelism between the standard curves and the serially diluted samples. For GC, inter-assay CV's were 7.1% (high pool) and 7.2% (low pool), and intra-assay CV was 7.4%. For androgens, inter-assay CV's were 12.9% (high pool) and 8.9% (low pool), and intra-assay CV was 6.0%.

### Fecal Sampling: Parasites

For parasite analysis, between January 2019 to July 2019. as much fresh fecal sample as possible was collected in the field, and 1.0g was measured upon return to the lab. Samples were stored at the Nabugabo Field Lab, where they were kept in 2.0mL 10% formalin solution (for helminth parasite fixation). Samples were then taken in batches to the Central Diagnostic Laboratory in the College of Veterinary Medicine at Makerere University in Kampala, where they were examined for parasite eggs and larvae by modified ethyl acetate sedimentation method

(Greiner & McIntosh 2009; Valenta et al., 2017). Sedimented feces were prepared as slides and examined under a light microscope at 10X magnification. Parasite species were identified to lowest possible taxonomic level and photographed for further identification and documentation (Chapman et al., 2016). Parasitism is reported as prevalence (percentage of infected samples from a given male) and richness (total number of parasite species observed from a given male, out of the 6 possible genera identified over the 2019 study period). We chose not to examine load (eggs/g) as an index of parasitism because it can be a problematic measurement; shedding of parasite eggs by a host varies with the life cycle of the parasite and with host defecation patterns and may therefore not be representative of actual infection intensity (Chapman et al., 2007; González-Hernández, 2014).

### Statistical Analyses: Intermale Colour Variation

Multicollinearity of all response and predictor variables was assessed for 2019 data. As no variable had a VIF greater than 4, there were no multicollinearity issues (Mason, 1987). For each colour response variable (i.e., blue hue, red hue, blue saturation, red saturation, blue luminance, red luminance, and red/blue contrast), a best linear model was chosen using the ‘dredge’ function in the MuMIn package in R (Barton, 2014). We included all predictor variables (i.e., parasite prevalence, parasite richness, minimum age, fARM, fGCM and ordinal rank), potential interaction terms as well as Group and Individual Male ID as random effects in the model search procedure. Top models were those with an AICc > 2; however, models with an AICc score within 2 were considered to be equally valid and in such cases, we selected the model with the highest R<sup>2</sup> value (Dormann et al., 2018). We tested normality of the residuals with a Shapiro-Wilk test for all top models. Testing the normality of the residuals is better than testing the normality of the raw data, as it is less likely to incorrectly decide that normality assumptions are not being met when they are and can account for variation in heteroscedasticity (Kozak & Piepho, 2018).

### Statistical Analyses: Intramale Colour Variation

Data from 2016, 2019 session 1 (Jan-March) and 2019 session 2 (May-June) were compiled, and a Relative Interaction Index (RII) value was calculated using the formula  $RII = (future\ value - past\ value) / (future\ value + past\ value)$ . While RII values are often used for plant

biology, they have a range of potential applications because they present change over time linearly and using basic arithmetic, with values close to zero (e.g.,  $-0.1 < RII < 0.1$ ) indicating relative stability over time, and values closer to -1 and +1 indicating high degree of decrease or increase, respectively (Armas et al., 2004). For each male, we calculated RII values for each variable in the short-term (i.e., 3 months: session 1 and session 2 in 2019), and long-term (i.e., 3 years: 2016 and 2019 session 2). From the individual male RII values, using the function `cor.test` in base R, we conducted Pearson's correlations based on short-term and long-term RII values of all response (hue, luminance, saturation and contrast) and predictor variables (fGCM, fAR, and ordinal rank). All statistical analyses were done in R (R Development Core Team, 2013).

## Results

We carried out a total of 352 photo shoots (27 in 2016, 167 in 2019 session 1, and 138 in 2019 session 2). We quantified blue scrotal colouration for 20 males in 2016, 19 males in 2019 session 1, and 15 males in 2019 session 2. We quantified red penile colouration for 18 males in 2016, 18 males in 2019 session 1, and 15 males in 2019 session 2. As there was some overlap of males participating between sessions, there were a total of 31 unique males for all years and sessions.

We identified six different taxa of parasites collected from 157 fecal samples between January and June 2019. This included cestodes, the nematodes *Strongyloides* spp. and *Trichuris* spp., an unknown trematode, the trematode *Schistosoma mansoni*, and the protozoan *Eimeria* spp. We collected 219 fecal samples for hormone analyses (62 from 2016 and 157 from 2019). Descriptive statistics, including range, mean and standard deviation of parasite prevalence and richness, rank, age and hormone data are presented in Table 3.

We carried out Spearman's rank correlations on all predictor variables (Table 4). Prevalence and richness were positively correlated (intermale  $\rho = 0.48$ ,  $p = 0.0045$ ; intramale  $\rho = 0.59$ ,  $p = 0.016$ ). Rank and prevalence were negatively correlated for intermale variation (intermale  $\rho = -0.41$ ,  $p = 0.0018$ ). Finally fARM and fGCM were positively correlated (intermale  $\rho = 0.61$ ,  $p = 0.00019$ ; intramale  $\rho = 0.81$ ,  $p = 0.00019$ ). All other correlations were not significant.

### Intermale Variation in Genital Colour Characteristics

Parasite prevalence was retained as a predictor in all models of intermale color variation, while species richness was only retained in five of them (Table 5). Ordinal rank was retained in six of the seven intermale variation models and minimum age in only one model (Table 5). None of the colour characteristics were predicted by either average fARM or fGCM ( $p > 0.05$ ).

Blue scrotal coloration ranged from a blue-green (minimum hue angle =  $225.03^\circ$ ) to a true blue (maximum hue angle =  $268.13^\circ$ ). Males with a lower parasite prevalence had more blue-green

scrota (lower hue angle) compared to more true-blue scrota (higher hue angle) in males with higher parasite prevalence ( $\beta = 34.48$ ,  $F = 1.16$ ,  $p = 0.028$ ). The blue scrotal hue of high-ranking males (i.e., those with a lower ordinal rank number) was blue-green compared to true-blue in lower-ranking males (i.e., those with a higher ordinal rank number;  $\beta = 5.69$ ,  $F = 14.096$ ,  $p < 0.001$ ; Figure 2). In addition, there was a trend towards an interaction between parasite prevalence and ordinal rank, in which males the effect of parasite prevalence on blue hue was more intense for low-ranking than high-ranking males (Figure 3). Neither blue saturation nor blue luminance were predicted by any of our models (see Table 5).

In contrast to blue scrotal hue, red penile saturation and luminance were predicted by our models, while hue was not (see Table 5). Red penile saturation was predicted by the interaction of ordinal rank and parasite prevalence ( $\beta = -2.057$ ,  $F = 6.76$ ,  $p = 0.015$ ; Figure 4), in which the relationship between parasite prevalence and colour was positive for high-ranking males, but negative for low-ranking males. Additionally, age was a predictor of red penile luminance, with younger males having more luminant red (i.e., pinker) penises than older males ( $\beta = -0.010$ ,  $F = 11.38$ ,  $p = 0.0024$ ; Figure 5). None of our models predicted the contrast between red and blue characteristics of colour (Table 5).

### Short-term Intramale Variation in Genital Colour Characteristics

When examining short-term variation (i.e., three months between session 1 and session 2 in 2019) we were able to compare blue scrotal colouration for 16 males and penile colouration for 16 males (Table 6). Within males, there was a small short-term (i.e., session 1 to session 2 in 2019) increase in blue luminance ( $R_{II} = 0.12$ ) and a small decrease in fGCM ( $R_{II} = -0.10$ ). Mean parasite prevalence and species richness remained relatively stable over the three-month short-term period, as did blue and red hue, blue and red saturation, red luminance, contrast, fARM and rank. In the short-term blue saturation was negatively correlated to parasite prevalence ( $\rho = -0.54$ ,  $p = 0.030$ ) and parasite species richness ( $\rho = -0.50$ ,  $p = 0.048$ ). Blue luminance was negatively correlated with measure of parasite richness and prevalence ( $\rho = -0.71$ ,  $p = 0.0021$  and  $\rho = -0.52$ ,  $p = 0.038$ , respectively). Rank and red hue were negatively correlated ( $\rho = -0.50$ ,  $p = 0.047$ ). Contrast of red and blue was negatively correlated with species richness ( $\rho = -0.59$ ,  $p = 0.017$ ).

