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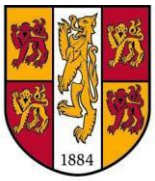

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Parasitism and Reproductive Effort in Male Rhesus Macaques: A Test of the Immunocompetence Handicap Hypothesis and the Stress-linked Immunocompetence Handicap Hypothesis

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Parasitism and Reproductive effort in male rhesus macaques: A test of the Immunocompetence Handicap Hypothesis and the Stress-linked Immunocompetence handicap hypothesis

A review of the relevant literature surrounding the Immunocompetence Handicap Hypothesis, and a study into the relationships between androgens, glucocorticoids, parasitism, and mating success in a group of free-ranging male rhesus macaques (*Macaca mulatta*)

Summary

A review of the literature showed, that across species, there is evidence of males experiencing lower immunocompetence than females. The Immunocompetence handicap hypothesis (ICHH) explains this by the occurrence of a trade-off between reproductive effort (via elevated androgens) and immunocompetence in males, specifically their defence against parasites. According to the ICHH, only high-quality males are able to withstand the immunosuppressive effects of elevated androgens, due to these elevations being energetically costly. The ICHH also proposes that female choice, is therefore, driven by the ability of the males to defend against parasites due to the benefits gained for their offspring. However, support for this hypothesis is inconsistent across and within species, possibly due to the need to include other environmental, social, or biological factors (due to their effect on parasite transmission), which is not always the case.

An alternative hypothesis tried to improve upon the ICHH by proposing the involvement of glucocorticoids (GCs) in the endocrine-parasite relationship. The Stress-Linked ICHH (SL-ICHH) proposes that GCs moderate the relationship between androgens and immunocompetence, however, again support for this hypothesis was inconsistent. Here I tested both the ICHH and SL-ICHH, on a group of free-ranging rhesus macaques, while trying to account for other confounding factors. This includes environmental factors such as rainfall and season, social factors such as

rank position, and biological factors such as age (*Figure 1*).

Overall, there was little support for both the ICHH and SL-ICHH. Androgens alone showed little relationship with male parasitism when accounting for other variables, and in the few cases where an interaction between androgens and GCs was found to affect parasitism, it was in the opposite way to predicted by the SL-ICHH; androgens were only negatively associated with parasitism when GCs were low. There was also more evidence for the role of rank in male mating success than male parasitism, suggesting that female choice is driven by the males' social status rather than their ability to defend against parasites. This study also highlighted the importance of testing different parasite species separately, as all species included here showed different results.

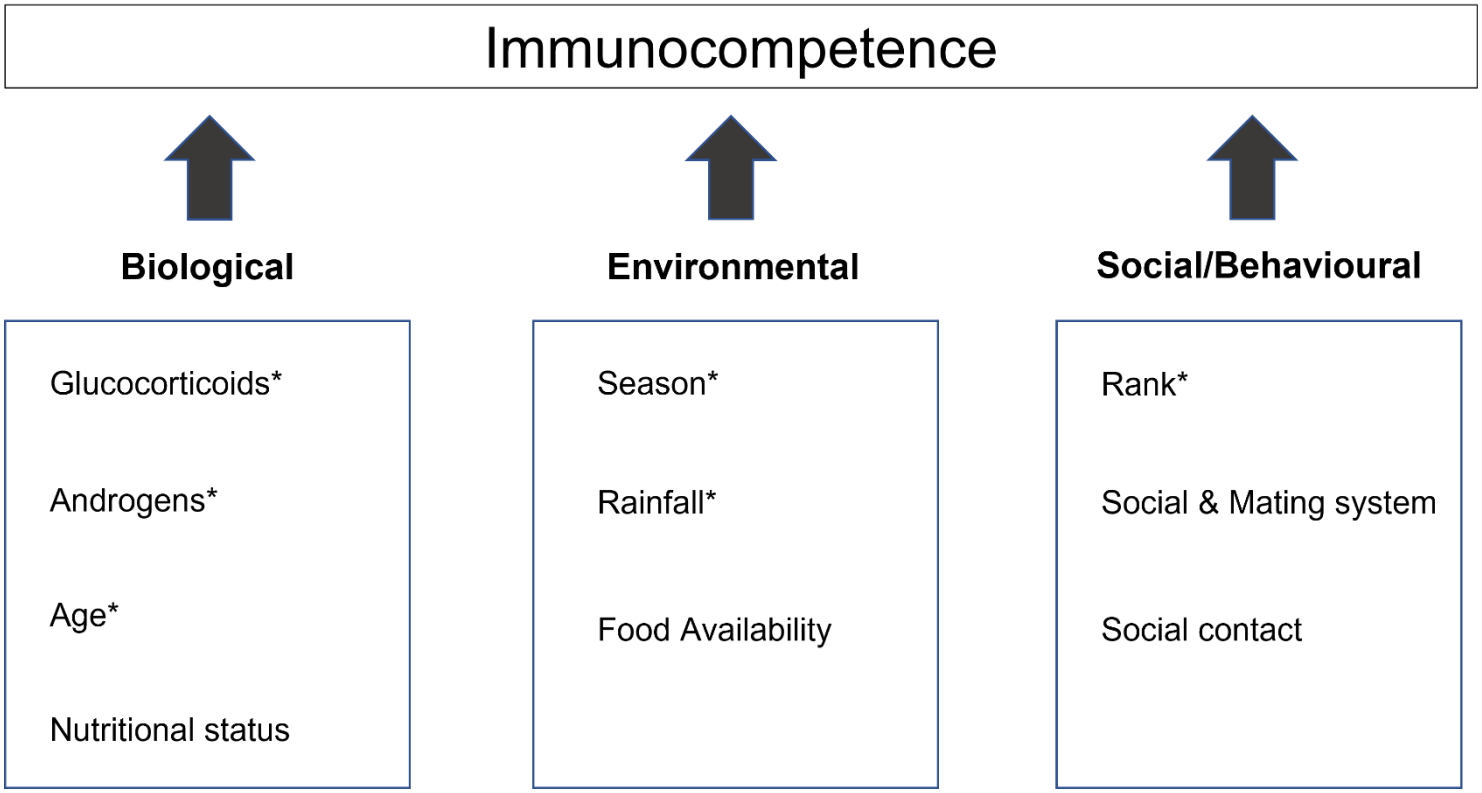

Figure 1*: A simplistic view of the biological, environmental and social factors discussed in this paper which may influence immunocompetence in male primates. Note that many factors are connected and can influence each other. For example, contact between individuals may occur more during the mating season due to increased competition, and rainfall and male rank can both affect how much food the male has access to and so impacts their nutritional status. Factors noted with * are explored in chapter 2.*

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Chapter 1: The Immunocompetence Handicap Hypothesis and factors affecting the endocrine-immune relationship in non-human primates – A Review

Abstract

The Immunocompetence Handicap Hypothesis (ICHH) proposes a trade-off between reproductive effort and immune function in males, due to the cost of developing secondary sexual characteristics, such as the diversion of energy away from the immune system. Previous research has so far found inconsistent support for the ICHH, both within and across many groups/species, and overall, the evidence is ambiguous. The Stress-linked ICHH offers an alternative hypothesis to try and resolve the ambiguous nature of the androgen-immunocompetence relationship by incorporating the effect of glucocorticoids on this relationship. The SL-ICHH proposes that glucocorticoids moderate the negative relationship between androgens and male parasitism, although the mechanism behind this remains unclear. However, support for this is also inconsistent within and across species. In this review I also look at other factors which could affect the endocrine-immune relationship in non-human primates and could therefore be contributing to the inconsistentsupport seen. Factors such as social/mating systems, seasonal differences and age are known to affect endocrine profiles of individuals, as well as parasite transmission, and other factors such as interaction with other individuals/environment and diet may increase the risk of parasite transmission and therefore affect immune responses. Due to the many of variables affecting parasitism of non-human primates identified here, future research investigating the ICHH should aimto include as many as possible to increase confidence in any endocrine-immune relationships discovered. As glucocorticoids have long been linked to variation in immune function, future research should include these hormones but also take care when interpreting the directionality between the relationship between infection and glucocorticoids.

ICHH Overview

The Immunocompetence Handicap Hypothesis (ICHH**)** produced by Folstad & Karter (1992) is based around two previous hypotheses, firstly Zahavi's (1975) idea that secondary sexual characteristics should act as a handicap for the males so that the females get an honest signal of their fitness. Secondly the hypothesis of Hamilton & Zuk (1982) which describes how female mate-choice is driven by the males' ability to effectively defend themselves against infection from parasites/pathogens. The ICHH proposes that due to the cost of developing secondary sexual characteristics, a trade-off occurs between male reproductive effort and immunocompetence (Folstad & Karter 1992). Immunocompetence refers to the overall function of an individuals' immune system and thus ability to effectively respond to pathogens (McCombe & Greer 2014). Measures of immunocompetence are often used in studies to look at factors which could be supressing the immune system (immunosuppression) and could thus be important in areas such as conservation to understand possible risk factors to species, but also for understanding human impact on wildlife (Wikelski & Cooke 2006). However, Immunocompetence can be measured in a variety of ways but often using antibody responses (Hasselquist et al. 1999) or endoparasite or ectoparasite load (Malo et al. 2008; Broche et al. 2017), White blood cell count (Zuk 1996) and other measures such as viral load after introduction to a virus (Muehlenbein et al. 2006).
Testosterone-dependent signals of fitness are honest because the strength of the signal depends on the likelihood of infection (due to immunosuppression) vs the likelihood of reproductive success (Folstad & Karter 1992). Each male is thought to have an optimal level of testosterone where the expression of secondary sexual characteristic is maximised with as little cost to their immune system as possible. Those with higher optimal testosterone and thus more developed sexual signals, should have higher immunocompetence.

Testosterone is known to influence of the development of secondary sexual characteristics (Mazur & Booth 1998; Schlinger et al. 2001; Setchell et al. 2008; Desprat et al. 2015), to aid reproductive effort. For example, testosterone was found to improve antler strength in Iberian red deer (*Cervus*

elaphus hispanicus) (Malo et al. 2008). In mandrills (*Mandrillus sphinx*), red facial colouration of males was also positively related to both faecal androgen levels and rank (Setchell et al. 2008) and in house sparrows (*Passer domesticus*) testosterone was found to influence the size of the patch of colour in their plumage (their "badge of status") (Evans et al. 2000). However, elevated levels of testosterone are also thought to suppress the immune system due to its role in the allocation of energy in vertebrates (Muehlenbein & Bribiescas 2005). The energetic resources that could be used for developing and maintaining the immune system, are used instead to develop the sexual characteristics (Wedekind & Folstad 1994; Muehlenbein & Watts 2010), to enhance reproductive effort at the cost of effective of immune responses. Therefore, it is thought that only individuals who are of high biological quality could withstand the immunosuppressive effects of testosterone, and thus via their testosterone-dependent traits, provide an honest signal of their phenotypic quality to females looking for a mate (Folstad & Karter 1992). Elevated testosteronelevels have been found to negatively affect immune function by increasing T-suppressor cells, reducing T- helper cells, inhibiting cytokine production and antibody release by B cells, and impairing the activity of natural killer cells and macrophages (Grossman 1990; Muehlenbein & Watts 2010).

There is a large amount of support for immunosuppressive effects of testosterone in birds, where testosterone implants were found to reduce immune responses and overall condition (Alonso-Alvarez et al. 2006; Fargallo et al 2007). However, in many immunocompetence studies it is important to closely examine the methods; for example, in Fargallo et al (2007), the results were recorded after 15 and 16 minutes after Eurasian kestrels (*Falco tinnunculus*) had the testosterone implant so can only represent the short-term effects of elevated testosterone, therefore we cannot assume that these effects would last long-term. In red jungle fowl (*Gallus gallus*), as predicted by the ICHH, female selection correlated to male ornamentation and these males had higher testosterone levels but appeared to have lower immunocompetence (lower lymphocyte levels) (Zuk 1996**)**. However, the use of only one immune parameter means we may not be seeing the full effectsof testosterone on the immune response**.** In contrast, higher testosterone levels were found to have no

effect on antibody levels in winged blackbirds (Hasselquist et al. 1999), thus reflecting the inconsistent support in birds. In mammals the support varies between species. In red deer (*Cervus elaphus hispanicus*) there was no relationship between testosterone and ectoparasite and endoparasite load (Malo et al. 2008). Whereas, in New Zealand fur seals (*Arctocephalus forsteri*), the highly territorial males who displayed high testosterone levels and reproductive effort, also showed higher parasite load (Negro et al. 2010).

According to the ICHH, females chose males for their genetic resistance to parasites so that these "good genes" are passed to offspring, however the use of parasites as a reflection of immunocompetence and likelihood of mortality is debated. Although controversial, parasites are often used as a non-invasive indicator of immune function in wild populations. A measure of parasitism may not be a direct reflection of immune performance, but they allow an insight into the host's ability to control infection (Muehlenbein 2006). This is especially important for these wild populations where invasive sampling cannot take place. Different parasites can also trigger the immune system of their hosts via various pathways and at different levels of intensity, making it important to look at all species present. Therefore, using both parasite richness (the number of unique parasites present) and the prevalence (the number of samples in which a parasite is present) of these parasites is a better measure of the hosts' overall ability to defend themselves against infection (Moller et al. 1999). Conversely, androgens may also affect parasites differently depending on the species of parasite. Some species may appear less affected by elevated testosterone, however, it is not certain whether this is due to direct immune suppression or behavioural differences limiting exposure to certain parasite species (Fuxjager et al. 2011).