## Long-term Intramale Variation in Genital Colour Characteristics

When examining long-term variation (i.e., three years from 2016 and 2019) we were able to compare scrotal colouration of four males and penile colouration of three males, two of whom matured from subadult to adult within that time (Table 7). There was a mean long-term increase in blue saturation (RII = 0.32), blue luminance (RII = 0.21), red saturation (RII = 0.21), and contrast (RII = 0.49), while red luminance decreased (RII = -0.38; Figure 6); blue and red hue, fARM, fGCM, and rank were all relatively stable. There were no significant long-term correlations associated with blue scrotal or red penile intra-individual variation.

Table 3: Descriptive statistics of all predictor variables from 2016 and 2019.

Year	Session	Predictor	Range	$\bar{x}$	s
2016		Rank	1-9	4.40	2.47
		fARM	4.35-28.56	14.32	6.29
		fGCM	3.42-12.28	6.87	2.49
2019	All sessions	Prevalence	0.34-1.00	0.85	0.21
		Richness	1-5	2.69	1.09
		Rank	1-9	4.65	2.81
		Age (months)	36-108	70.16	19.29
		fARM	5.36-26.21	10.72	4.45
		fGCM	4.24-20.30	8.49	3.94
	Session 1	Prevalence	0.5-1.00	0.89	0.17
		Richness	1-5	2.94	1.16
		Rank	1-9	4.22	2.53
		Age (months)	36-105	68.61	19.42
		fARM	5.36-26.21	11.71	5.25
		fGCM	4.24-20.30	9.33	4.23
	Session 2	Prevalence	0.34-1.00	0.79	0.23
		Richness	1-5	2	0.98
		Rank	1-11	5.11	3.07
		Age (months)	42-108	72	20
		fARM	5.60-16.94	9.74	3.35
		fGCM	1.07-13.55	12.48	3.54

Table 4: Spearman's correlation coefficients ( $\rho$ ) and associated p-values (p) of all associated predictor variables for both intermale data (above mid-line) and short-term intramale data (below mid-line).

		Prevalence	Richness	Rank	fARM	fGCM	Age		
Prevalence	$\rho$		<b>0.48</b>	<b>-0.41</b>	0.26	0.13	0.288	Correlations of intermale data	
	p		<b>0.0045*</b>	<b>0.0181*</b>	0.14	0.45	0.104		
Richness	$\rho$	<b>0.59</b>		-0.19	0.027	0.21	0.23		
	p	<b>0.016*</b>		0.25	0.89	0.26	0.2		
Rank	$\rho$	-0.48	-0.085		-0.32	0.029	<b>-0.78</b>		
	p	0.062	0.75		0.068	0.87	<b>p&lt;0.001*</b>		
fARM	$\rho$	0.47	0.039	-0.31		<b>0.61</b>	<b>0.41</b>		
	p	0.069	0.89	0.24		<b>0.00019*</b>	<b>0.017*</b>		
fGCM	$\rho$	0.36	0.084	-0.29	<b>0.81</b>		-0.011		
	p	0.17	0.76	0.27	<b>0.00019*</b>		0.95		
		Correlations of intramale variations from RII values							

Table 5: Results of linear models of intermale variation for each colour variable with the predictors selected from model selection process. Bolded predictors indicate  $p < 0.05$ , and italicized  $0.05 < p < 0.10$

Response	Predictor	Intercept	Estimate ( $\beta$ )	F	p
Blue Hue	<b>Prevalence</b>		34.48	1.16	<b>0.028*</b>
	<b>Ordinal rank</b>	203.509	5.687	14.095	<b>0.00092*</b>
	<i>Prevalence:Ordinal</i>		-4.46	3.51	<i>0.072</i>
Blue Saturation	Prevalence		3.23	0.061	0.81
	Richness	18.7976	0.32	0.031	0.86
	Ordinal rank		-0.54	0.58	0.45
	Prevalence:Richness		0.034	0	0.99
Blue Luminance	Ordinal rank		-1.033	2.83	0.11
	Prevalence	37.2871	-14.53	2.32	0.14
	Richness		2.33	0.19	0.67
	Prevalence:Richness		-1.81	0.04	0.84
Red Hue	Richness		-15.23	2.85	0.11
	Prevalence	373.6064	-6.94	1.24	0.28
	Ordinal rank		-0.64	0.33	0.57
	Richness:Prevalence		12.091	0.55	0.47
Red Saturation	Prevalence		10.63	0.024	0.88
	Ordinal rank	2.3264	1.44	0.028	0.87
	<b>Prevalence:Ordinal</b>		-2.057	6.76	<b>0.015*</b>
Red Luminance	<b>Age</b>		-0.1	11.38	<b>0.0024*</b>
	Richness	16.7623	0.93	1.85	0.19
	Prevalence		-2.06	0.21	0.66
Contrast	Ordinal rank		-1.29	2.79	0.11
	Prevalence	10.312	-8.22	0.016	0.89
	Richness		-2.26	0.018	0.92
	Prevalence:Richness		2.42	0.043	0.84

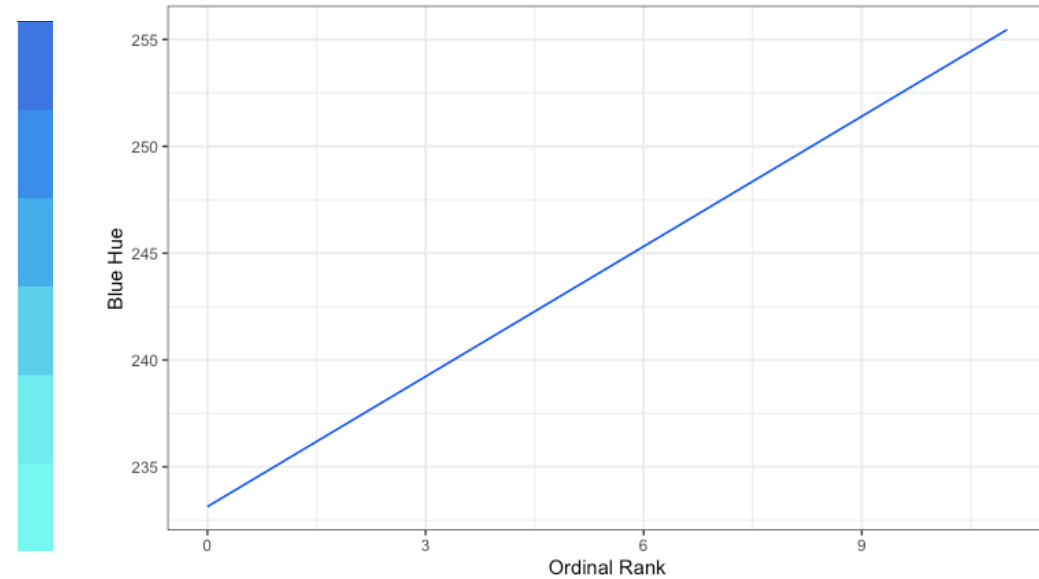


Figure 2: The effect of ordinal rank on blue hue based on the linear model with Individual ID and Group as random effects. Each dot represents an individual male that was recorded in 2019. This includes both session 1 and session 2. Higher ranking individuals are on the left (highest rank is represented by an ordinal value of 1).

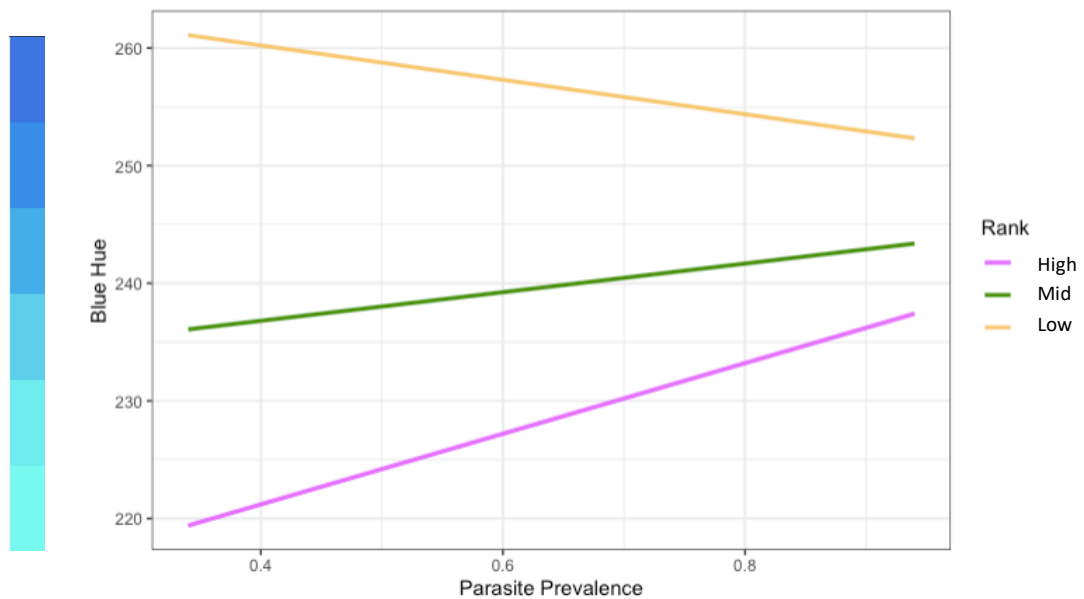


Figure 3: The influence of parasite prevalence on blue hue was negative for low-ranking males and shifted to positive for high-ranking males. Rank categories were determined using Jenks natural breaks.

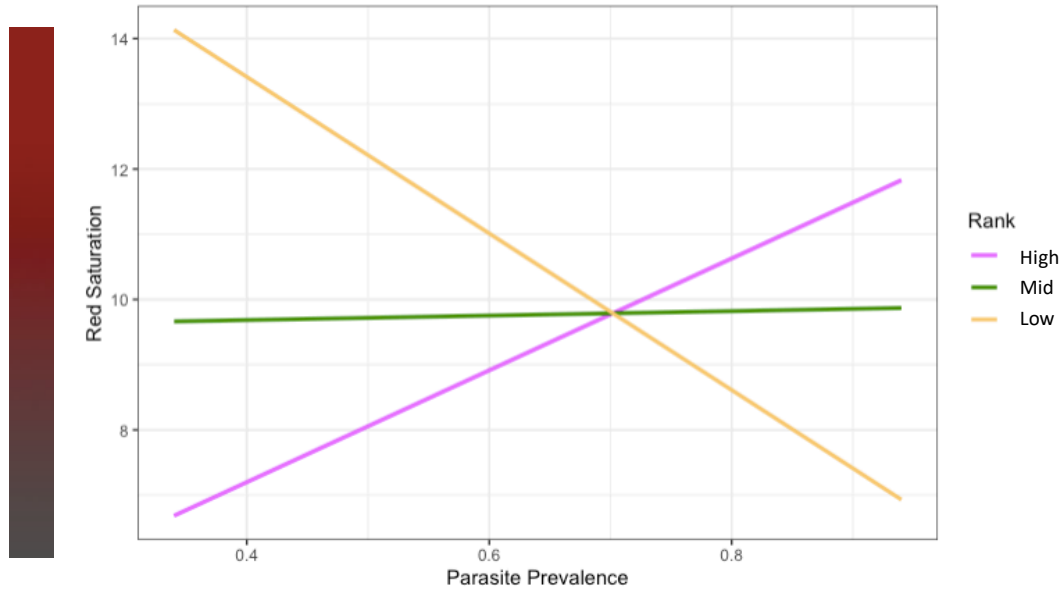


Figure 4: The influence of parasite prevalence on red saturation was negative for low-ranking males and shifted to positive for high-ranking males. Rank categories were determined using Jenks natural breaks

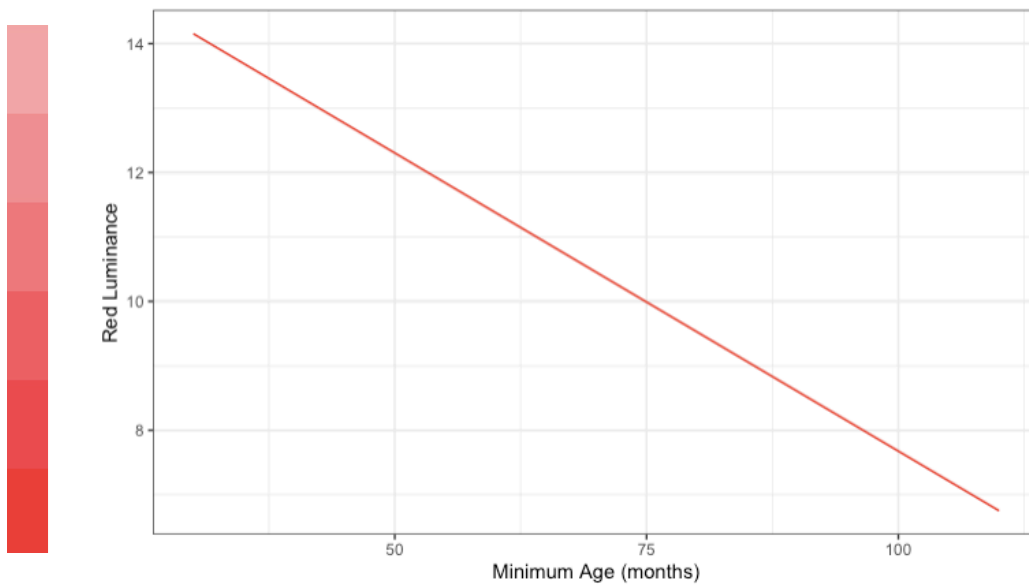


Figure 5: The effect of minimum age in months on red luminance. Younger males had lighter penises.

Table 6: Short-term (3 month) Spearman's correlation coefficients ( $\rho$ ) and p-values of individual male RII values

		Prevalence	Richness	Rank	fARM	fGCM
Blue	Hue	$\rho$ -0.04	0.17	0.34	-0.39	-0.015
		p 0.87	0.52	0.20	0.14	0.96
	Saturation	$\rho$ <b>-0.51</b>	<b>-0.61</b>	0.25	0.18	0.30
		p <b>0.044*</b>	<b>0.012*</b>	0.35	0.49	0.25
	Luminance	$\rho$ <b>-0.68</b>	<b>-0.59</b>	0.18	-0.058	-0.10
		p <b>0.0041*</b>	<b>0.016*</b>	0.52	0.83	0.71
Red	Hue	$\rho$ 0.099	-0.060	<b>-0.57</b>	0.012	-0.12
		p 0.71	0.82	<b>0.021*</b>	0.97	0.65
	Saturation	$\rho$ -0.15	-0.33	0.079	0.032	0.062
		p 0.58	0.22	0.78	0.91	0.82
	Luminance	$\rho$ 0.13	-0.16	-0.063	0.38	0.31
		p 0.62	0.55	0.82	0.15	0.25
Blue & Red Contrast		$\rho$ -0.42	<b>-0.69</b>	0.048	0.074	0.085
		p 0.11	<b>0.0032*</b>	0.86	0.79	0.75

Table 7: Long-term (3 year) Spearman's correlation coefficients ( $\rho$ ) and p-values of individual male RII values

		Rank	fARM	fGCM
Blue	Hue	$\rho$ -0.80	-0.40	0.80
		p 0.33	0.75	0.33
	Saturation	$\rho$ 0.80	0.40	-0.80
		p 0.33	0.75	0.33
	Luminance	$\rho$ 1	0	-0.40
		p 0.083	1	0.75
Red	Hue	$\rho$ -1	-0.5	-0.5
		p 0.33	1	1
	Saturation	$\rho$ -0.5	0.5	-1
		p 1	1	0.33
	Luminance	$\rho$ -0.5	-1	0.5
		p 1	0.33	1
Blue & Red Contrast		$\rho$ 0.5	1	-0.5
		p 1	0.33	1