Evidence for the ICHH and testosterone's effect on susceptibility to parasites, although inconsistent, is found in a wide variety of species, even in species with very different endocrine systems such as reptiles (Olsson et al. 2000), fish (Cowan et al. 2017) and insects (Nunes et al. 2020). This hypothesis may therefore apply to many species across different groups in some form or another, where males compete for mates. In scorpionflies, females had stronger immunocompetence but no difference

between the immunocompetence of offspring depending on the fathers' ornamentation (Kurtz & Sauer 1999). The study found that overall, the estimated effect of male ornamentation on offspring immune function was small but suggested it could still be enough to drive female mate choice. This study haslimited comparability to other studies in vertebrates due to the secretion of different androgens by invertebrates. However, the supporting evidence suggests the ICHH could act via different pathways according to the biological differences in invertebrates compared to vertebrates.

In reptiles, male sand lizards with elevated testosterone had higher tick loads and males treated with testosterone also had greater mobility leading to higher mating success (Olsson et al. 2000). Elevated testosterone during development may also affect future immunocompetence in reptiles as when male common lizard eggs were treated with testosterone, although they experienced a higher growth ratethan the control, they suffered a significant decrease in growth rate when exposed to ticks (Uller & Olsson 2003).

Evidence for the ICHH: Meta-analyses

There have been several meta-analyses looking into the ICHH and although most of them show some evidence for support of the ICHH, much of it is weak or mild (Roberts et al. 2004; Boonekamp et al. 2008; Foo et al. 2016; Habig et al. 2018). For example, testosterone was only found to be related to immunosuppression in reptiles but not in mammals or birds, therefore providing little support for the ICHH (Roberts et al. 2004). They did find some evidence for a relationship between testosterone and immune function but suggest androgens may not directly affect immunocompetence as proposed bythe ICHH. It is possible androgens affect behaviour, which indirectly affects the immune system through immunosuppression or immunoredistribution, (Roberts et al. 2004). The "priority of access"and "condition dependent" models (Habig et al. 2018) explain how testosterone may affect male behaviour and aid the acquisition of high status. This causes differences between individuals in their access to resources which can lead to exposure to infection (priority of access) or a higher physical condition making them less susceptible to infection (condition dependent) (see section below on rank effects).

Another meta-analysis finding a significant interaction between testosterone and immunocompetence, suggeststhe interaction between testosterone and the immune system occurs in the opposite direction to what the ICHHproposes, whereby the activation of immune responses from infection suppresses the secretion of testosterone (Boonekamp et al. 2008). For example, it is possible that production of cytokines affects neuroendocrine function (Roberts et al. 2004) and could thus affect testosterone levels. Previous research suggests that male reproductive effort, via testosterone elevation, could have a negative impact on immunocompetence; however, further exploration is needed to understand the relationship between testosterone and immune system, and the variation in results across studies. Testosterone may have varying strengths of influence over different immune branches which could mean parasites are affected differently depending on how they impact the immune system. One possible explanation could be due to the lack or inconsistent inclusion of other ecological (e.g., rank (Muehlenbein & Watts 2010), social interaction (MacIntosh et al. 2012; Balasubramaniam et al. 2016; Balasubramaniam et al. 2019), diet (Chapman et al. 2006; Prall & Muehlenbein 2014), season (Lee 2006; Gesquiere et al. 2010) and biological (e.g. glucocorticoids (Sapolsky 2004) and age (Haberthur et al. 2010)) factors which have been found to affect parasite transmission, immune function and testosterone levels. Below I introduce and explain how and why these factors are important to include in ICHH testing.

Stress-Linked ICHH

Similarly, to androgens, stress hormones such as glucocorticoids (GCs)are important for energy allocation (Sapolsky et al. 2000; Wingfield & Romero 2000; MacLeod et al. 2018), which could explain how theyalso affect how the immune system functions. GCs are produced by adrenal glands, and when elevated they initiate the 'fight or flight" response, where energy is diverted away from non-essential processes like digestion, to the more immediate needs for responding to a stressful event/period, such as increasing levels of glucose (Buchanan 2000; Emery Thompson & Georgiev 2014). The secretion of GCs allows the mobilisation of energy for the muscles which helps to initiate a physical response to a stressor (Sapolsky 2003). Short-term elevations of GCs are adaptive for

avoiding or dealing with danger and injury and may even enhance immune responses. For example, inducing migration of immune cells to an injury site to prepare for potential challenges/stressors they are about to face (Dhabhar & McEwen 1999; Buchanan 2000; Wingfield & Romero 2000; Sorrells & Sapolsky 2010). Whereas chronic or repeated acute elevations are thought to negatively affect the immune system and lead to HPA dysregulation (Wingfield & Romero 2000; Buchanan 2000; Sapolsky et al. 2000; Sapolsky 2004; Dhabhar 2009). Decreased number of immune cells and their function and promotion of pro- inflammatory responses are thought to be some of the immunosuppressive effects of chronic elevations of GCs, such as corticosterone (Dhabhar 2009).

Research has shown support for a relationship between GC levels and parasitism in animals, however the relationship is complex as we cannot be sure of the direction of this. Those with higher glucocorticoid levels can suffer from a reduction in their body condition which could then lead to increased rates of parasitism. However, parasitism is also known to raise glucocorticoid levels (Chapman et al. 2006). A recent meta-analysis (Defolie et al. 2020) found evidence of positive relationships between parasitism and GCs. Parasites may directly increase levels of these stress hormones by producing proteins that trigger an increase in their production, as well as increasing glucose in the blood (Defolie et al. 2020). Parasites are thought to induce stress via negative effects on nutrition (parasite infection may reduce absorption of nutrients – see below) and immune system (Snaith et al. 2008).

Supporting evidence for the ICHH often finds that T's negative relationship with immunocompetence is accompanied by higher GC levels (Kurtz et al. 2007). This, combined with the above evidence for the role of GCs in immunocompetence, suggests there could be some sort of interaction between testosterone and glucocorticoids influencing immunocompetence and a male's ability to defend against parasites. The stress-linked ICHH (SL-ICHH) proposes that GCs may moderate T's immunosuppressive effects whereby the negative relationship between androgens and male parasitism, only occurs when GCs are elevated. The way these two hormones may interact to affect

immunocompetence is complex and remains unclear (Rantala et al. 2012). Previous research has suggested the possibility that androgens and GCs compete for binding sites (binding globulins), so that an increase in one may cause an increase in free levels of the other (Klukowski et al 1997) and so could result in an interaction between the two hormones. In humans, it is thought that glucocorticoids may also moderate the relationship between testosterone and facial attractiveness as well as immune function (Rantala et al. 2012). However, others suggest that testosterone may indirectly suppress the immune system via testosterone's relationship with glucocorticoids (Roberts et al. 2004; Zang & He 2014). Elevations in testosterone levels may trigger an increase in glucocorticoids which directly suppress the immune system and reduces the ability for males to defend against parasites. In contrast, it is suggested that suppression of the whole immune system would be "wasteful and maladaptive" (Braude et al. 1999) so androgens may be immunomodulatory rather than immunosuppressive (Nowak et al. 2018). Rather than full suppression of the immune system, testosterone acts with corticosteroids, to redistribute immune cells to areas of the body where they are needed (Braude et al. 1999). Elevated testosterone could be a good indicator of possible injury (or stressful encounters), due to its link with dominant behaviour and reproductive effort, which activates corticosteroid production which in turn triggers immunoredistribution. This is where the HPA-axis is activated and in response, leukocytes move from blood to tissues where they may be needed (Braude et al. 1999). This redistribution may not be detected in studies investigating the testosterone-immune system relationship due to being very short-term, and what may have actually been immunoredistribution, was thus interpreted as immunosuppression, which would have made the ICHH appear more likely (Braude et al. 1999). Previous research shows that the interaction between testosterone and glucocorticoids andtheir relationship with parasite defence is clearly very complex and the mechanisms driving this arestill ambiguous.

ICHH and Non-Human Primates

Research into the relationship between testosterone and immune function is often difficult in non-human primates, possibly owing to logistical difficulties obtaining long term data especially in wild

populations. There is some evidence for the immunosuppressive effects of both testosterone and glucocorticoids separately in primates. Elevated testosterone has been linked to higher levels of viremia when long-tailed macaques were administered with Venezuelan equine encephalitis (Muehlenbein et al. 2006). Glucocorticoids have been linked to higher mortality and morbidity due to the physiological effects elevated levels have on the body (Sapolsky et al. 2000; Sapolsky 2004). When energy is constantly being mobilised due to chronic stress and high glucocorticoids, processes such as growth and immune function may be negatively impacted, as well as causing physiological effects such as increased bloodpressure (Sapolsky 2004). These could result in the individual to be more susceptible to infection. In two different studies on lemurs, those with higher levels of glucocorticoids were also found to have a higher mortality rate or lower survival probabilities (Pride 2005; Rakotoniaina et al. 2017).

In terms of the stress-linked ICHH in primates, like in most other groups, evidence is again inconclusive, especially evidence for the immunosuppressive role of T. Research has even shown that androgen supplementation may actually help reduce some aspects of age-related immune decline (immune senescence) in rhesus macaques (Rais et al. 2017). In terms of male parasitism, little correlation between both testosterone and cortisol and parasite eggs per gram (EPG) of faeces was found in Yakushima macaques (*Macaca fuscata yakui*); they suggested that suppression of the immune system throughcortisol may be too subtle to pick up (Broche et al. 2017). Results even indicated that testosterone may have a positive effect on immune function. However, it should be noted that sampling took place over 5 days giving a very small sample size. However other studies provide support for the SL-ICHH; testosterone was found to be associated with levels of viremia after exposure to Venezuelan equine encephalitis in long-tailed macaques when controlling for cortisol (Muehlenbein et al. 2006) and both testosterone and GCs were positively correlated to parasite richness in chimpanzees (Muehlenbein 2006). However, also in chimpanzees both testosterone and glucocorticoids were correlated with helminth richness but not protozoan richness (Muehlenbein & Watts 2010) showingagain how inconsistent the support for the SL-ICHH is

currently.

Methodological Considerations

It is often hard to reach conclusions regarding endocrine-immune relationships because of difficulties when comparing studies, usually due to methodological differences. As briefly mentioned previously in Zuk's (1996) study on red jungle fowl, a common issue of research into endocrine-immune function is that often, the focus is only on one area of immune function. For example, in the study mentioned above, results were only provided for humoral immune responses due to the type of immune measure used and do not show the possible effects of testosterone on cell-mediated immune responses. Therefore, as highlighted by Prall & Muehlenbein (2014), the most accurate way to test the relationship between testosterone and immune function would be to use multiple measures of the immune system. Using multiple measures would reflect more than just one branch of immune response so giving a more accurate indication of overall immunocompetence. This would also aid comparison between studies, which is often made difficult due to the variation in the immune response measured (Foo et al. 2016). However, it must be noted that Interspecies differences mean that one method of testing the ICHH is not always applicable for all, so some species may not be easily comparable to others.

This can relate to parasites specifically too, as the use of ectoparasites or endoparasites can be subject to different factors and may draw different inferences from the results depending on which is used. In studies, (e.g., Olsson et al. 2000; Uller & Olsson 2003), using ectoparasites as an immune measure, could lead to skewed results due to other ecological/environmental factors which can impact their transmission. Ideally studies should therefore control for these factors.

The method in which variables are measured may also impact results. Differences between measuring glucocorticoids through urine/faeces and blood can make it hard to compare results. Caremust be taken when comparing research as those using blood samples to measure GC secretion mayhave stress of capture effect which makes it more difficult to identify a true baseline

of an individuals' GC levels (Sheriff et al. 2011). This could affect the results, especially if some have experience of capture and some individuals don't (Hones & Marin 2006). As Cortisol shows circadian pattern of secretion, the time the blood samples were obtained must also be considered, preferably so there is little variation in sampling time across individuals, to provide more accurate results (Sorwell et al. 2015). The amount of stress an individual suffers from parasite infection may also depend on whether theindividual has been previously exposed to it. The parasite may have less intense effects on glucocorticoid production if the individual has built up a tolerance to it and already has a targeted response so energetic cost of responding to infection is lower (Defolie et al. 2020). If possible, it could be beneficial to try to measure changes in GC levels when the host is introduced to a new parasite to account for the effects of previous exposure. Research investigating the SL-ICHH shouldtry to account for new versus common parasites if both are present in the study, as responses to parasitism could differ and affect endocrine-immune relationships.

Using measures of stress can also give rise to issues of directionality, which can be common when testing endocrine-immune relationships; studies showing correlations or interactions cannot always be certain of the direction they are occurring. For example, it is often hard/impossible to identify if stress is causing a reduction in immunocompetence or if infection is causing a stress response. This is highlighted in the meta-analysis by Roberts (2004) where it is explained how activation of the immune system may also influence hormone production/levels.

The study by Foo et al. (2016) also highlights the importance of considering the type of study used when comparing research in endocrine-immune relationships. For example, in the meta-analysis, testosterone was found to have a significant moderate immunosuppressive effect across experimental studies and the effect appeared stronger in studies comparing to castrated individuals, however this effect was not significant in correlational studies. We must also consider the environment the subjects inhabit in the study; results based on captive or free ranging individuals will have limited applicationto wild populations due to different pressures being faced

(e.g. possible lower levels of competitiondue to a controlled diet and less fear of predation).Testosterone dependent immunosuppression could be related to differences between in access to food/resources, as well as the trade-off between reproductive effort and immune response (Habig et al. 2018) meaning that studies using provisioned populations may not pick up the same results.