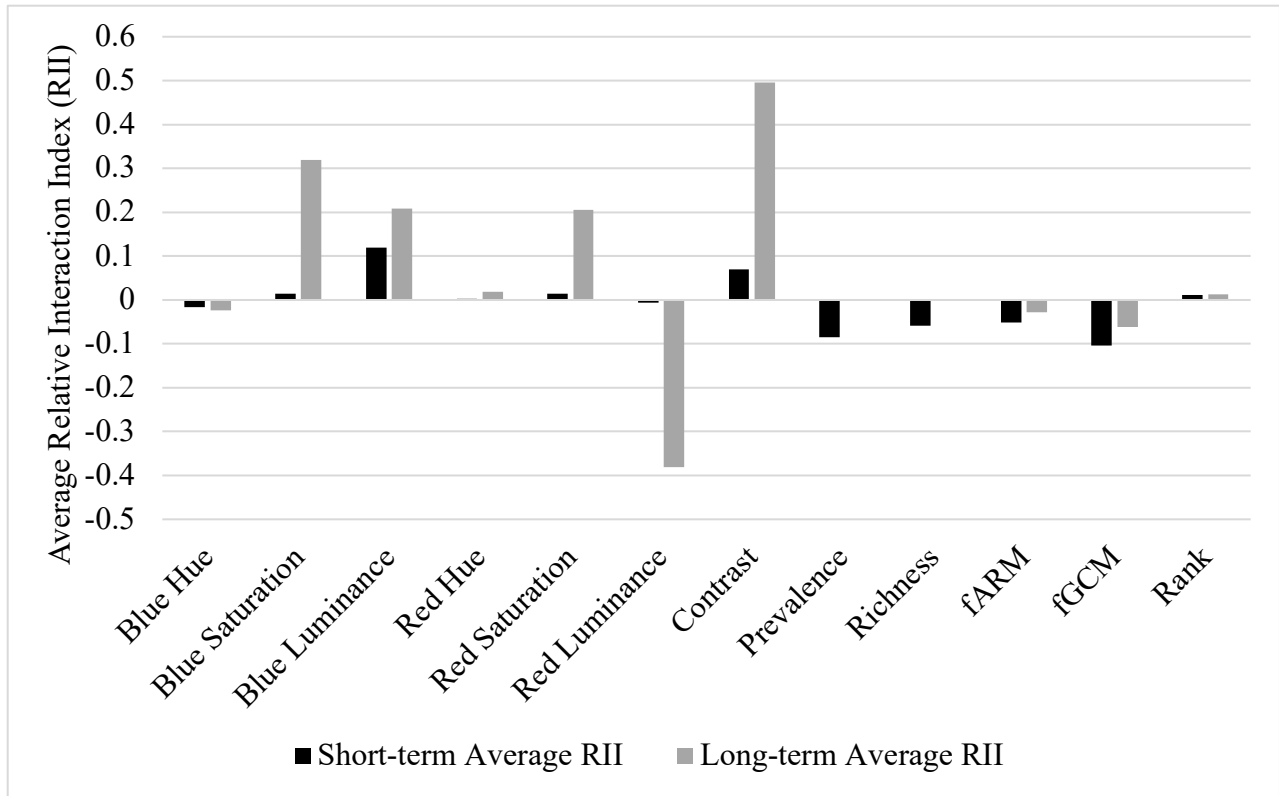


Figure 6: Average RII values of each individual male RII value for all short-term and long-term comparisons

## Discussion

Our study of intermale and intramale variation of scrotal and penile coloration in male vervets found partial support for the Hamilton-Zuk hypothesis (1982), but no support for the stress-mediated hypothesis or the immunocompetence handicap hypothesis (Folstad & Karter, 1992). Our results indicate that different aspects of both intermale and intramale variation in blue and red vervet genital colour are associated with measures of parasitism, dominance rank, and age, but not with androgens or glucocorticoids.

### The Hamilton-Zuk Hypothesis

According to the Hamilton-Zuk hypothesis, females will prefer males with exaggerated secondary sexual characteristics, as these traits are honest signals of individual quality (i.e., less parasitized). Thus, according to the Hamilton-Zuk hypothesis, we would expect males with increased trait expression to have fewer parasites; our results support this prediction, lending credence to the idea that the vervet male genital signal could play a role in intersexual selection.

We found an effect of parasite prevalence and ordinal rank on blue scrotal hue in vervets, where blue-green color was predicted by low levels of parasitism and high dominance rank. Therefore, blue hue is a signal of both parasitism and dominance rank. In addition, we also found a trend towards an interaction between parasite prevalence and rank, suggesting that the Hamilton-Zuk hypothesis was better supported in high- and mid-ranking males, but not low-ranking ones. While high-ranking males always produce an elevated blue hue signal in relation to low-ranking males, within each rank category our results suggest that blue colouration may be an honest signal among high-ranking, but not low-ranking individuals (Figure 3). We base our interpretation of the results on the assumption that a blue-green signal is the elevated variant of the signal, which is supported by previous findings indicating that higher ranking males are more likely to display a blue-green or aqua signal (Henzi, 1985; Gerald et al., 2001). Although we found that parasite prevalence was the only measure of parasitism that predicted intermale scrotal colouration, both prevalence and species richness were significant correlates of intramale variation. Consistent with the Hamilton-Zuk hypothesis, when a male's parasite prevalence and species richness changed

over the short-term, their blue scrotal colour also changed. For example, the scrotal color of males who became more parasitized were both greyer (less saturated) and darker (less luminant). This exploration of intramale variation addresses some of the challenges highlighted by Higham (2016), in which the true cost of a signal can only be determined by the cost an individual would face if they increase their individual signal expression. Few studies in mammals have examined the relationship between blue skin coloration and parasites. However, Isbell (1995) reported that scrotal blue faded in vervet males with poor nutrition. Nevertheless, it is difficult to equate the qualitative colour descriptors used by Isbell (1995) and others (e.g., ‘lighter’) to quantifiable color characteristics such as hue, saturation, and luminance. Thus, the blue signal may provide information about how parasitized an individual is based on their rank.

Surprisingly, we found the reverse interaction of parasitism and rank on red coloration. Specifically, we found that among low-ranking males, individuals with low levels of parasite prevalence had high red saturation, while among high-ranking males, individuals with high parasite prevalence had the least saturated red penile coloration. There is support for the Hamilton-Zuk hypothesis reflected in low-ranking males penis colouration but not in high-ranking male penis colouration; these results suggest that red colouration may be an honest signal among low-ranking, but not high-ranking individuals (Figure 4). We are assuming that saturated red is an exaggerated signal, given that higher ranking male mandrills and geladas have more saturated red faces and chest patches, respectively, and males become “redder” following a rank increase (Setchell et al., 2008; Bergman et al., 2009). Furthermore, in humans saturated reds may elicit a more salient emotional response than less saturated red and may therefore be more perceptible (Wilms and Oberfeld, 2018; Witzel, 2019). An effect of parasitism on red colouration has also been found in bald uakari (*Cacajao calvus*), where red facial skin fades with increased parasite load; parasitized uakari’s have been observed to have paler red faces, indicating that for the bald uakari, red colouration is a signal of health status (Ayers, 1996; Hill, 1965). In contrast to our findings and those for uakaris, Setchell et al. (2009) found no relationship between red mandrill nose colouration and measures of parasitism (while controlling for male age and rank). One reason for the different findings may be that we used a different method for quantifying colour; Setchell *et al.* (2009) measured red colour by dividing red intensity (metric determined in Adobe Photoshop) by luminance. Therefore, differences in findings may be due to methodological

differences or true species differences in signal information content. As an added layer of signal saliency, vervet male red penis hue varies from pink-red to a more true-red within males as they increased in rank. Perhaps the more true-red hue enhances perception of the red saturation, thereby acting as a signal of dominance rank, or competitive ability. The association between red skin colour and rank is also evident in both mandrills and geladas. Mandrills with redder noses were more dominant (Setchell et al., 2008), and geladas with redder chest patches were more dominant (Beehner et al., 2009). However, in macaques, red is more likely involved in mate choice, rather than signalling dominance rank (Higham et al., 2013; Dubuc et al., 2014).

We found that within males, blue saturation and luminance were negatively correlated with measures of parasite prevalence and richness in the short-term, and signal contrast decreased with increased parasite richness. Males with a higher number of parasite species richness may experience higher survival costs (Nunn & Altizer, 2006), and therefore higher costs associated with elevating their signal, so only males with lower parasite richness can increase their signal expression. Perhaps this is because the number of infections by unique parasite species has a greater influence over male colour variation, or that certain species of parasites are more costly than other species of parasites. Examining the prevalence of each parasite taxa of each individual may help to assess if parasites have differential effects on their hosts.

There are some challenges associated with parasite sampling. Since each male had between 2 and 8 fecal samples to calculate parasite prevalence from, calculating prevalence with small sample sizes presents challenges, as minute variation cannot be detected with smaller sample sizes (Jovani & Tella, 2006). We did not to examine load (eggs/g) as an index of parasitism because it can be a problematic measurement; shedding of parasite eggs by a host varies with the life cycle of the parasite and with host defecation patterns, and may therefore not be representative of actual infection intensity (Chapman et al., 2007; González-Hernández et al., 2014). Finally, our study only examined gastrointestinal parasites as a measure of health. Other measures of individual health (i.e., genetic, hematological) could yield different results. Since pathogen virulence and transmission mode may play a role, expansion of parasite sampling techniques could be very interesting, although some methods such as blood sampling is more invasive to the animals (Ezenwa, 2004).

## Sexual Signalling Reproductive Strategies and Rank

One potential interpretation of the finding that blue hue and red saturation are expressed differently in high and low ranking males, is that blue hue and red saturation aspects of the signal expression are primarily rank-condition dependent and secondarily parasite-condition dependent. Since conspecifics can likely already discriminate male rank (possibly by the blue signal portion as described above, or possibly through individual recognition; Borgeaud et al., 2019) conspecifics may assess high-ranking males differently than low-ranking males. Conflicting male tactics regarding the Hamilton-Zuk hypothesis between the blue and red signal components suggests that there are different strategies being employed between high- and low-ranking monkeys, that also differ for each signal aspect (i.e., red penis and blue scrota). For high-ranking males, an aspect of producing the blue signal may be more costly than producing the red signal, therefore high-ranking males are able to withstand the costs associated with cheating the red signal but not the blue signal. Blue and red skin are relatively simple to produce (Prum & Torres, 2004), therefore not a direct cost. Since the blue signal portion may also be communicating information directly about individual rank, the cost of signal elevation may be social, as elevating the blue signal may leave an individual more exposed to agonistic encounters with high-ranking males than elevating the red signal would. Thus, high-ranking males may be prioritizing energy investment in elevating the blue signal, while low-ranking males may prioritize energy investment into elevating the red signal portion at higher parasite levels.

Since low-ranking males possibly cannot withstand the costs of elevating the blue signal, they may be more able to withstand the costs of elevating the red signal instead. Low-ranking males are already producing a diminished blue scrotal signal in relation to high-ranking males, which may reduce some underlying costs involved in elevating blue signal expression. Since within low-ranking males there is an exaggerated blue signal at high levels of parasites (the signal is not honest), perhaps an aspect of exaggerating the blue signal within the category of low-ranking males is less costly or may be worth the reproductive benefits. In vervets, Cheney *et al.* (1988) reported that lower-ranking males experienced more harmful effects of gastrointestinal parasite infection than higher-ranking males, which may constrain low-ranking males from expressing

elevated red saturation with higher parasite infection. Future studies should consider the influence of rank when testing the Hamilton-Zuk hypothesis, and could seek to understand underlying costs associated with both the red and blue signal expression by examining predictors of colour within each male rank category.

The red portion of the signal may convey information to conspecifics about short-term changes, while the blue portion may be involved in more long-term stable aspects of signalling. Red skin colouration in primates can exhibit short term changes (Freeman-Gallant et al., 2009). Meanwhile, blue skin may be less subject to short-term variability than the red portion (Dixson, 1998). Setchell & Dixson (2001) found that blue luminance did not change within male mandrills over a year-long period, further suggesting blue skin colouration is relatively stable over time (Setchell & Dixson 2001). It is also possible that shifting hue is more restricted than shifting luminance or saturation. This is supported by our long term results, in which red and blue hue exhibit less intramale variation than luminance and saturation. Future research is needed to determine physiological mechanisms involved in producing each characteristic of colour, and whether hue is indeed a more constrained aspect of colour signalling.

### Stress-Mediated Hypothesis & Immunocompetence Hypothesis of Signalling

We did not find any support for either the stress-mediated or androgen-mediated immunocompetence handicap hypothesis of colour signalling. Fecal glucocorticoid metabolites (fGCM) were not included as a predictor of intermale variation, nor were glucocorticoid levels correlated to any measure of parasitism. In contrast, Cramer and colleagues (2014, cited in Turner et al., 2019) reported that “bluer” and “lighter” males had lower cortisol concentrations. Setchell *et al.* (2010) found that in mandrills, glucocorticoid levels did not predict red nose colour, but glucocorticoid levels increased with increasing parasite diversity, indicating that high parasite infections are costly in mandrills. In some species, including vervets (Schoof et al. 2018), elevated parasites have also been linked with increased glucocorticoid levels (Chapman et al., 2007; Setchell et al., 2010; Friant et al., 2016), which have in turn been linked to higher mortality (Pride 2005). In contrast with Schoof et al. (2018), we found no relationship between parasites and GCs, thus GCs may not be the mechanism by which parasites mediate colour signals in vervet monkey males.

We also did not find any support for the immunocompetence handicap hypothesis (Folstad & Karter, 1992), a hypothesis that posits elevated testosterone to be the mechanism mediating parasite resistance. Our results indicate that fecal androgen metabolites (fARM) were not a predictor of genital colour between or within males. Even though we did not find a direct connection between androgens and colour characteristics, this does not mean it is not directly implicated; perhaps other variations of androgen steroids are involved instead (Maney 2020). We found that intermale measures of fARM and age were negatively correlated, such that younger males had lower fARM levels, which has also been observed in white-faced capuchins (*Cebus capucinus*) (Jack et al., 2014). We also found that intermale measures of age and ordinal dominance rank were negatively correlated, such that younger males were lower ranking. While fARM did not directly predict genital colour, the correlation between fARM and age, as well as age and rank may indicate that there is an influence of fARM on signal expression (Higham 2016). Since we included sub-adult males in our study, this fARM increase could reflect males reaching reproductive maturity. Once reaching reproductive maturity, the reproductive benefits of both increasing rank and producing an elevated signal would likely increase, as now there is the potential for mating. Finally, a signal increase may occur after a change in dominance rank (Setchell & Wickings, 2005). Thus, the role of androgens in signal expression may be indirect (e.g., via increased aggression leading to rank attainment; Muller, 2017) rather than directly involved in signal production. This is supported in rhesus macaques, in which androgens and red facial luminance were not correlated (Higham et al., 2013), however experimental elevations of testosterone in rhesus macaques faces induced facial reddening (Rhodes et al., 1997), further indicating that androgens likely play a role in signal expression, yet the mechanism is indirect in wild populations. Future studies could look at rank and hormone levels within age categories in relation to signal production to help further elucidate the role of androgens in signal production, if any.

### Rank and Age Predict Genital Colour

Red luminance was predicted by age, in which younger males had more luminant (or lighter) penises than older males. We found similar results when examining long-term intramale

variation in penile coloration, where average red luminance decreased over the three-year period. Grueter et al. (2015) found that the red lips of black-and-white snub-nosed monkeys (*Rhinopithecus bieti*) were a signal of individual age. However, contrary to our results, Grueter et al (2015) did not find any variation in red luminance, finding instead that the variation came from the red-green opponency channel. Our results may differ from Grueter et al. (2015) because we used different methods for quantifying colour, and possibly also because our measure of age was an estimate of minimum age. As such, in our intermale analysis, it is likely that some males – the ones who immigrated from unknown groups or who were born into our study groups before research began – are in fact older than our estimated minimum age. Nonetheless, our findings from long-term intramale comparisons, in which males serve as their own control, show that red luminance decreases within individuals as they age. Cramer et al. (2013) also found that age was communicated by the vervet signal, but contrary to our results they found that blue skin becomes ‘bluer’ (from the blue-yellow opponency channel) and lighter with age.