This can also be an issue for studies investigating humans. For example, free testosterone was positively correlated to greater immune responses to an influenza virus vaccination, (opposite to the predictions of the ICHH) (Nowak et al. 2018). However, this study only sampled men from an affluent, society between the ages 18.9–36.7, so it is impossible to say that using men from a less developed area or outside this prime age group, with differing levels of stress/competition/overall physical condition, will produce the same results. Testosterone's regulation of energy allocation to the different processes is thought to also depend on the risk of infection in their environment. Therefore, those in places with low risk, such as the males in the study above, may afford to invest less in immunocompetence without suffering increased infection and so a negative endocrine-immune relationship may not be as apparent. It is thus beneficial to sample from a range of risk level environments to get a more accurate representation of the endocrine-immune relationship.

Sex-differences in Immunocompetence and Endocrine Levels

Much of the evidence for the ICHH comes from the differences seen in immunocompetence between males and females (Prall & Muehlenbein 2014). Although testosterone is produced in females in the adrenal cortex and ovaries, a much larger quantity is produced in the testis of males (Mazur & Booth 1998), which according to the ICHH is the main cause of differences in immunocompetence in males and females (Zuk & McKean 1996; Prall & Muehlenbein 2014; Foo et al 2016; Klein & Flanagan 2016). Past studies have looked at the possible explanations as to why, despite the obvious negative health/survival impacts, factors such as higher testosterone levels, leading to immune suppression would be selected. One possible hypothesis is the "Susceptible male

hypothesis" which, based on Bateman's principle (Bateman 1948), explains the differences in the method to increase fitness by the sexes (Rolf 2002). According to this hypothesis overall survival is more important to females so they can reproduce and raise their offspring successfully, whereas males gain more from investing less in immunity and more in reproductive effort (Stoehr & Kokker 2006). The benefits of increasing "attractiveness" to females outweigh the costs of reduced immune function, because by the time these costs have had an effect, they may have received the benefits of higher reproductive success compared to other males who invest more in immune function but are less "attractive" (Zuk & McKean 1996). High levels of parasitism do not necessarily lead to mortality in all species and low levels do not necessarily predict survival, so it could be questioned as to why females would select for males with low parasitism (Rakotoniaina et al. 2017). However, in mammals there appears to be a positive correlation between male-biased mortality and male-biased parasitism suggesting that overall, in mammals it would be beneficial for females to select for low parasitised males (Moore & Wilson 2002). Although an old study, a meta-analysis across vertebrates did find that males tend to be more susceptible to infection (Poulin 1996).

As with many species, evidence for sex-biased immunocompetence in primates is mixed. In Japanese macaques, males were significantly more likely to die from infectious diseases compared to females, however this is likely due to displaying more "risky behaviour", (often related to higher testosterone levels), rather than reproductive effort (Fedigan & Zohar 1998). Older male milne-edwards sifakas (*Propithecus edwardsi*) appeared to have higher mortality rates than older females despite no differences in testosterone. This again suggests that testosterone dependent immune suppression is not solely responsible for reduced likelihood of survival (Tecot et al. 2013). In wild populations of the monogamous Azara's owl monkeys (*Aotus azarae*), there was no difference in age-dependent mortality between males and females (Larson et al. 2015), but according to the ICHH this would be because of less competition for mates in monogamous species. Similar to other vertebrates (Reeder & Kramer 2005), female primates are thought to have a higher baseline of glucocorticoid level (Smith & French 1997). However, females have also shown higher levels of immunity than males; for

example, they tend to have a higher concentration of antibodies and higher B cell numbers and activation (Prall & Muehlenbein 2014). From this it could be inferred that glucocorticoids alone may not be immunosuppressive.

Parasite infection rates also vary between the sexes in primates; however, although it is thought generally males have higher susceptibility to parasites, evidence (even within studies) is inconsistent. For example, in wild Japanese macaques (*Macaca fuscata*) there was male-biased parasitism found in parasites directly transmitted but not in parasites indirectly transmitted. Also, EPG (eggs per gram) was significantly higher in males during the mating season but higher in females during the birthing season (MacIntosh et al. 2010). This suggests that environmental and behavioural sex-differences may affect sex-bias in parasitism in primates. In contrast, no sex-differences in parasite richness were found in Yakushima macaques, although the study period only took place during a few days in the mating season (Broche et al. 2017).

The degree of sexual dimorphism in a species may also affect sex-biased infection rates, as it may account for other behavioural factors affecting transmission. For example, if one sex is much larger in overall size than the other, they are likely to consume more food which would increase their exposure to pathogens/parasites (Nunn et al. 2003; Parr et al. 2013). Associations have been found between degree of sexual dimorphism in primates and male-biased parasitism, supporting the role of sex dimorphism on parasitism possibly though differing behaviour (Moore & Wilson 2002).

Sex-differences in immunocompetence are not limited to one group or species and these differences have been recorded across a number of different groups, not just primates. This has even been recorded in invertebrates; for example, female scorpionfiles (*Panorpa vulgaris*) were found to have a stronger immune system compared to males. However, offspring from the males who displayed more developed “handicaps” showed no significant difference in immunocompetence, thus does not support the ICHH despite the sex-differences seen (Kurtz. & Sauer 1999, Kurtz et al. 2000). Invertebrates have extremely different endocrine and immune systems compared to vertebrates

and so provides evidence for an evolutionary aspect to sex- differences in immunity. In amphibians, no difference in the level of parasitism was found between the sexes in non-breeding northern leopard frogs. However, there was female-biased parasitism for one parasite during breeding season (Dare & Forbes 2009), hinting that seasonality could play a role in sex-biased susceptibility to infection in other species, as well as primates (see section below).

It is possible that factors other than differing sex hormones could be responsible for sex-differences in immune function. In Siberian hamsters, females displayed greater immunocompetence compared to males when sex-hormones had been removed (Bilbo & Nelson 2001), suggesting that the sex-differences in immunity in research may not be dependent on sex-hormones but on another factor or on different hormones not tested. Some suggest that sex-differences in parasitism are more likely caused by ecological differences than differences in reproductive hormones. In threespine sticklebacks *(Gasterosteus aculeatus*), sex-biased parasitism appears inconsistent, where males had significantly higher parasitism of two parasites, but females had higher rates for the other two species (Reimchen & Nosil 2006). It is likely that sex-differences in habitat use and diet could be driving the differing levelsof parasitism.

The life-history traits of a species can also play a role in the allocation of resources to different processes/systems. Species that are slow-living (long lifespan with slow developmental rate, low reproductive rate and invest more into parental care) would benefit from investing more in survival than reproductive effort, compared to fast-living species (similar to females in the Susceptible male hypothesis) (Lee 2006). This may account for inter-species differences in males and females, where ifa whole species is “slow-lived”, males may not adhere to the “Susceptible male hypothesis” and viceversa with females. That said, the differences found in immunocompetence between sexes and slow/fast living species may just be reflecting differences in the type of immune responses being used. Females or slow living species may invest more in Th-2 adaptive responses which are non- inflammatory and so less costly than Th-1 responses (Lee 2006). This could possibly account for some of the inconsistent support for the

ICHH across species and why some show higher levels of sex-bias in immunocompetence.

It is also important to note that sex-differences only show that there is a difference in immunocompetence between males and females, not what causes this difference, so support for the ICHH from these types of studies are limited. Sex-differences in immunocompetence may be a result of testosterone's influence on behaviour, e.g., inducing more risky behaviour, rather than having a direct immunosuppressive effect (Poulin 1996; Fedigan & Zohar 1998; Zuk & McKean 1996; Braude et al. 1999).

The effect of Social/Mating System on the Relationship Between Testosterone and Immune Function

The Susceptible male hypothesis was updated to include the role of the strength of sexual selection, where males invest less in immunocompetence when sexual selection is high (Stoehr & Kokker 2006). However, males may invest more into immunocompetence if resulting parasite infection has a large impact on their physical condition, as this could negatively affect their reproductive success as they appear less "attractive". We would therefore expect to see that sex-differences differ depending on the mating system of a species due to the different levels of sexual selection in the different mating systems (Moore & Wilson 2002). Studies in vertebrates support this, where sex-differences in immunosuppression are linked to the type of social/mating system adopted by a species (Loehle 1995; Zuk & McKean 1996; Moore & Wilson 2002; Altizer et al. 2003). Male-biased parasitism could be linked with degree of polygyny, sexual size dimorphism (Moore & Wilson 2002) as well as the transmission type of the parasite. For example, sexually transmitted parasites would favour males in a polygynous group (Zuk & McKean 1996). According to the ICHH, males will display significantly lower immunocompetence compared to females in polygyandrous groups, due to the high level of mating effort needed by the males. The social system used could also influence immunefunction of individuals. If the group is hierarchical, individuals may suffer differences in the amount of physical contact with each other and contaminated substrates depending on their status

(Snaith et al. 2008; MacIntosh et al. 2010). Within species which live by hierarchies, the relationship between rank and the effect of hormones on immune function would also differ depending on how rank position is weighted (the amount of benefits/ costs from being at a certain rank) as well as the type of hierarchy present e.g., a top-down "despotic" or a bottom-up "egalitarian" (Habig et al. 2018). Weight of rank positions varies between species but, in despotic groups, rank position is usually weighted highly, where the alpha gets more access to mates/resources, but lower ranks get very little. These differences in access to recourses across ranks would affect the amount of stress suffered by the individuals (Sapolsky 2005), which would in turn, impact their hormone levels and possibly their immunocompetence. Furthermore, if individuals immigrate to new groups, they may bring with them new foreign pathogens/parasites and also themselves be exposed to new pathogens/parasites in their new group which could have an adverse effect on both themselves and the new group members (Freeland 1976; Altizer et al. 2003). Those who are introduced to new pathogens/parasites could likely have more difficulty effectively responding compared to ones to which they have previously exposed.

Due to the competition for mates and the need for males to signal their fitness, support testosterone dependent immune suppression is expected to be stronger in polygamous groups. However, support for the ICHH has been found in monogamous bird species such as Dark-eyed juncos (*Junco hyemalis*) (Grieves et al. 2006; Deviche & Parris 2006). In addition, some studies havefound little evidence for the effect of social/mating systems on parasite infection or immunocompetence (Foo et al. 2016; Habig et al. 2018). For example, a recent meta-analysis foundno link between parasitism and rank in males in both polygynous hierarchy systems and coop breeding systems (Habig et al. 2018). It is possible that endocrine-immune interactions show little variation across mating systems because the cost of male ornamentation/sexual characteristics in polygynandrous species is replaced by the costs of parental care in monogamous species (Foo et al. 2016).

The Effect of Primate Rank Position Within a Hierarchy on the Relationship Between

Testosterone and Immune Function

Dominance hierarchies are important in many primate species allowing benefits such as mates and access to resources for dominant individuals. According to the "Challenge Hypothesis" (Wingfield et al. 1990), dominant males will exhibit higher levels of testosterone than their subordinates. Furthermore, in seasonally breeding species, males would display high levels of testosterone during times of increased competition e.g., the mating season or times of social instability, especially if these males show little parental care. Male testosterone levels are also thought to be even more exaggerated in polygynous species compared to monogamous (Wingfield et al. 1990). In social species which live by a hierarchy, according to the "Challenge Hypothesis", higher ranking males should display high levels of testosterone due to the dominance required to maintain their status, in species where rank is position is obtained/maintained through aggression and predictive of mating success, and where the hierarchy is unstable (Muller 2017). According to the ICHH, these high-ranking males would be expected to display more developed secondary sexual characteristics to reflect their higher biological quality and so should have the ability to withstand the immunosuppressive effects of elevated testosterone. In primates, it was found high ranking males had generally higher testosterone levels and increased helminth richness compared to the lower ranks (Muehlenbein & Watts 2010), which suggest a possible link between testosterone and reduced immune defence. However, due to high-ranking individuals having greater access to resources and mates, they may also experience greater exposure to parasites/pathogens which could account for this pattern ("Priority-of-Access Hypothesis" Habig et al. 2018). For example, due to the higher reproductive success expected with a high status, they are more at risk from sexually transmitted diseases (Altizer et al. 2003). Despite having higher parasitism rates, these high-ranking males may be able to better defend against effects of the parasites due to their apparent better biological quality.

There is also evidence that rank differences may incur different levels of stress which could also accompany differences in immune function. It is possible that high ranking males suffer more stress

during social instability due to the need to protect their position (Stress of dominance), but lower ranks have higher stress due to limited resources access and being targets of aggression (Stress of subordination) (Emery Thompson & Georgiev 2014). However, differences in stress hormone levels between individuals may differ depending on how individuals maintain their rank; whether they are born into their position, or they achieve and maintain it aggressively (Abbott et al. 2003; Sapolsky 2005). In species that often use dominance and aggression to maintain a high rank, lower ranks may suffer higher stress (and higher glucocorticoid levels), whereas, in species where rank is maintained through only rare instances of aggression, those at a higher rank suffer comparatively higher glucocorticoid/stress levels (Abbott et al. 2003) due to status protection**.** This may account for the variation of results in studies investigating stress and rank across primate species. For example, Kalbitzer et al. (2015) found that high ranked males showed higher glucocorticoid levels in chacma baboons (*Papio ursinus*), but not in guinea baboons (*Papio papio*). However, other research suggests that most primate species, show the higher ranked males to have higher (or equal) levels of glucocorticoids compared to the lower ranks (Cavigeli & Caruso 2015). Those at a low rank may use strategies to reduce stress (and glucocorticoid levels) such as affiliating with other low ranked individuals, or in other words, creatingbonds with others for social support (Abbott et al. 2003). According to the SL-ICHH, high ranking males should be able to withstand the immunosuppressive effects of testosterone if they also display low levels of glucocorticoids. However, this is not consistently found in primates, e.g., in wild yellow baboons (*Papio cynocephalus),* although high-ranking males showed both higher levels of testosterone and glucocorticoids, their wounds healed faster than the lower ranks' wounds (Archie et al. 2012). This instead offers support for a positive correlation between testosterone and glucocorticoids and immunocompetence. However, comparability to this study is limited due to using wound healing as the measure of immunocompetence. Although this measure does allow insight as to how efficient the immune system is at healing after a wound has occurred, this is different to the processes behind defending against parasites.