As noted above, blue scrotal hue was predicted by rank, where males with more blue-green (rather than true-blue) scrota were higher ranking. Our results appear to conflict with Gerald *et al.* (2001), who found that ‘darker’ males became dominant while ‘paler’ males became subordinate, as well as with Young *et al.* (2020) who found that male dominance rank was not a predictor of scrotal color. The differences with Young et al. (2020) may result from using different ways to model colour (i.e., CIE  $L^*a^*b^*$  vs. colour opponency channels). Additionally, our contrasting findings with Gerald et al. (2001) may be due to the lack of clarity about which aspect of blue color was being perceived by male competitors, since that study used observer qualifications of colour described by ‘pale’ and ‘dark’ blue colour. Nonetheless, the findings of our study, Gerald et al. (2001) and Young et al. (2020) all support the idea that vervet genital scrotal blue colouration is indicative of an individual’s rank, or competitive ability. Further supporting the idea that vervet males are advertising competitive ability in their genital signal, Young et al. (2020) found that more dominant males displayed the red-white-and-blue (RWB) display more frequently.

Rank and age can be connected. In our study population, we found that younger males were lower ranking. One reason we might expect younger males to be lower ranking is because young adults and subadult males may not have reached optimal body size to be competitive with larger

males (Cowlshaw & Dunbar, 1991). In geladas, redness of the male chest patch was predicted by both rank and age, in that leader males (i.e., those who control one-male multifemale units) and prime-aged males had redder chests (Bergman et al., 2009), as prime-aged males are more competitive. We also found that over the short-term, intramale penis colour correlated positively with rank; males become redder (as opposed to pink-red) with increased rank, and older males were less luminant (darker) than younger males. Possibly, the red signal is changing in relation to both rank (via hue) and age (via luminance), therefore conspecifics may assess males in terms of prime age (darker red) and having successfully increased in rank (redder hue as opposed to red-pink hues). Whether this is an intersexual or intrasexual signal would be an interesting avenue for further research, possibly by assessing the signal in terms of rates of female solicitations or matings and bouts of male-male aggression.

The vervet genital signal may function as a badge of status in relation to rank or reproductive maturity. Badge of status symbols are useful when receivers have no previous knowledge of an individual (Bergman & Sheehan, 2013). Since male vervets disperse to new groups roughly every five years (Cheney & Seyfarth, 1983), conspecifics in the new group do not have any social information about new immigrant males. Since signals evolve to benefit the sender, signalling competitive ability may benefit the incoming male. For example, when the male blue signal was experimentally manipulated, similar-coloured males would aggress one another (Gerald et al., 2001). Therefore, in vervets the blue signal may function to signal competitive ability to other males to avoid unwanted aggression from more competitive males. This is supported by our results, as blue hue was predicted by male rank. This has been observed in both geladas and mandrills (Setchell & Wickings, 2005; Bergman and Sheehan, 2013). The signal may also be a signal of intersexual selection, or mate choice, and signallers may receive more sexual solicitations from females. To further examine the function of the badge of status signal and how it is involved in intersexual and intrasexual selection, focal and scan data could be used to determine rates of male-male aggressions and sexual solicitations from females towards new males compared to males that have had a longer tenure in the group.

### Multicomponent Signalling: Red and Blue Contrast

By signalling a blue component next to a red signal component, vervet genital coloration can be considered a multicomponent signal, and the two colours beside one another may increase signal saliency by increasing contrast (Renoult et al., 2011). While contrast was not predicted by any variables in our intermale models, parasite species richness was negatively correlated with short-term intramale red and blue contrast. Since both both parasite prevalence and richness were moderately negatively correlated with blue saturation and luminance in the short-term, it is possible that blue colour characteristics adjust within the individual to maximize contrast with the red penis, which is signalling for changes of parasitism within that individual. Unlike our findings, Renoult et al. (2011) noted that mandrill red and blue facial skin exhibits maximized contrast based on individual rank, however they did not look at any measures of parasites. Since contrast decreases within vervet males as parasite richness increases, males with a higher parasite species richness appear to produce a less salient signal. Even though contrast in mandrills and vervets were predicted by different characteristics, it is likely that similar to mandrill faces, vervet genital sexual signalling is driven by both red and blue colours in tandem. Additionally, contrast increased on average among males in the long-term, while rank, fGCM and fARM were relatively stable over time, indicating that contrast could be a signal within males of age or reproductive maturity. However, this warrants further investigation, as the sample size for long-term variation was too low to determine any significant relationships.

Whether an individual's penis is erect or not may provide an extra layer of social information all within the same signal package. Additionally, the red-white-and-blue (RWB) display may also act as another component of the signal. Young et al. (2020) found that more dominant males engaged in RWB display more frequently than more subordinate males. Similar to our findings, this indicates that dominance rank may be conveyed by the signal. Yet in contrast to our findings, Young et al., (2020) reported that in their population of vervets, none of the male genital colour characteristics were predicted by dominance, age, tenure length, injuries, or fGCM. Unlike this study, Young et al. (2020) used a different metric for describing colour (see above), which may explain some differences in our results. Also, the red region of interest differed between the two studies, with a focus on penile red colouration in our study and the red perianal region in the Young et al. (2020) study. Finally, we included parasites and fARM as predictors in our model,

including the role of parasitism which we have shown is correlated with rank and age, which Young et al. (2020) did not include, so this could also account for some differences in our results.

## Colour Perception and Modelling Colour

While the properties of colour are perceived in concert with one another, varying characteristics of colour may influence emotional responses in humans. It was found that when viewing more saturated and luminant colours, humans would experience increased arousal (characterized by increased heart rate and skin conductance response) (Wilms & Oberfeld, 2018). In the case that this effect may be similar in primates as in humans, then increased saturation and luminance could increase emotional response, which may influence receiver perception of the signal. For example, in mandrills increased blue and red saturation was related to higher ranking males, thus males with increased saturation may have a more detectable signal of rank (Renoult et al., 2011). Observer-based descriptions of colour likely conflate colour characteristics given that color is perceived through the combination of hue, saturation, and luminance (Wright, 1984).

We chose to use CIE  $L^*a^*b^*$  (or CIE LCh) model because it is a quantitative, well-established and uniform way to describe characteristics of colour (Schloss et al., 2018). We chose the CIE  $L^*a^*b^*$  space because it attempts to model how colours subjectively appear at the end stages of colour processing, while opponency channels or LMS values model an earlier stage of colour processing (Fairchild, 2005). However, since CIE  $L^*a^*b^*$  is modelled based on the human visual system, animals with vastly different visual systems would require a different model approach. Since humans and vervets have similar visual systems, which is relatively conserved for most catarrhine species (Jacobs, 2008), CIE  $L^*a^*b^*$  colourspace is useful (Renoult et al., 2011). Colour vision is often thought to tie directly to linkage of cone types and number. Even if at the cellular levels they appear quite similar, other neurological and social processes may influence colour perception (Cuthill et al., 2017). In fact, later stages of neural processing of colour and psychophysical processes relating to colour detection, discrimination and interpretation remain poorly understood (Cuthill et al., 2017).

## Future Directions

While we were able to look at some preliminary patterns of long-term variation over the three years, all long-term variation was sampled between three males for red colour variation and four for blue colour variation. Given the lack of statistical power in these long-term analyses, we provided qualitative patterns, but it would be valuable to repeat this study in a few years with a larger sample size. It would be very interesting to pursue further research into long-term variation within males to understand what social and behavioural factors are influencing signal production within males, although certainly difficult data to acquire.

There may be other factors influencing the signal that I have not addressed in this research. For example, fARM and fGCM levels are subject to fluctuation due to social and ecological influences. Response to reproductive competition or interaction with females may influence fARM levels (Goymann et al., 2019) and social and ecological factors (e.g., time spent grooming, predation risk, hierarchy instability, ecological and mating season) may influence fGCM levels (Sapolsky, 2005; Higham 2016). Future research should aim to include these factors, in particular social dynamics and season variation of dispersal and conceptions. In vervets at Lake Nabugabo there is some season variation of conceptions that is timed with male dispersals (Schwegel et al., in prep.), and this may be reflected by short-term seasonal changes of genital colouration. Applying a social network analysis to this research could help to elucidate the role of the signal in social contexts and provide further knowledge of vervet male communication networks (Snijders & Naguib, 2017).