Those at different rank positions often display differences in behaviour due to the different stressors associated with the different positions. This could mean that certain parasites, where their transmission is heavily affected by a certain behaviour, may appear more prevalent at some levels in the hierarchy, but this could be due to behavioural differences rather than differing hormone levels. A recent meta-analysis found parasitism to be associated with high rank (males) but only in species were rank was predictive of reproductive success (Habig et al. 2018**)**. However, they also found evidence that status-related behaviours and access to resources affect parasitism of the hosts. Differences were found between parasitism and rank but only in parasites transmitted through contact but not for parasites from flying vectors. This indicates that it is more likely that the rank differences in parasitism were a result of the behavioural differences displayed at the different rank positions. Therefore, the transmission pathway of the parasites is also an important factor to consider, when investigating endocrine-parasite relationships, to determine why an individual was susceptible to the parasites. The type of parasite and its' effects/ intensity of effects may also affect the trade-off between reproductive effort and immune effort (Stoehr & Kokker 2006). If parasites have little effect on body condition, males will have less need to invest in immune responses so may alter the apparent trade-off with testosterone. It is therefore important to have as much knowledge about the parasites present as possible when conducting research for host endocrine-parasite relationships in primates.

The Effects of Social Interactions on the Relationship Between Testosterone and Immune Function

As mentioned previously, physical contact with other individuals is also likely to play a role in the transmission of parasites in primates. In social primates, those who have more interactions via grooming may be more susceptible to infection due to the increased physical contact with others (MacIntosh et al. 2012). It is thought that this relationship may also be influenced by rank, as grooming is usually directed upward in social primate societies (MacIntosh et al. 2012), so that the higher-ranking individuals spend more time being groomed (Watts 2000; Schino 2001; MacIntosh et

al. 2012). This could be a strategy to establish social bonds (Cooper & Biernstein 2000) and assert dominance for the higher-ranking males or for lower-ranking individuals to form allies with the higher-ranking ones to get aid in future agonistic competitions (Watts 2000). As well as enhancing the development of social bonds, grooming may also have health implications such as reducing glucocorticoid levels (Shutt et al. 2007) and lowering heart rate (Aureli et al. 1999), as well as decreasing the levels of ectoparasites, such as lice (Akinyi et al. 2013). These could all amount to a better physical wellbeing in those with a high frequency of grooming behaviour. Grooming can also differ between the sexes and so could account for some of the differences in ectoparasite load in males and females (Akinyi et al. 2013). Therefore, grooming could have both positive and negative implications for parasite levels, depending on the type of parasites being measured. Research in rhesus macaques (*Macaca mulatta*) suggests that social connectedness can both mediate (via contact-mediated transmission, especially in those with more aggressive contact) or impede pathogen transmission (via grooming and the reduction in stress due to social bonds (Balasubramaniam et al. 2016). It may also depend on where the individual is positioned in the social network, those who are central may act as "superspreaders" and transmit pathogens to those nearby (Balasubramaniam et al. 2019).

This link between social contact and rank may also be influenced by endocrine levels. In line with the "Challenge Hypothesis" (Wingfield et al. 1990), as mentioned previously, there is much evidence for a link between high rank and higher levels of testosterone, especially in times of competition/instability, due to its effect on dominance and mating success (Abbott et al. 2003; Muehlenbein et al. 2004; Muller & Wrangham 2004; Beehner et al. 2006; Cristóbal-Azkarate et al**.** 2006; Setchell et al. 2008). Therefore, we would expect these higher ranked males to come into more contact with others through agonistic displays and copulation, which could increase their chance of contracting parasites/pathogens (Muehlenbein 2009). Furthermore, in species where a lot of weight is placed on rank, more dominant males will get significantly more access to food than the subordinates. Although this may have a positive effect on the dominant males' overall body

condition, it could also increase their exposure to possible parasites/pathogens that are trophically transmitted through their diet and water (Hernandez et al. 2009).

There is also evidence that, as mentioned earlier, group size and intergroup interactions in primates may have a role in the transmission and susceptibility of parasites/pathogens. Although there is some disagreement about group size effects (Snaith et al. 2008; Nunn 2012), it is considered that group living in primates may result in higher frequency and intensity of parasites/infectious diseases (Freeland 1976; Davies et al. 1991; Ezenwa 2004; Nunn & Heymann 2005; Nunn & Altizer, 2006; MacIntosh et al. 2012). This could be because the frequency of social grooming/interactions increases with group size (Dunbar 1991) while proximity to others decreases (Coté & Poulin 1995). There is some evidence that a close proximity to an anxiety-inducing individual may lead to chronic elevation in stress which could affect susceptibility to disease/infection (Sapolsky 2005). However, recent research suggests the relationship between group-size and infection, may not be as previously thought (Nunn et al. 2000; Snaith et al. 2008; Chapman et al 2009) due to behavioural adaptations (Freeland 1976; Snaith et al. 2008) and even that genes related to immunity are positively selected in larger groups (Wlasiuk et al. 2010).

Sub-grouping could be one of the behavioural adaptations which may help to reduce the risk of infection in large groups (Nunn et al. 2015) or individuals may spread out more to reduce contamination (Snaith et al. 2008). Nevertheless, if the risk of disease transmission is proportional to the number of individuals interacted with (Freeland 1976), it could be inferred that the level of contact with other groups of primates can also affect the transmission rates (Altizer et al. 2003; Snaith et al. 2008). For example, it was found that gorillas suffered higher transmission rates due to increased contact with other gorilla groups (Walsh et al. 2009). However, transmission may not just be influenced by intra-species interaction, but also by proximity to other species who may be carriers of parasites/pathogens (Walsh et al. 2009). Those with large home ranges are expected to come into more contact with parasites/pathogens (Gillespie et al 2005) which could be because of crossing paths with other species. For example, although rare, there is potential for the transmission

of Ebola from bats to chimpanzees due to shared territory and shared diet preferences (Walsh et al. 2009).

There could also be a possibility of anthropological effects on transmission of infection in non-human primates where they may have close or even share home ranges. Increased proximity to humans, through human population growth and habitat destruction, could aid the spread of disease to primate populations because primates are forced to live in a closer proximity to humans (Dupain et al. 2009; Vitazkova 2009). Furthermore, human activity could also indirectly affect disease transmission; for example, apes living in closer proximity to roads had reduced rates of Ebola transmission as they were hunted more intensely resulting in lower ape densities (Walsh et al. 2009). This also provides further support for the relationship between group size/ social interactions and transmission. Furthermore, transmission from domestic animals may also affect primates living in a close proximity to humans (Vitazkova 2009). This area of research could also have implications for conservation of primate species and the actions and measure conservationists use to control for disease transmission from humans. There is also evidence for the direct transmission of pathogens from humans to non-human primates and it is thought that the more closely related they are to humans, the higher the number of pathogens that can be exchanged (Wallis & Lee 1999). Therefore, the proximity to humans may have different levels of impact on parasitism of primates depending on the species. This could then also affect the inter-species differences in parasitism, especially in species who may inhabit areas close to humans.

Overall, due to differing forms of transmission of parasites, there are many social considerations to account for then looking at endocrine-parasite relationship, especially when comparing between different species. Where differences in sociality, group size and contact with others occur between or within species being studied, care must be taken to understand the impact and the intensity of the impact they have on the parasitism of the hosts being sampled.

Seasonal Effects on the Relationship Between Testosterone and Immune Function

In primates, as in many other groups, there is evidence to suggest that individuals go through seasonal changing in their endocrine levels (Ostner et al. 2008; Gesquiere et al. 2010) as well as other ecological/environmental fluctuations. Thus, studies that only collect data during one season may not be getting the full picture and can only relate their results to that specific period (Broche et al. 2017). “The Challenge Hypothesis” proposes that during the mating season, males will display higher levels of testosterone due to the increased competition for mates (Wingfield et al. 1990). According to the ICHH this would be accompanied by a reduction in immunocompetence and may leave them more susceptible to pathogens/parasites. This would be because, during this season, it is more useful for males to invest in reproductive effort in order to outcompete the other males and be more likely to be selected as a mate.

In rhesus macaques, testosterone, prolactin and leptin all showed significant seasonal fluctuations, but cortisol varied littles across the seasons (Mann et al. 2000(a)). Testosterone was highest in late Autumn then declined in the Winter, during which their sexual activity is highest. They also found that Th1-type cytokine production decreased in the winter (breeding season), but lymphocyte proliferation increased. During times of higher demand (e.g., food shortages or high reproductive effort), males may invest more in these less costly immune responses (antibody- mediated responses/ Th-2 cytokine production) (Lee 2006). However, in a later study, rhesus macaques, high ranking-males appeared to have a stronger immune system at the start of their mating season (Georgiev et al. 2015). Unlike the former study (Mann et al. 2000(a)), most species are thought to exhibit a peak in baseline glucocorticoid secretion in the breeding season (Romero 2002). It could be that the differences in types of breeding systems may mean different species have different seasonal variations in immune function (Lee 2006), meaning not all species may show the same patterns. In most environments food/resource availability also fluctuates across the seasons which can influence immune function via varying energy budgets (Lee 2006), endocrine levels and behaviour of males (see section below). This may also differ across species and the environments they inhabit which could affect how their hormones, behaviour and immune function vary.

Males' behaviour is thought to vary across the seasons depending on competition need and food availability. During the mating season, males will have higher levels of activity and contact with others (via male-male competition and copulations) so could be more exposed to parasites and pathogens via contact transmission (Zuk & McKean 1996). This could also explain inconsistencies in sex-differences in parasitism. For example, in desert rodents, male biased parasitism was found in several rodent species in winter and only in one species in summer, but female-biased parasitism was only found for one species of rodent in winter (Krasnov et al. 2005)**.** Despite these inconsistencies in how season affects endocrine immune relationships, it is clear seasonal differences do occur, especially in seasonal breeders, like many primates. Behavioural habits and overall condition, due to fluctuating resource availability, vary with season resulting in varying susceptibility to parasites and immunocompetence.

The Effects of Nutrition on Immune Function and Testosterone Levels and Glucocorticoids

There is much research on the effects of feeding behaviour and available energy on the immune function of primates (Mann et al. 2000(a); Mann et al. 2000(b) Sapolsky 2005; Snaith et al. 2008; Rothman et al. 2009; Prall & Muehlenbein 2014). It is thought that those with limited access to food will result in a lower overall condition which could leave them more susceptible to infection from pathogens (including parasites) due to a compromised immune system as less energy is available for immune defence (Chapman et al. 2006; Prall & Muehlenbein 2014). Levels of testosterone are known to decrease during times of fasting and when food is scarce (Rothman et al. 2009; Prall & Muehlenbein2014), suggesting energy is being reallocated from the costly production of testosterone to be used for survival. Often in species with strict hierarchies, lower ranked individuals may suffer from this as they get less access to resources than higher ranked individuals (Sapolsky 2005; Czoty et al. 2009;Emery Thompson & Georgiev 2014**),** possibly causing nutritional deficiencies and lower immunocompetence (Chapman et al. 2006; Rothman et al. 2009; Prall & Muehlenbein 2014). In contrast, these rank differences could be due to testosterone's effect on

behaviour where those with elevated testosterone may get priority of access to food allowing them to be of a better condition and have higher immunocompetence than those with low testosterone and less access to food (Evanset al. 2000). The food availability to primate groups as a whole could also impact infection rates, as it can influence their competition for food (especially in larger groups) and ranging behaviour which could increase their interaction rates (Freeland 1976; Dunbar 1991) and, as mentioned above, this could then increase their exposure parasites/pathogens (Snaith et al. 2008).

Glucocorticoids are also known to vary with food availability (Cheney & Seyfarth 2009), as noted in birds where, during food deprivation, GCs were elevated (Pérez-Rodríguez et al. 2006). This pattern was also seen in female primates (Pride 2005) and ring-tailed lemurs (Cavigelli 1999) in times of food shortages or high feeding effort. This could explain some of the seasonal variation seen in the endocrine-immune relationship, and how, due to less nutritional pressures, the ICHH and stress-linked ICHH are not well supported in humans inhabiting affluent societies (Prall et al. 2011; Nowak et al 2018). This suggests that maybe a relationship between testosterone and immune response is only revealed when available resources are scarce.

The nutritional status of individuals in the group may also affect their levels of leptins; a hormone secreted by adipocytes (fat cells), which help to regulate metabolism and control the feeling of hunger (Mann et al. 2000(b)). However, leptins are also known to regulate both innate and adaptive immune responses, where deficits in leptin levels are thought to increase susceptibility to infections (Lam & Lu 2007; Maurya et al. 2018). Leptins also function as a cytokine and can activate macrophages and prevent the apoptosis of immune cells (Maurya et al. 2018). Studies in humans have found that those with lower levels of leptins had reduced immune responses (Cason et al. 1986; Farooqui et al. 2002). There is also evidence of a relationship between leptins and immune responses in non-human primates (Mann et al. 2000a; Mann et al. 2000b). A study using several non-human primate species, found that testosterone and leptins were elevated during the season when immune response of PBMC were also high, but when NK (natural killer) activity and Th1 function were

reduced (Mann et al. 2000b). This may suggest there is an interaction between testosterone and leptins which relates to both humoral and cell-mediated immune responses, although the type of relationship may vary for each type of immune response. Cell-mediated immune responses via T-cells are also sensitive to nutritional deficits due to high cost of resulting inflammation when this response triggered. During times of shortages individuals may switch to Th-2 and antibody/ B-cell responses which are less costly (Lee 2006). This highlights why it is it important to have multiple immune parameters when investigating nutritional effects on immune function.

In primates, diet composition may also have an effect on their immune function (Hernandez et al. 2009; Rotham et al. 2009). For example, literature reviews on chimp and gorilla diets show that they consume many plants which are also used by humans for treatment of parasites/infection (Hernandez et al. 2009). However, we cannot say for certain that they would have the same beneficial properties in non-human primates. Research has shown that compounds found in foliage consumed by primates (e.g., tannins) may influence defence against parasites (Rotham et al. 2009). Tannins are a form of defence in plants which reduces their palatability as they inhibit the digestion of starch and cellulose when consumed (Glander 1982). However, tannins have also been found to supress intestinal parasites in domestic ruminants (Rotham et al. 2009). There are two hypotheses for the role of tannins in the defence against parasites which are: 1) Condensed tannins can affect the biological processes of parasites as they have a high affinity for two of the amino acids found in the reproductive anatomy of nematodes. This inhibits their development from free-living to parasitic by preventing the removal of its outer sheath (needed for development); 2) Tannins are thought to improve absorption of high-quality proteins by the small intestine which increases the host's nutritional status, as well as preventing protein deficiency caused by parasites and reducing their negative impact on a host (Rotham et al. 2009).

However, less severe infections and gastro-intestinal parasites are known to reduce the absorption of nutrients (Chapman et al. 2006; Muehlenbein 2009), making it hard to distinguish the direction of correlations between nutrition and infection. A poor body condition may result from nutrient

deficiency from infection, but also can lead to compromised immune responses which increase susceptibility to infection. The presence of parasites may also exacerbate the effects of nutritional deficits from limited food availability (Chapman et al. 2006). Overall, the nutritional status has a complex relationship with susceptibility to defend against infection in that it could be both a factor of and a result of infection. Similarly, testosterone and glucocorticoid levels may also affect the individuals' overall condition and their immunocompetence, but again this is complex, as infection can also influence hormone levels.

The Effects of Ageing

As individuals age, their immune system changes and they come at a higher risk of mortality from infection (High 2004). The term "Immune Senescence"/ "Immunosenescence" describes how the immune system declines with age (Haberthur et al. 2010). The different branches of the immune system are thought to age differently, with adaptive immunity changing more severely than innate immunity (Weiskpof et al. 2009, Haberthur et al. 2010). Some of the effects of ageing include decreased macrophage activity and dysregulation of cytokines and chemokines (Haberthur et al. 2010). In humans, as age increases, the thymus decreases in overall size meaning less unspecialised T cells are being produced by the thymus (Weiskpof et al. 2009). The type of immune responses relied upon may also change depending on age. In some species it is important for young males to develop quickly to compete for mating success, so they may rely less on costly inflammatory responses compared to already well developed, older males (Lee 2006). However, studies in humans do not always account for age which could be contributing to the inconsistent results (Rantala et al. 2012; Nowak et al. 2018).

In primates, testosterone levels follow a similar pattern to immune function in terms of ageing. Testosterone levels will gradually increase leading up to pubescence, then peak and gradually decrease with age (Martin et al. 1977; Aujard & Perret 1998; Folstad & Karter 1992; Beehner et al. 2009; Urbansk & Sowell 2012). In ring-tailed lemurs, younger males had significantly lower testosterone levels and lower rates of male–male agonism during mating season compared with the

"prime-age" older males (Gould & Ziegler 2007). However, according to the ICHH, we would expect this decline in testosterone to coincide with an increase in immunocompetence. Therefore, age must be considered when testing the ICHH across a group/population to account for any age - related differences that could be inferred incorrectly. Age appears to have less of an impact on glucocorticoids in primates. Basal glucocorticoid was found to show little variation across ages in rhesus macaques and baboons. However older males may suffer from longer periods of elevated glucocorticoids as normalisation of the HPA axis after a stimulus takes longer in older males (Goncharova & Lapin 2006). Overall, it seems age may not hugely affect glucocorticoids' influence on the testosterone-immune relationship.

In contrast to the ICHH, ageing could influence parasitism via a different pathway. Ageing may cause changes in behaviour which affects their exposure to pathogens/parasites. For example, older capuchins were more likely to be infected with *Filariopsis barretoi* parasites and spent more time on the forest floor (Parr et al. 2013), where they would have more contact with more soil-borne parasite larvae than the younger individuals. This study also found the older individuals also had higher overall parasite richness due to long-term exposure to a range of parasite species. The aging process may therefore impact susceptibility to infection, through either reduction in immunocompetence and/or changes in behaviour. Longer living species may alsoshow higher levels of parasite richness just due to a longer time of exposure to them and so parasiterichness may increase with age (Nunn et al. 2003). The evidence above highlights the importance of including age in any research investigating differences in immune function or parasite defence.

Concluding Comments

The ICHH has been well debated across a variety of groups/species, but research has constantly produced inconsistent results. The endocrine-immune relationship is complex with many confounding factors, making it hard to accurately test and could explain why there are alternative hypotheses for these relationships (Figure 2). Despite inconsistent results, research does show the possibility of arelationship between testosterone and immunocompetence, whether directly or

indirectly, however the exact mechanisms/pathways behind this relationship are still unknown. There is also evidence for the role of glucocorticoids in endocrine-immune relationships and the SL-ICHH offers an alternative explanation to the ICHH, where GCs moderate this relationship between androgens and immunocompetence. As research has shown elevations in GCs may also have a negative impact on immune function, as well as the possibility of an interaction with androgens. Future research should include GCs in their studies to ensure correct interpretation of results and relationships.

The endocrine-immune relationship may differ depending on environmental factors, such as the infection risk in that environment, season and resource availability, and biological factors such as age. Sociality among primates differ drastically. These social and mating systems differences can also affect the need for sexual signals and so relationships may appear weaker in species where males compete less for mates. However, one of the biggest issues facing the ICHH is whether reduced immunocompetence is due to testosterone induced behaviour or direct testosterone-dependent immune suppression. Therefore, to be able to investigate testosterone-dependent immunosuppression accurately, as many of the variables mentioned (as logistically possible) should be included in future research.

Handicap Principle (Zahavi 1975)	**Mate Choice Hypothesis** (Hamilton & Zuk 1982)	**Immunocompetence Handicap Hypothesis** (Folstad & Karter 1992)	**Stress-linked Immunocompetence Handicap Hypothesis** (Møller 1995)
Secondary sexual characteristics act as a handicap were only the highest quality males can afford to display more developed characteristics and thus be an honest signal of male fitness/quality.	Females chose males based on their ability to defend against parasite. So good genes are passed to offspring so they will have better defense against parasites and males will be able to invest more in parental care. Both increasing the offsprings' chance of survival.	Testosterone used in the development of secondary sexual characteristics act as a handicap and reduce the immunocompetence of the individual. Only the high-quality males can afford to display these signals. It is therefore an honest signal of male fitness and their ability to defend against parasites, which females use to chose their mates.	Same as the ICHH however, there is an interaction between glucocorticoids and testosterone which result in immunosuppression.
Condition Dependent Model (Habig et al. 2018)	**Priority of Access Hypothesis** (Habig et al. 2018)	**Immunoredistribution** (Braude et al. 1999)	**Non-handicap Principle** (Higham 2014)
Those at a higher rank will have better access to food which means they have better nutritional status/ overall condition allowing better defense against parasites. They would also have more access to mates compared to lower-ranking males	The differing levels of testosterone affects the males' behaviour which means some individuals will display behaviour that creates more contact with parasites.	Elevated testosterone does not cause immunosuppression but causes a temporary redistribution of the immune cells to an area of that body that is in need.	Instead of testosterone as a handicap, each male has an optimum level where they can develop maximum secondary sexual characteristics without suffering the immune costs.

Figure 2. The different hypotheses discussed in this paper. Note that "Non-handicap Principle" is not an official title, this is just what I have termed it as for ease of reading in this paper.

Chapter 2: Parasitism and Reproductive Effort in Male Rhesus Macaques: A Test of the Immunocompetence Handicap Hypothesis and the Stress-linked Immunocompetence Handicap Hypothesis

Abstract

Sex-differences in immunocompetence have long been documented and are thought to be related to sex difference in reproductive effort and associated endocrine physiology. The Immunocompetence Handicap Hypothesis (ICHH) suggests there is a trade-off between male reproductive effort and immune function due to the cost individuals incur by maintaining the high testosterone levels needed for the development of secondary sexual characteristics. The hypothesis posits that the resources used in the development and maintenance of these secondary sexual characteristics are diverted away from use in the males' immune system and this affects their ability to defend against pathogens and parasites. According to the ICHH, these secondary sexual characteristics may therefore act as an honest signal of the males' ability to defend against parasites, as only high-quality males can afford to display the signals without compromising their immune system. Female mate choice is thus proposed to be driven by these signals, so they gain the benefits of having a high-quality mate who experiences less parasitism. However, evidence for the ICHH is weak with varying levels of support across studies and species. To account for some of the discrepancies in evidence, the stress-linked ICHH (SL-ICHH) proposes that glucocorticoids (GCs), due to their effects on immunocompetence, interact with androgens to influence the males' ability to defend against parasites. I tested these hypotheses in a group of free-ranging male rhesus macaques, using multiple measures of parasitism (richness, prevalence and abundance (measured using eggs per gram of faeces (EPG) here). Although parasite richness was highest during the mating season (when androgens were elevated), and the alpha male exhibited highest parasite richness, copulation rates, androgen and GC levels, providing support for immune-related costs of reproductive effort via androgens and GCs, the results across the male dominance hierarchy show

little support for either the ICHH or SL-ICHH. Androgens alone mostly showed no relationship with the parasitism measures, whereas often males with high GCs exhibited lower parasitism, although this was not consistent across all parasite species. GCs and androgens only appeared to interact to influence two of the parasitism measures, but this was in an opposite way to the prediction of the SL-ICHH. This pattern suggests those with elevated androgens suffer higher levels of parasitism but only when GCs are low, whereas when GCs were high, males with elevated androgens exhibited either a lower level of parasitism or showed no relationship with male parasitism. Overall, rank was not significantly related to many of the parasitism measures, suggesting each male has an optimum balance where they achieve their maximum reproductive effort without incurring the associated immunosuppressive effects. The results show the importance of analysing each parasite species individually as there was no recurring pattern across the different parasites. Males with high rank also achieved higher copulation rates irrelevant of parasitism or hormone levels, suggesting male parasitism had little effect on their attractiveness as mates.

Introduction

The Immunocompetence Handicap Hypothesis

The Immunocompetence Handicap Hypothesis (ICHH) (Folstad & Karter 1992) proposes that males develop secondary sexual characteristics as an honest signal of their ability to defend against parasites. The maintenance of elevated testosterone, for reproductive effort and the development of these characteristics, is thought to use resources which could instead be used in the immune system and so can lead to reduced immunocompetence (Folstad & Karter 1992). Originally the hypothesis was based on Zahavi's (1975) Handicap principle, which stated that only high-quality males would be able to withstand the immunosuppressive effect of maintaining high androgens for the development of these secondary sexual signals, thus making it an honest signal. However, this has been disputed in more recent years and the altered view suggests that "high-quality" males are able to withstand the reduced immunocompetence as a consequence of more developed signals,

which lower quality males are unlikely to incur as they would display less intense signals (Higham 2014). Instead of these signals acting as handicaps, as the original hypothesis suggests, each male may have an optimum balance where they achieve maximum signal display without suffering a compromised immune system as a result (Penn & Számadó 2020).

According to the ICHH, the males who would experience a decrease in immunocompetence due to the trade off with reproductive effort, would be more susceptible to infection and so may exhibit higher parasite loads. The hypothesis posits that females could therefore use these signals to identify males with less parasitism, thus driving female mate choice (Hamilton & Zuk 1982). It would be advantageous for females to select males with fewer parasites as it allows their genetic resistance to parasites to be passed down to their offspring to aid survival. Males free of parasites are also suggested to be better at providing resources for their mate and offspring, as well as pose low risk of passing parasites to their partner (Jacobs & Zuk 2012; Martinez-Padilla et al. 2012).

Testosterone is a gonadal steroid hormone, mainly produced in the testes and known for promoting growth and influencing dominant behaviour, as well as aiding the development of secondary sexual characteristics (Setchell et al. 2008). In line with the ICHH, studies have shown that testosterone can impair the activity of natural killer cells and macrophages, inhibit cytokine production and antibody release, reduce T helper cell numbers and increasing T suppressor cells (Grossman 1990; Muehlenbein & Watts 2010). Studies in birds have found that males suffered decreased immune responses after receiving a testosterone implant which was thought to be due to the abnormal testosterone levels increasing above their optimum (*Taeniopygia guttata*: Alonso-Alvarez et al. 2006; *Falco tinnunculus*: Fargallo et al. 2007).