## Conclusion

These results provide compelling support for the Hamilton-Zuk Hypothesis. For the red signal, parasites have less effect on colour production in high-ranking males than in lower-ranking males, while the blue signal provides an honest, elevated signal in high-ranking males, and a diminished and dishonest signal in low-ranking males. Based on the results of blue and red variation, this suggests that male rank is a good predictor of male quality. This signalling is likely not mediated by glucocorticoids, however androgens increase with age, and older males

are higher ranking, so androgens may represent one underlying cost associated with elevating the signal. We found that the signal is likely a multicomponent badge of status symbol that is conveying information about rank, age and male quality. It could be that individuals are using both the colour signal and social information to make decisions about mate choice and influence male-male competition

### Broader Significance

Animal communication occurs over multiple modalities, with the visual colour signal displayed in male vervet monkey genitals. Signalling and communication can play an important role in functions such as territory defence, conflict resolution and avoidance, mate choice, individual recognition, group recognition and discrimination, and social contexts (Halliday & Slater, 1983). Signalling can help to balance reproductive investment of internal resources, such that it may offset reproductive costs (such as risk of injury) by advertising competitive ability or quality (Marler, 1965). Comprehensive understanding of animal sexual signalling and its connection to endocrine, environmental, social and immunological costs can help us to elucidate more factors involved in animal communication. Understanding how a primate reacts within its environment and with conspecifics is a useful tool for primate conservation, for example predicting the behavioural reactions of a species to novel social or ecological environments. Additionally, examination of endocrine foundations of behaviour in primates can provide insight into human behaviour and endocrinology.

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## Appendix A: Ethogram

### **Nabugabo Vervet Ethogram for Focal Follows**

#### **LETTER 1: Type of data to be entered**

**Self (S)** – focal, self- or generally-directed behaviour

**Contact (C)** – focal in physical contact = 0m

**Proximity (P)** – focal within <1 body length from interactant

**Direct (D)** – focal gives, or directs, the behaviour

**Receive (R)** – focal receives the behaviour

**Mutual (M)** – mutual behaviour

**OOO** – out of sight

#### **LETTERS 2&3: Behaviour Codes**

##### **SELF or GENERALLY DIRECTED (S)**

**Rest (RE), state** – individual is sitting in a relaxed (i.e., non-vigilant) manner; may also be lying on side or straddling a tree branch (Struhsaker 1967a)

**Scan (SC), state** – individual is looking around, turning its head, in the farther visual distance >2meters; often occurs as a standing pause during a “move” (included visual foraging only during summer 2016 project); differs from “rest” because the animal is usually not sitting or lying down.

**Visual Forage (VF), state** – individual is slowly moving or standing still while looking around in foreground <2m (on the ground, in tree or shrub branches, grasses); differs from “Move” because “Visual Forage” is often includes locational displacement less < 1 meter.

**Move (MV), state** – walking or galloping, occasionally including a hop, and may be preceded or interrupted by vigilance (Struhsaker, 1967a); locational displacement greater than 1 meter and excludes positional changes/adjustments with locational displacement of less than 1 meter (Schoof).

**Vigilant (VG), event** – standing erect on hindlimbs staring in one directing or glancing in several directions (Struhsaker, 1967a); if in tree, the actor stands quadrupedally or sits with chest forward and stares or glances (Schoof)

**Self-Groom (SG), state** - an individual combs through its own pelage using fingers and/or mouth, and may place foreign particles in mouth (Schoof 2016, included scratching)

**Scratch (SK), event** – includes scratching (*sensu* “self-cleaning” in Struhsaker 1967a).

**Feed (FE), state** – individual is manipulating and/or ingesting food.

- When possible, the observer should note the food species and part in the “comments”
- Food parts include: Ripe Fruit (RF), Unripe Fruit (UF), Unknown Fruit (FR), Flower (FL), Young Leaf (YL), Mature Leaf (ML), Leaf Bud (LB), Young Leaf Petiole (YLP), Mature Leaf Petiole (MLP), Bark, Dead Wood, Pine Needles, Seeds, Seed pods, Pith, Soil, Tuber/Root/Potato

**Drink (DR), state** – individual is ingesting water from a ground or tree source (natural or man-made), normally involves lowering head towards standing water from water holes or rain pools; individuals may also lick water from hands, fingers, tree branches, or pelage of others (Struhsaker 1967a).

**Yawn (YW), event** – Mouth open all the way, briefly, in the vertical rather than horizontal plane; head may go back a bit. Lips may cover teeth or roll back, exposing top and bottom canines.

**Other (OT), state/event** – any behaviour not described in the ethogram; use “comments” section to describe behaviour

**Out of sight (OO), state** – focal animal is out of sight of the observer, usually resulting from substantial visual obstruction such as a building or dense vegetation, or because the focal animal has been “lost”.

### **SOCIOSEXUAL (S,P, C, G or R; occasionally M)**

**Hip grab (HG), event** – actor will stand behind female and grab at her hips pulling her in as if to mount but does not mount.

**Inspect (IN), state** – actor visually inspects, touches, licks or smells the genital region of the recipient; this may include looking from a short distance (<1ft), manipulating with hands or mouth, touching with face and includes various forms of “muzzling” as described by Struhsaker (1967a). Self licking or manipulating (males genitals) is S,P or C

**Present (PR), event** – female actor stands quadrupedally and orients her hindquarters towards the male recipient, sometimes glancing over shoulder; female may stop to present if followed by a male or male grabs hindquarters of a sitting female (i.e., behaviour of “estrous female” as described in Struhsaker 1967a).

- Note: a male focal animal can therefore Receive a Present (RPR) from a female Interactant.

**Mount (MT), state** – actor holds mountee hips with its hands, and legs with its feet (i.e., foot claspings); mount with no thrusting; may be associated with grooming and individuals may alternate between role of actor and recipient; note: mount may be incomplete, with grasping of only one body part Struhsaker 1967a)

**Thrusting mount (TM), state** – actor mounts (see above) recipient with pelvic thrusts; thrusts may be relatively rapid and short and/or relatively slow and long (Struhsaker 1967a); may be followed by a pause before dismount. (Field assistants call this copulation or mating (CO).

- In the “comments” section, observer should attempt to (1) note whether the thrusts are rapid/short or slow/long, (2) count the **number of thrusts**, (3) note if the presence/absence of a “**pause**” before dismount, and (4) note the presence/absence of **ejaculate** on the male and female’s genitals.

**Mating refusal (MR), event** –female actor who is being grabbed by male remains sitting, lies down, crouches low, or walks away; note that female may also hit, lunge, or make “anti-copulatory squeal-scream” vocalizations (i.e., behaviour of “anestrous female” as described in Struhsaker 1967a).

**Penile erection (PE), event** – actor has a conspicuously erect red penis; may occur during 1) grooming when recipient is male, 2) intragroup agonism, usually by the aggressor (Struhsaker 1976a).

### **AFFILIATIVE (G or R; occasionally M)**

**Approach (AP), state** – One monkey moves into contact or within 2 meters of a second monkey (Jack 1998).

**Leave (LE), state** – One monkey moves out proximity (2 meters) of another monkey (Jack 1998).

**\*Breast feed (BF)** – actor suckles from one or both nipples simultaneously (Struhsaker 1967a); this is a behaviour directed by an infant (sometimes a young juvenile) to a female recipient; a.k.a., nursing or suckling.

**Carry (CA)** – actor has another individual, usually an infant, clinging to its ventral surface or sitting/straddling on its back while engaged in locational movement.

**Entwine tails (ET; Mutual behaviour)** – two individuals sitting in close proximity on a branch criss-cross their tails (Struhsaker 1967a)

**Groom (GR), state** – actor combs through the fur of recipient using fingers and/or mouth, and may place foreign particles in mouth; may be accompanied by lipsmacking or teeth chattering (Struhsaker 1967a).

**Groom solicit (GS), event** – actor presents a specific body part to recipient for grooming (Struhsaker 1967a)

**Ignore (IG), event** – actor presents a groom solicit that is not acted on by the recipient. (Event)

**Hug (HU), event** – actor wraps their arms around the recipient while sitting dorso-ventrally or ventro-ventrally (recipient-actor) and may be associated with grooming; a.k.a. embrace (Struhsaker 1967a).

**Play (PL; D, R, or M behaviour; or S for rare self-play), state** – Play behaviours are quite variable and encompass a large number of behaviours described elsewhere (e.g., grab, chase, wrestle, mount, groom, embrace, hop, etc...)