Although testosterone-dependent immune suppression has been explored for many years, there seems varying levels of support. There have been several meta-analyses investigating this, with conflicting results. Although most of these studies do show some support for the hypothesis, much of it is weak (Roberts et al. 2004; Foo et al. 2016). For example, testosterone was not found to be

immunosuppressive after controlling for studies using the same species (Roberts et al. 2004). However, it is possible that much of the inconsistent results seen may be due to differences in methods, especially issues resulting from the use of different immunological parameters (Roberts et al. 2004; Prall & Muehlenbein 2014). Some disputed the ICHH by noting that parasitism does not directly reflect whole immunocompetence, however it can still prove useful as they allow an insight into the individuals ability to cope with infection (Muehlenbein 2006). Therefore, using parasitism is still considered a relevant measure to include when investigating immunocompetence. The ICHH focuses on parasites due to the proposed influence it has on female mate choice.

Studies in a range of species have yielded inconsistent results, within and between studies, for the relationship between testosterone and parasitism in males. Some show different results within the same study for different parasite measures (richness/abundance etc.) (*Junco hyemalis* – Deviche & Parris 2006), type of immunity measured (adaptive/innate) and species of the parasites (*Nanger granti*- Ezenwa et al 2012). These inconsistent results could be due to an indirect relationship where testosterone influences male behaviour which influences their parasitism levels (Roberts et al. 2004) due to transmission via contact with the environment/ other individuals. The relationship between testosterone and parasitism in males may differ depending on the effect of testosterone on behaviour in that species. The

"Priority of Access" model (Habig et al. 2018) explains how testosterone aids the acquisition of high status and access to resources, which can lead to an increased exposure to infection. Therefore, to accurately investigate endocrine-parasite relationships and rule out this indirect effect of testosterone, behavioural and social factors must be taken into account, especially when studying species with complex social systems.

Stress – Linked ICHH

The stress-linked ICHH (SL-ICHH) was first proposed to in response to the ICHH being referred to as "oversimplified" due to the absence of consideration given to other factors e.g., other hormones which are known to also influence immune defence and secondary sexual characteristic

development (Møller 1995). In more recent research, the negative relationship between parasitism and testosterone posited by the ICHH, has often been accompanied by high levels of glucocorticoids (GCs) (Kurtz et al. 2007), indicating that GCs may also be involved. The stress-linked version of the ICHH proposes that GCs moderate the relationship between testosterone and the immune system. However, the exact mechanism behind this interaction is still unclear. Birds (*Junco hyemalis*) that were implanted with testosterone also experienced a rise in corticosterone. This suggests a link between GCs and androgens, where an increase in one, is related to in an increase in the other (Klukowski et al 1997). This study also found the possibility of androgens and GCs competing for binding sites (binding globulins) where increases in one may cause an increase in free levels of the other which could offer some insight as to how they interact. Whereas others suggest that testosterone has an indirect immunosuppressive effect via its relationship with glucocorticoids, where an increase in testosterone may increase GCs, which in turn negatively impacts immune function (Evans et al. 2000; Zang &He 2014). Due to the immunosuppressive effects found for both hormones (see below for GCs), it is plausible that elevated levels of both, could lead to a decrease in immunocompetence and the males' ability to defend against parasites.

GCs are produced via the HPA (hypothalamic- pituitary adrenal) axis in response to stress which helps the individual launch a response to the stressor. Although acute elevations may have some health benefits, chronic elevation of glucocorticoids has been linked to immunosuppression such as a decrease in number and function of immune cells (Dhabhar 2009). Prolonged exposure to stressors may, therefore, interfere with immune function due to diversion of resources away to other processes, resulting in less available for defence against parasites. Therefore, a trade-off exists where immediate risk from the stressor, competes with future risk from chronic glucocorticoid elevation (McNamara & Buchanan 2005). Although not limited to males, a recent meta-analysis found a positive relationship between glucocorticoid levels and parasitism. However, the study highlights the complexity of this relationship as the direction it occurs could not identified (Defolie et al. 2020). The activation of the HPA axis in response to infection can cause chronic elevations of

glucocorticoids (Sapolsky 2000; Zang & He 2014; Defolie et al. 2020) which reduces resources allocated for display of sexual signals. Glucocorticoids appear to play a role in energy allocation impacting both the immune system and signal display, so it is feasible that they may interact with androgens to influence male parasite defence. However, this interaction and the effect it has on male parasitism is complex and is still not fully understood (Rantala et al. 2012).

ICHH and SL-ICHH in Primates

Similar to other species, previous studies have shown inconsistent results between hormones and parasitism in primates. There is evidence for the effect of both testosterone and glucocorticoids on immunocompetence in non-human primates. For example, in lemurs, elevated glucocorticoid levels have been linked to a reduced likelihood of survival (Pride 2005; Rakotoniaina et al. 2017), and in long-tailed macaques, elevated testosterone was linked to higher levels of viremia for Venezuelan equine encephalitis (Muehlenbein et al. 2006). Recently, there was also evidence that elevated GCs may affect ability to defend against parasites in western lowland gorillas (*Gorilla gorilla gorilla*) (Shutt-Phillips et al. 2021). However, evidence for both the ICHH and SL-ICHH remains inconsistent. For example, in chimpanzees, there is support for the relationship between both hormones (T and GCs) and parasite richness (Muehlenbein 2006), but another study showed that these hormones were only correlated with helminths (Muehlenbein & Watts 2010).

Some have also found no relationship between these hormones and parasitism in primates, (Yakushima macaques (*Macaca fuscata yakui*): Broche et al. 2017), and even opposing results where there was (insignificant) negative relationship between GCs and Trichuris egg counts (baboons (*Papio cynocephalus*): Habig et al 2019).These inconsistent results could suggest that more factors (e.g. environmental or social etc.) may need to be considered, especially when investigating endocrine-parasite relationships in species with complex life-histories, to ensure that any results obtained are accurate and are not being caused by other factors not accounted for.

Many unresolved questions about these endocrine-immune relationships remain. Although the

immunosuppressive effects of androgens have been well documented, it is still unclear if or how GC levels might moderate the relationship between androgens and immune function. To try to provide new evidence for these relationships, I tested both the ICHH and SL-ICHH in rhesus macaques.

Rhesus Macaques

I tested both the ICHH and SL-ICHH in rhesus macaques (*Macaca mullata)* which, due to their complex social system and breeding seasonality, make them a suitable study system. They live in multi-male, multi-female groups where both sexes mate with multiple partners (polygynandrous) (Bercovitch et al. 1997; Georgiev et al. 2016). They are seasonal breeders so have a distinct mating season and birthing season. Males have a linear dominance hierarchy where higher-ranking males tend to benefit from higher mating success and better access to resources (Bercovitch et al. 1997). Males emigrate away from their natal group just after puberty and enter a new group at the bottom of the hierarchy, where they typically queue for rank (Vessey 1984; Georgiev et al. 2016). As older males die or younger males emigrate to new groups, the new males will move up in the hierarchy resulting in little direct competition. The facial colouration of males tends to become brighter in the mating season (Bercovitch et al. 1997) and is thought to act as a sexual signal. Those with darker/redder faces may achieve higher mating success (Dubuc et al. 2014) and may reflect their overall physical condition (Petersdorf et al. 2017).

Hypotheses and Predictions

Hypothesis 1 (ICHH):

Males with higher reproductive effort (as indexed by elevated androgens) will show lower immunocompetence.

Predictions:

a) Parasitism will be higher during periods of competition when androgens are at their highest.

Despite direct competition being rare (Bercovitch 1997; Higham & Maestripieri 2014), previous studies on rhesus macaques (Higham et al. 2013), as well as studies supporting the challenge hypothesis (Wingfield et al. 1990), have found that during times of reproductive competition or social instability, males will be expected to exhibit a rise in testosterone, so they are able to compete and be ready for possible agonistic interactions. In terms of this study, the times of high competition where androgens would be highest would be during the MS (mating season) and possibly the overthrow period (OT), a period of heightened social instability and dominance competition, (*see Methods*), so this is when parasitism is expected to be highest.

b) Males with high androgens will display higher levels of parasitism

Based on the ICHH, maintaining high androgen levels for reproductive effort will divert resources away from the immune system and leave them more susceptible to parasite infection (Folstad & Karter 1992).

Hypothesis 2 (SL-ICHH):

GCs moderate the relationship between androgens and immunocompetence. The negative relationship between androgens and parasitism will only occur when their GC levels are elevated.

Predictions:

a) Parasitism will be higher during times of competition when androgens are higher, in individuals also experiencing high GCs levels

In addition to the prediction outlined above (for the ICHH) for the relationship between androgens, competition and parasitism, during competitive periods glucocorticoids are also expected to rise due to their role in energy mobilisation to aid responding to the stressors/challenges occurring during times of competition (Sapolsky et al. 2000). As the SL-ICHH predicts that GCs moderate the relationship between androgens and parasitism, in that the positive relationship will only occur when GCs are also high, parasitism is expected to be highest where males experience high levels of

both androgens and GCs which is likely to be times of high competition. In terms of this study, the times of high competition included the mating season and the overthrow period, so this is when parasitism is expected to be highest.

b) The positive relationship between androgens and parasitism will only be present when GCs are high.

According to the SL-ICHH, GCs moderate the relationship between androgens and the individuals' susceptibility to parasites, such that males will exhibit the trade-off between androgens and immune function only in times when GCs are also high (and also that at the within-individual level this relationship will only be evident for males that have high GCs). Conversely, when glucocorticoids are low, the relationship between androgens and parasitism will not be apparent. The mechanisms behind this are still largely unclear, but there has been evidence that these hormones share binding receptors. In birds, both androgens and cortisol are known to bind with corticosteroid binding globulins (CBG) whereby the increase in plasma levels of cortisol due to a stress response increases the levels of free testosterone (Deviche et al. 2001). This highlights the possibility of an interaction occurring between androgens and GCs.

Shared Prediction for Both the ICHH and SL-ICHH:

a) Higher-ranking males will exhibit lower levels of parasitism

Based on the original ICHH which used the handicap principle, where higher-ranking males may be of a higher biological quality (Georgiev et al. 2015), they are able to withstand the immunosuppressive effects of maintaining a high reproductive effort via elevated androgens, and stress via elevated GCs, that may accompany high rank (Higham et al. 2013).

b) Males who are better at defending against parasites will be more attractive to females.

Females gain benefits from mating with males displaying low parasitism. Their offspring may inherit

the males' genetic resistance to parasites, enhancing their chance of survival. Also, the females will have a lower chance of contracting parasites from their mate which could allow them to better raise their offspring, as males show little parental care in rhesus macaques, (Maestripieri & Hoffman 2012). Also, in rhesus macaques, alpha males cannot effectively monopolise the receptive females, however the high-ranking males usually achieve higher mating success or sire more offspring than the lower ranks (Bercovitch et al. 1997; Dubuc et al. 2011; Higham & Maestripieri 2014; Georgiev et al. 2015). According to the ICHH and SL-ICHH, these high-ranking males would be a higher biological quality allowing them to appear more attractive to females without suffering from an increased parasite load.

Method

Study Population

To test these hypotheses, data were collected from a group of free ranging rhesus macaques on Cayo Santiago Island as used in Georgiev et al. (2016). The group consisted of 15 males aged between 5 – 13yrs. Data was collected between February – November 2013. For this study, the mating season(MS) was estimated to occur between 21st February and 28th June, based on the frequency of observed copulations in the field and known fertile windows of females in the population. Therefore, any data collected after 28th June, was considered to have occurred in BS (birthing season). However, between 17th – 23rd July, there was a period of social instability where the alpha male (11Z) was overthrown by a coalition of other lower-ranking males and was replaced by the second highest ranking male (53N) as alpha. This period is referred to as the overthrow period (OT) and allowed comparison of results between, not only the mating season and birthing season, but also to see the effect a period of social instability has on hormone and parasite levels. This population on Cayo Santiago suffers no predation threat or limitations of food supply as they are provisioned with food and water each day. They are provisioned with commercial monkey chow and the "hoppers" in which the food is dispensed are cleaned regularly. Usually, all food is consumed so there is little issue of food waste. Male ages were obtained from the records at

Caribbean Primate Research Center (CPRC) of University of Puerto Rico. In this study group, previous research found that higher ranked males had stronger innate immune responses before the start of the mating season and that males with better innate immune responses mated with more potentially fertile females (Georgiev et al. 2015), suggesting a possible link between immunocompetence and male copulations.

Vet checks for the rhesus on Cayo Santiago are not common would only occur for some individuals (for research purposes) during trapping season (Hernandez-Pacheco et al. 2016). During these checks none were given any drugs or de-worming medicine that would have influence their parasitism. They have only been given a vaccination against tetanus which has not been noted to have any long term-impacts on parasitism (Kessler et al. 2015).

Behavioural Sampling

Behavioural data were collected between March – August 2013 using focal and behavioural sampling on Cayo Santiago Island as in Georgiev et al. (2016). The field data collection was conducted by the researchers visiting/based at Cayo Santiago who were working on Georgiev et al. (2016). Focal follows were conducted 4-5 days a week between the hours 0730 – 1400hr. These follows of specific males lasted 5 minutes and total observation time for the study was 430.8hrs. Researchers conducting the behavioural sampling had all been trained at identifying the males and had time to familiarise themselves with the males in the group to ensure accurate observations. Interactions between males were also recorded to calculate Elo-ratings (using EloRating (version 0.43) package in R) which continuously scored individuals based on the outcome of these interactions and the rating of the individuals involved (Albers & de Vries 2001). A mean Elo-rating for each male (one meanfor before and during the OT, and one mean for after the OT) was used to give the rank position ofthe males before and after the hierarchy shift (Georgiev et al. 2016). A higher Elo-rating equalled ahigher rank, whereby the alpha male would have the highest mean Elo-rating.