- in play, these behaviours – such as chase or groom – are “alternated with one another in rapid sequence” and “may be the major distinctions between play and non-play encounters, rather than uniquely different behavior patterns” (Struhsaker 1967a: 33)
- intergroup play between juveniles and juvenile males of different groups may occur (Struhsaker 1967a).

**\*Wean (WE)** – female actor refuses breast-feeding attempt, or nips and/or pushes away a suckling infant (Struhsaker 1967a); this is a behaviour directed by a female to an infant or young juvenile.

**Mouth to mouth (MM), event** – actor approaches recipient and the two put their mouth close together touching (or nearly).

## AGONISTIC

In “comments”, note the stimulus or context prior to the agonistic event(s), such as copulation/mount, male, infant, grooming, food, space, or unclear; do not leave blank (i.e., if you didn’t notice anything, write “unclear”).

### Aggressive/Dominance Behaviours (G or R; occasionally M)

**Bite (BI), event** – as described, usually accompanied by grabbing (Struhsaker 1967a)

**Bob (BB), event** – Jerking or bobbing of head or entire body in up and down motion (on sagittal plane); for body includes torso bobbing from quadrupedal to bipedal position – often associated with eyelid flash (*sensu* “jerking” in Struhsaker 1967a)

**Branch-shaking (BS), event** – Actor deliberately bounces once or twice on a branch, sometimes associated with racing through trees creating noticeable noise, normally associated with intergroup encounters (Struhsaker 1967b, Henzi 1982)

**Broadside (BD), event** – Actor stands perpendicular to receiver, pauses, and then moves on; actor's tail may be raised. Note: Receiver is usually sitting (Henzi 1982; *sensu* “sideward-display” in Struhsaker 1967a)

**Chase (CH), state**– Actor moves rapidly toward the recipient, who runs away (*sensu* “chase-and-attack” in Struhsaker 1967a); recipient frequently looks back at actor while running away, and often emits submissive vocalizations

- note: some chases may be “false” (*sensu* “false-chase” in Struhsaker 1967a), wherein the actor gallops slowly and hesitatingly towards the recipient that is never caught → actor generally subordinate to the recipient

**Coalitionary Display (CD)** – any agonistic interaction involving three or more monkeys; the initial aggressor(s) is the “actor(s)”, while the initial victim(s) is the “recipient(s)”; additional details on the behaviours involved should be noted in the “comment” section.

**Eyelid flash (EF), event** – actor exposes lighter coloured eyelids by retracting brow while maintaining eye contact or staring at the recipient (Struhsaker 1967a); may also be submissive (“defensive”) in nature if actor is crouching (Struhsaker 1967a)

**Hit (HI), event** – actor hits, or slaps, the recipient with its hand (Struhsaker 1967a)

**Lunge (LU), event** – Actor leaps or jumps towards the recipient. Forward lurching of the chest.

**Penile Display (PD), event**– Male actor displays red and erect penis while standing bipedally or sitting upright with his hands on the recipient, whose face is directed towards the actor's genitals (Struhsaker 1967a).

**Red-white-and-blue display (RB), state** – Male actor displays the red perianus, white pelage between anus and scrotum, and blue scrotum to the recipient; male encircles or paces next to recipient with tail lifted, or briefly stands on his hindlimbs while oriented towards the recipient, thereby exposing genitals; actors include only adult, subadult, and older juvenile males (Struhsaker 1967a; Henzi 1982). Note: Receiver is usually sitting and vocalizing. Like a male presenting/ may involved movement around receiving individual.

**Solicit Assistance (SA)** – aggressor or victim of aggressive behaviour solicits assistance from others by “head flagging”, glancing back and forth between opponent and individual assistance is being solicited from, or vocalizing (e.g., chuttering)

**Supplant (SU), event** –the actor moves towards the recipient and occupies the space, eats the food, takes copulatory position, or assumes the grooming position of the recipient; the recipient or “supplantee” generally moves away, sometimes engaging in submissive behaviours such as lip-smacking or submissive vocalizations (Struhsaker, 1967a); a.k.a. displacement.

- Note: indicate in “comments” what the actor removed: food, grooming, or space (note that because “space” is generally taken when removing food or grooming from the recipient, “space” should only be used if neither food nor grooming were removed from the supplantee)

### **Submissive (G or R; occasionally M)**

**Avoid (AV), event**– at the approach of another individual (i.e., the recipient of the avoidance behaviour), the actor spontaneously vacates and moves away without any threat or aggression from the other individual; the recipient may just be passing by and does not occupy the space vacated by the actor (i.e., the individual doing the “avoiding”)

- note: if the space (or food/grooming partner) is then occupied by the approaching individual, this behaviour should be coded as a supplant directed by the approaching individual.

**Cowering (CW), event** – Actor lowers head/body by crouching towards the ground and avoids eye contact with recipient; actor may be moving away from recipient; usually associated with submissive vocalizations/lipsmacking (Henzi 1982; sensu “crouching” in Struhsaker 1967a)

**Fear grimace (FG), event** – actor opens mouth about halfway and exposes teeth by retracting lips on horizontal plane (resembles a “smile”); often accompanied by submissive vocalizations and staring at the recipient (*sensu* “grimacing” in Struhsaker 1967a).

### **VOCALIZATIONS (G or R; occasionally M)**

**Alarm call (AC), event** – Alarm calls are often multi-syllabic barks can be emitted in response to a real or perceived threat, most notably for “other vervet group”, snakes, eagles (i.e., *rraup*), leopards (i.e., *chirp*), & dogs and humans at Nabugabo ()

**Bark (BK), event** – low-pitched and gruff uni-syllabic exhaled vocalization emitted by males during intergroup encounters and occasionally during intragroup agonism; given towards other vervet monkeys who are fighting, it is emitted to stop the fighting (Struhsaker, 1967b).

**\*Chutter (CT), event** – low-pitched, monotonal and staccato vocalization emitted by females and juveniles to express aggression and solicit assistance; mouth is closed and the teeth are covered (Struhsaker, 1967b).

**Submissive vocalizations (SV), event** – lipsmacking, teeth-chattering, purring (Struhsaker 1967b, Henzi 1982), and also includes:

- **Woof woof (WW)**: This call is non-tonal, deep, and has a guttural sound (Struhsaker, 1967) emitted with closed or slightly-open mouth to indicate submission (Struhsaker, 1967b).
- **Wa (WA)**: This call is a continuous tonal exhalation that occurs with a grimace and indicates submission (Struhsaker, 1967b); may be combined with as “Woof-wa” vocalization.
- **Rraugh (RR)**: For this call the mouth is closed or partially opened and the teeth are covered (Struhsaker, 1967b). This call is emitted by yearlings when they approach older members of the group, and is a signal of nonaggression (Struhsaker, 1967b); includes both the long and short rraugh, and the aarr-raugh.
- **Lipsmack (LS)**: Moving the lips together quickly, opening and closing the mouth repeatedly.

**\*Squeal-Scream (SS)**: high-pitched, piercing calls usually are emitted by females and juveniles that are seeking help from threats by an aggressor, and may be anti-copulatory (Struhsaker, 1967b).

### **INTERACTANT(S)**

For identified monkeys, write their name or code in the Interactants (or NN) column;

For unidentified monkeys, use the following two or three letter codes; if there are multiple individuals of the same age/sex category, add number identifiers. For example, AM1, AM2

AM – Adult male

AF – Adult female without infant

AFI – Adult female with infant

AD – Adult, unsexed

SM – Subadult male  
SF – Subadult female  
SB – Subadult, unsexed  
JM – Juvenile Male  
JF – Juvenile Female  
JV – Juvenile, unsexed  
INF – infant (always unsexed)  
XX – Unidentified vervet  
ZZ – other species (if known, indicate the species in the “comments”; e.g., snake, bird, dog, cow)  
OO – observer  
OH – other human

- If there are multiple interactants, use a comma to separate their IDs

**ADDITIONAL NOTES:**

**Copulatory harassment** of a male or a mating pair has been observed, but the behaviours comprised in copulatory harassment include other behaviours described herein (e.g., biting, lunging, grabbing, etc...). As such, if copulatory harassment is observed, this should be noted in the “comments” of the associated behaviour.

**\*behaviours** identified with an asterisk (\*) are not usually performed by adult or subadult males; e.g., Breast feed, Wean, Chutter and Squeal-Scream.