The last day of mating season was considered as 28th June and the first day data was collected in the birth season was the 1st July. Any copulations observed during the focal follows were also recorded throughout the study period. There were four instances of copulation recorded in July; however, because these did not occur in the designated mating season they were not included in the analyses. These would not be successful copulations due to occurring outside the fertile windows of all the females in the group (Georgiev et al. 2016).

Selection of Variables

Investigating parasite susceptibility in primates is complex as many factors can influence parasite transmission and the ability of an individual to defend against them. Therefore, to ensure the hypothesis is being tested accurately, other confounding variables should be included. This should also allow to differentiate between the indirect (via behavioural differences) and direct effect of androgens on parasitism.

Rhesus macaques are thought to experience immune senescence in a similar way to humans (Haberthur et al. 2010), so it could be possible that older males are less able to defend against parasite infection. However, previous research has found that age can have a large influence on parasite richness in wild primates, where parasite richness may increase with age up to a peak when they reach sexual maturity but then declines after (Benavides et al. 2012). The system of queuing for rank in rhesus macaques also means that often older males tend to occupy higher ranks (Georgiev et al. 2016) and therefore may achieve higher levels of mating success than some of the younger post-puberty males. Therefore, age-related immune decline and mating bias will be accounted for by including age as a variable in the models. The age value included in analysis was males' age as of 1st March 2013.

Lastly, the environmental conditions of their habitat may also influence the abundance and thus the transmission of parasites in primate populations. Wet conditions may aid parasite transmission (Chapman et al. 2010) so during times of high rainfall males could be more at risk of parasite

infection. Rainfall could cause more humid conditions or cause flooding where faeces are more likely to spread, possibly into puddles/ sources of drinking water. However, this may also vary depending on the parasite. Some parasite egg counts were found to increase after a dry period, but some showed the opposite and individuals were more likely to be infected after a period of heavy rainfall (Habig et al. 2019*).* Therefore, rainfall is included in the model to account for any climate effects on parasite transmission. Rainfall data was obtained from the Caribbean Primate Research Centre, who check the rain gauges daily.

Hormone Measures

Faecal sample extraction (completed by Alexander Georgiev and Kevin Rosenfield), and the hormone assays (completed by Melissa Emery Thompson) were done at the University of New Mexico's Comparative Human and Primate Physiology Center (CHmPP). Faecal samples were obtained to provide androgen (to reflect testosterone levels) and GC levels for the males. This faecal sampling took places between February – November 2013 (n=1620). The use of faecal samples is known to be an accurate method of measuring hormone levels while minimising stress to the individual (Shimizu 2005). Usually, the time taken for the hormones being excreted must be accounted for, however as the lag is most likely within 26hrs for primates of their size (Bahr et al.2000), accounting for this lag is not necessarily due to the use of monthly averages in analysis.

All the samples were extracted following the protocol set out by Palme (2005). Samples collected in the field were kept in cool bags with ice packs before being stored at -20° within 8hrs of their collection. Glucocorticoid levels were obtained, as described by Milich et al. 2018, and assayed (at the laboratory at the University of New Mexico) using the ImmunoChem double-antibody radioimmunoassay kit, following the protocols described by Beehner & McCann (2008). This antibody has previously been shown to be reliable at detecting elevations of faecal glucocorticoids in macaques (Wasser et al. 2000). This corticosterone assay was compared to a group-specific assay for 3α,11ß- Dihydroxy cortisol metabolites, which has been used for rhesus macaques in previous studies (Higham et al. 2013), but the corticosterone assay was more consistent. Glucocorticoids had

an intra-assay CV mean of 5.51% and a mean inter-assay CVs of 11.0% and 11.8% for low and high samples.

Faecal sample extracts were assayed for immunoreactive testosterone using enzyme-immunoassay protocols and reagents provided by the University of California at Davis Clinical Endocrinology Laboratory. The polyclonal antibody R156/7 cross-reacts 100% with testosterone, 57.4% with 5alpha-dihydrotestosterone, and less than 0.3% with other androgens. Inter-assay CVs were 12.0% for a low faecal control and 11.1% for a high faecal control. Intraassay CV, assessed as the average CV of duplicate determinations was 5.9%. For both androgens and GCs, samples with intra-assay CVsof 15% or higher were excluded from analysis or they were re-run.

Parasite Measures

Faecal parasite analysis was completed at the Institute of Parasitology, Biology Centre, by Klara Petrzelkova, Barbora Pafčo. Parasite data was also obtained from faecal samples (n=451), collected using the same method as used for the faecal samples for the hormone data. To obtain the parasite data from the faecal samples, roughly 2g of faecal matter was extracted from the centre of the faecal bolus and was weighed in the field on an electronic scale. The sample was then placed in 10 ml of 10% formalin solution. The sampletube was then shaken by hand to immerse the faecal matter in formalin and stored at roomtemperature until analysis at The Institute of Parasitology, Biology Centre, The Czech Academy of Sciences, Ceske Budejovice, Czech Republic.

To prepare samples for parasitological analyses, they homogenised each sample and strained it through a sieve into Falcon conical tubes (50 ml) to minimise the effects of feeding residues or varying water content in the faeces on the parasite quantification. The final sediment was weighed after centrifugation and re-suspended it up to 10 ml with 4% formaldehyde. They used a modified sedimentation procedure, which was used for both parasite identification and quantification (Pafčo et al. 2017). Briefly, they took 2ml of faecal suspension, centrifuged and examined the whole remaining sediment by microscopy (Olympus BX41, Olympus CX40) while counting parasite stages found.

Parasite taxa were identified based on characteristics of observed stages, e.g., shape, size, external and internal structures of eggs, cysts or trophozoites (Ash & Orihel 2007; Modrý et al. 2018). The number of parasite stages per gram of sediment was calculated according to the following formula: n= N/(m), where n = number of parasite stages/g of sediment, N = number of parasite stages in examined amount of sediment and m = weight of examined sediment (Pafčo 2017).

There are limitations of using only one type of parasitism measure; for example, using only parasite richness is not always thought to be accurate because determining the species/genus of the parasites is often difficult using coproscopic methods (Modrý et al. 2018). Using EPG (eggs per gram)alone is not considered highly accurate as it may not reflect the intensity of parasite infection at the time of testing, especially when reproductive cycles and details of egg shedding of the parasites are unknown (Gillespie 2006; Muehlenbein & Watts 2010). Also, it should be noted that not all parasitesare density dependent (Defolie et al. 2020), therefore, EPG may not be an accurate measure of severity of infection for all parasite species. The use of parasite prevalence also lacks showing how intense the infection is so again, provides limited results. Therefore, in this study I calculated parasite richness and prevalence for the faecal samples, as well as using the EPG data provided) to try to give the most accurate representation of male parasitism.

Analysis

To provide a descriptive summary of the patterns in the data over the study period, I first conducted some simple comparison and correlation tests using either parametric or non-parametric tests based on the results of the Shapiro-Wilk tests of normality, (Ghasemi &Zahediasl 2012). To examine differences in parasite and endocrine measures between seasons, Friedman tests were used and for relationships between variables either Spearman rho or Pearson's correlation coefficient test depending on normality of data. All tests were performed in R (Version 1.2.5033). All Figures were made using the "ggplot2" package in R (Wickham 2011). Where there were multiple correlational tests being performed, a Bonferroni correction was used to ensure results were interpreted accurately. Mixed models were used to identify variables that significantly correlated with male

parasitism and copulation rate. All models were computed using glmmTMB() in the “gmmTMB” package and then compared using anova in thecore R package. The best fitting model was determined using a backward stepwise method where the least significant predictor was removed from the model. The models that had the lowest AIC value and were significantly different from the null model were deemed the best fit (Symonds & Moussalli 2011). In the models, season (MS, OT, BS) and male I.D were included as random effects with levels. Season uses the BS as the base to compare the other two seasons against. Studies investigating the interaction between testosterone and glucocorticoids, often categorise glucocorticoid levels as either high or low to use in models investigating how they might moderate testosterone dependent relationships. However, due to the limitations of categorising continuous data (Mehta & Josephs 2010), where possible, I chose to use an interaction term instead to investigate the relationship to give more accurate results and then only used categorised glucocorticoids (High = above median, Low=below median) to further investigate the results gained fromthe models or when the mixed model method was not possible.

In these models, male monthly mean values were used for androgens, GCs and rainfall but each faecal sample was used as an observation to give parasite richness (N=366). Parasite richness referred to the number of different parasites present in each one of these samples. Male I.D and month were included as random effects to account for repeated testing of the same male across the months. The fixed effects for these models included androgens, GCs, season (MS, OT, BS), rank (1=alpha male, 15=lowest ranking male), Age (years), Rainfall (mm), and an androgen*GC interaction term. All the numerical variables in the model were centred using a z-score to allow comparison of variables (Schielzeth 2010). Parasite richness was modelled using a gaussian distribution. This same format was also used for the parasite prevalence models, but prevalence was modelled as a binary variable, where the presence or absence of the parasite was recorded for each sample collected (Zuur et al 2009).

The models for parasite prevalence thus use a binomial distribution to account for this binary response variable (1=present, 0=absent). Male I.D and month were again included as random effects

to account for repeated testing of the same male across the months.

Due to the nature of the EPG data, where some samples were much higher than the average, the models would not converge. As a result, the data was analysed by visually inspecting the relationships graphically.

The number of copulations achieved each month by a male in the MS was used as the outcome variable for this model, monthly-male average parasite richness (in the MS) was used as the parasitism measure (N=59). In the copulation models, month was included as a fixed effect to account for the higher number of copulations which usually occurs at the start of the mating season. Observation time was also included as a fixed effect to account for the differences in the time each male was observed for, which would affect how many copulations were recorded for them.

Model Validation

All models were compared against the null model (containing just the random effects) using anova and the models with the lowest AIC that were also deemed significantly better than the null, was selected as the best fitting model.

All the variables in the models were tested using the "performance" package in R and in all the models, all vif's = <2.6, meaning there were no issues of multicollinearity. As parasite richness and copulation models consisted of many 0's, these models were tested for zero-inflation using "DHARMa" package, but results showed neither of the models were zero-inflated. Model diagnostics were also completed using the package "DHAMa" and "ggplot2". Using "DHARMa", the simulated residuals and qq-plots were checked for all models and deemed fit (Hartig 2020). The residuals were also double checked using qqnorm(residuals() and plot(residuals() in the"ggplot2" package.

Results

Seasonal variation of Androgens, Glucocorticoids, and Parasitism

To compare differences across the periods, means from only 9 males (for each season) were used in

the analysis due to no samples being collected for some males in the OT (overthrow period). Overall, across the whole study period, there was no significant correlation between mean male androgen and mean male glucocorticoid levels (*rho= 0.493, n=15, p=0.064*). Androgen levels did not vary significantly across the three periods (*x^2=4.22, N=27, p=0.121; Figure 3.A*). Androgens (*mean 92.13 ± SE 2.14 ng/g*) were highest in the MS (mean 97.17 ± 6.52 *ng/g*) compared to the BS (*mean 86.20 ± SE 6.11 ng/g) and OT (mean 85.65 ± SE 9.82 ng/g, p=0.5).* However, paired Wilcoxon tests showed*, these differences were not significant when using male means (MS vs BS: p=0.1;6 MS vs OT: p=0.5).* Glucocorticoid levels (*mean 197.16 ± SE 3.45 ng/g*) also did not vary significantly across the three periods (*x^2*=8.22, N=42, p= 0.062; *Figure 3B.*). Glucocorticoids were higher in the OT (*mean 260.99 ± SE 33.80 ng/g*) than in MS (*mean 209.47 ± SE 14.49* ng/g) and in the BS (mean 181.82 ± SE 12.63ng/g) although paired Wilcoxon tests showed these differences were not significant. (OT vs MS: p=0.5, OT vs BS: p=0.059)

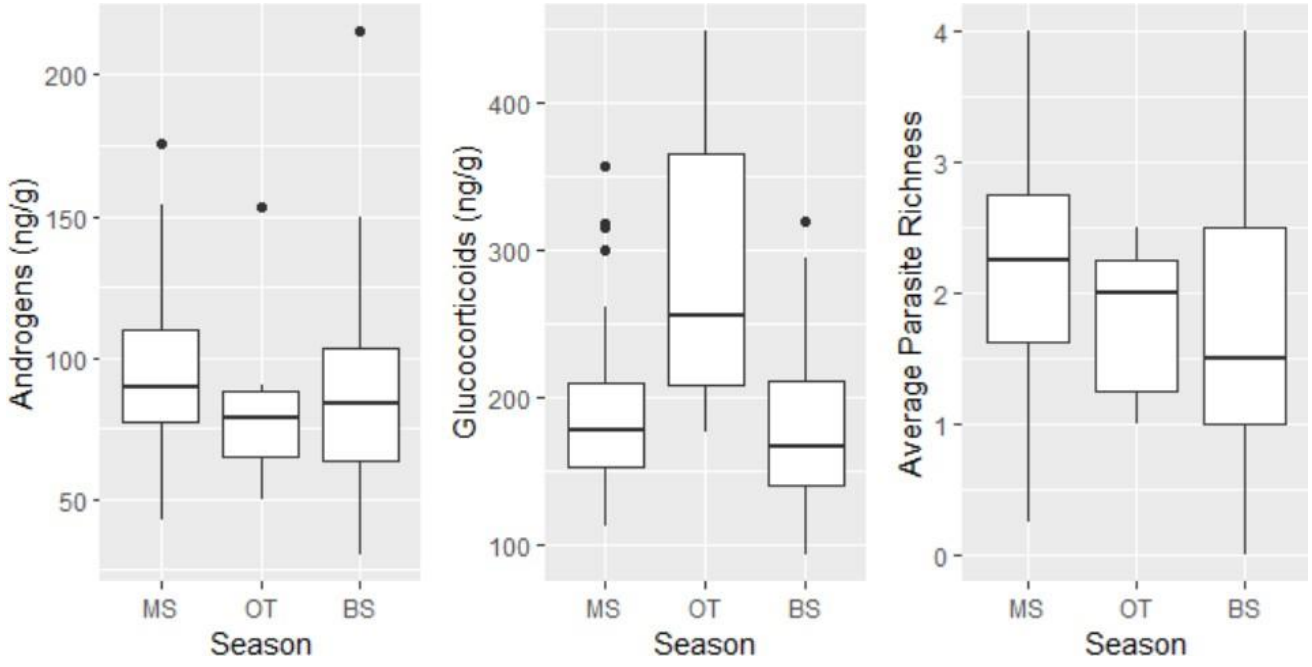

Figure 3: *Variation in male monthly means of A) Androgens B) Glucocorticoids C) Parasite Richness. Boxplots depict variation in male-monthly means of androgens and glucocorticoids (N = 108 male-monthly means in total, N=59 for MS, N=7 for OT, and N=42 for BS; line shows median of mean male-monthly values; box = upper and lower of the interquartile range; whisker extended to the highest value within 1.5 interquartile range of the box).*

There were seven different parasite species identified in the faecal samples collected including:

Helminths: (Trichuris (whipworm) & Strongyloides (roundworms) & Strongylida (hookworms)) and the majority of all parasites were Protozoa. This included amoebas (Entamoeba & Iodamoeba), Ciliates (Balantidium coli) and flagellates (Chilomastix). Parasite richness varied less than either hormone across the three periods (*Figure 3.C*) but was slightly higher in the BS (*mean 2.19 ± SE 0.20 ng/g*) followed by in the MS (*mean 2.13 ± SE 0.14 ng/g*) and lowest in the OT (*mean 1.86 ± 0.19 ng/g*). These minor differences however were not statistically significant (x^2*=5.378, N=36, p= 0.0680*).

Within the MS, parasite richness appeared peak towards the end of the MS (May and June) and then decrease in the following two months (supplementary *Figure 1).* However, it must be noted that there were many more samples collected in the MS compared to the other seasons.

Table 1: *Means in each season and Friedman test and posthoc (paired Wilcoxon test) results for comparing androgen, glucocorticoids, and all parasite measures across the three periods (N=15). For the endocrine analysis only 9 males were included as some gave no samples during the OT (n=27 male-season means). MS= Mating Season, OT = Overthrow period, BS= Birthing Season.*

	MS	*OT*	*BS*	*P-value (Friedman test)*	*Posthoc Paired Wilcoxon Test*
Androgens (ng/g)	**97.166**	85.65	86.20	0.122	-
Glucocorticoids (ng/g)	197.205	**268.991**	184.355	0.0622	-
Average Parasite Richness (no. of unique parasites)	2.128	1.861	**2.192**	0.0680	-
Entamoeba Prevalence	0.338	**0.514**	0.396	0.717	-
Iodamoeba Prevalence	**0.094**	0.0417	0.069	0.191	-
Chilomastix Prevalence	0.198	0.083	**0.230**	0.066	-
Balantoid Prevalence	0.671	0.875	**0.931**	0.0013	Higher in BS compared to MS (p=0.021)
Balantoid EPG	973	5169	**4899**	0.017	Higher in BS compared to OT (p=0.026)
Strongyloides Prevalence	**0.411**	0.292	0.332	0.486	-

Strongyloides EPG	**61.868**	52.792	36.882	0.060	-
Trichuris Prevalence	**0.254**	0.056	0.148	<0.001*	Higher in MS compared to OT (p=0.012), Higher in MS compared to BS (p=0.022)
Trichuris EPG	**18.441**	0.889	4.358	<0.001*	Higher in MS compared to OT (p=0.010), Higher in MS compared to BS (p=0.010)
Strongylida Prevalence	**0.161**	0	0.086	0.006	Higher in MS compared to OT (p=0.027)
Strongylida EPG	**9.562**	0	1.819	0.002	Higher in MS compared to OT (p=0.027),

Notes: Values in bold are the highest out of the three seasons. Significant results are marked with an *. Only Trichuris prevalence and EPG were significant after correcting for multiple testing of male parasitism (p=0.05/12 = 0.004).

The majority of parasite measures were highest in the mating season (*Table 1*), with significant differences for the prevalence of Iodamoeba, Chilomastix, Strongyloides, Trichuris, Strongylida and the EPG for Chilomastix, Strongyloides, Trichuris and Strongylida. However, after accounting for multiple testing, only Trichuris prevalence and EPG were still significantly higher in the MS than the OT and BS (Trichuris prevalence and EPG) (*Table 1*). Entamoeba prevalence was highest in the OT (*Table 1*), but this difference was no longer significant when accounting for multiple testing (*Table1*). Unlike the other parasites, Balantidium prevalence and EPG were highest in the BS (Table 1*),* however, this again was not significant after correcting for multiple testing (*Table 1*). Mean rainfall was lowest in the MS (133.51mm) slightly higher in the BS (264.16mm) and highest in the OT period (308.36mm). However, the difference in rainfall across the three seasons was not significant when using an average for each season, (*x^2=2, df=2, p=0.368*).

Do Androgens and GCs influence Male Parasitism?

Parasitism Measure: Parasite Richness

Parasite richness was best predicted by model e (*Table 2*) which included season, rank, age, rainfall and the interaction between androgens and GCs.

Table 2: *Comparison of the linear mixed effects models for parasite richness*

Comparison of Parasite Richness	***AIC***	***P-Value***
Model 1. Parasite Richness		
a) Null: (1|Male) + (1|Month)	1225.822	-
b) Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	1232.831	0.7014
c) T + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	1230.276	0.2733
d) GC + Season + Rank + Rainfall + (1|Male) + (1|Month)	1227.865	0.1752
d) T + GC + Season + Rank + Age + Rainfall + (1|Male) (1Month)	1229.578	0.1752
e) T*GC + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	**1222.083**	**0.01137**

Notes: Parasite richness = the sum of different parasites in each faecal sample (n= 366). T = Androgens, GC = Glucocorticoids. This model was conducted using glmmTMB() in the "glmmTMB" package. All were modelled using a Gaussian distribution Models were compared using anova (core R). Significant p-value= <0.0125 after correcting for multiple testing are marked in bold (p=<0.05/5).

As seen in *Table 3,* there was no relationship between parasite richness and either androgens or GCs individually. Only the interaction between mean androgens and glucocorticoids significantly predicted average parasite richness. From *Figure 4*, it appears that androgens have a slight positive relationship with parasite richness when glucocorticoid levels are low (below the median GC value), but there is a stronger negative relationship when glucocorticoid levels are high (above the median GC value).

Rank was included in the best fitting model but was not significantly associated with parasite richness when accounting for the remaining variables. Using averages for each rank position, rank did not significantly correlate with parasite richness overall (*rho=0.218, n=15, p=0.434*) or during any period individually (*MS: rho= 0.059, n=15, p=0.835, OT: rho=-0.537, n=9, p=0.107, BS: rho=0.059, n=15, p=0.835*).

Table 3: *Summary of predictors for the best fitting model for predicting male parasite richness (N=366)*

Summary: Model 1. Parasite Richness ~ T * GC + Season + Rank + Rainfall + Age + (1|Male) + (1|Month)				
Predictors	*Estimates*	*std. Error*	*t-value*	*P*
MeanT	0.1108504	0.1228639	0.902	0.367
MeanGC	-0.1416853	0.1048611	-1.351	0.177
Season: MS	0.1241664	0.2250350	0.552	0.581
OT	0.0003362	0.3968044	0.001	0.999
Rank	0.0911284	0.1136676	0.802	0.423
Age	-0.0708408	0.1264901	-0.560	0.575
Rainfall	-0.0001310	0.1051160	-0.001	0.999
T*GC	**-0.1235246**	**0.0399644**	**-3.091**	**0.002**

Notes: Rank: 1= Alpha, 15= lowest ranking male, T = Androgens, GC = Glucocorticoids, MS = Mating Season, OT= Overthrow period.

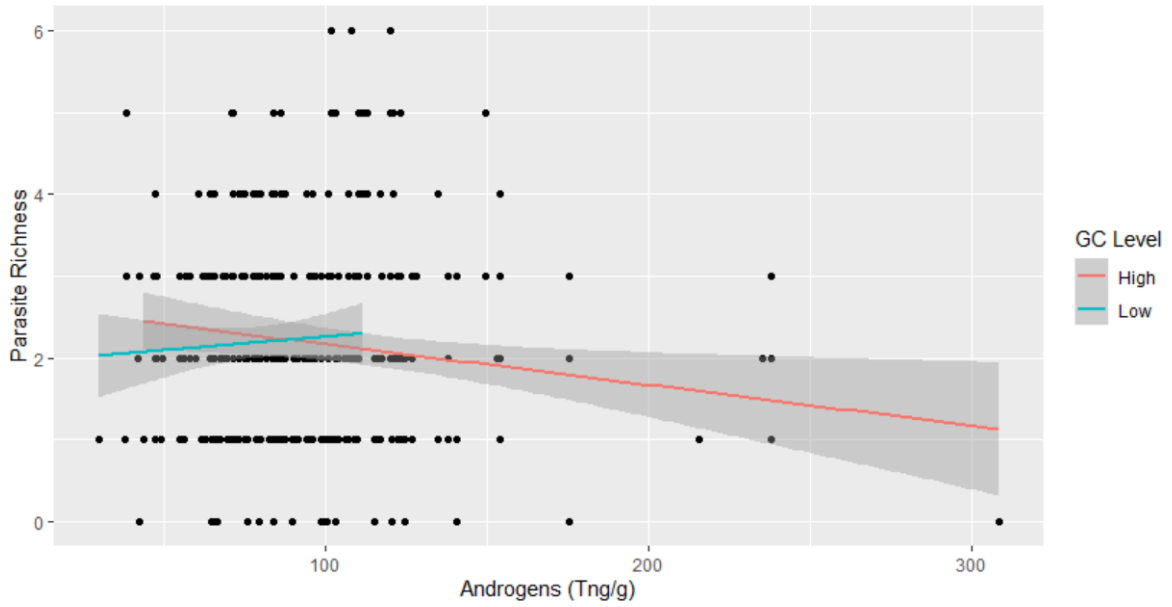

Figure 4: *The relationship between average parasite richness and average androgen levels for each male each month when glucocorticoids are high or low. The blue line shoes the relationship when glucocorticoid levels are low (below the median) and the pink line shows the relationship when glucocorticoids are high (above the median).*

Parasitism Measure: Parasite Prevalence

Every faecal sample had at least one parasite present, and the majority contained multiple parasite

species. Balantidium was the most prevalent parasite (*supplementary Figure 2.D*); they were present in 95% of the samples (275/366). Whereas Iodamoeba was the least prevalent parasite overall, as they were only present in 8.2% of the samples (30/366) (*supplementary Figure 2.B*).

Overall, when predicting parasite prevalence, models with the interaction between androgens and GCs were the best fitting model for 3 out of the 7 species (*Table 4).* This was the case for Entamoeba, Trichuris and Strongylida. However, after accounting for multiple testing, the model containing the interaction was only significant for Entamoeba and Strongylida prevalence (*Table 4. A&G*). The model with no endocrine measure was the best fitting model for Iodamoeba prevalence, even after correcting for multiple testing (Table 4.B). The best fitting model for Balantidium prevalence was the model containing glucocorticoids but not androgens (*Table 4.D).* This was still significant after correcting for multiple testing. The model that was the best fit for Chilomastix prevalence consisted only of GCs and the random effects; however, this was no longer significantly better than the null after correcting for multiple testing (*Table 4.C*). The best fitting model for Strongyloid prevalence consisted only of season and male rank, however this was not significantly better than the null (*Table 4. E*).

Table 4. *Comparison of generalised mixed models for predicting the prevalence of each parasite species.*

Comparison of Parasite Prevalence	***AIC***	***P-Value***
Model A. Entamoeba Prevalence		
T*GC + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	**467.32**	**0.007**
Null: (1|Male) + (1|Month)	472.48	-
Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	478.81	0.599
T + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	478.94	0.477
GC + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	480.80	0.720
T + GC + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	479.94	0.478

Model B. Iodamoeba Prevalence		
Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	**187.65**	**0.004**
T + **Season +** Rank + Rainfall + Age + (1|Male) + (1|Month)	189.01	0.006
GC + **Season +** Rank + Rainfall + Age + (1|Male) + (1|Month)	189.53	0.007
T + GC + **Season +** Rank + Rainfall + Age + (1|Male) + (1|Month)	189.95	0.008
T * GC + **Season +** Rank + Rainfall + Age + (1|Male) + (1|Month)	191.02	0.010
Null: (1|Male)	195.12	-
Model C. Chilomastix Prevalence		
GC + (1|Male) + (1|Month)	**351.16**	**0.02087**
T + GC + (1|Male) + (1|Month)	353.15	0.06904
T + (1|Male) + (1|Month)	354.21	0.1304
Null: (1|Male) + (1|Month)	354.49	-
T * GC + (1|Male) + (1|Month)	356.99	0.186
Model D. Balantidium Prevalence		
GC + Rank + Season + Age + Rainfall + (1|Male) + (1|Month)	**365.32**	**0.006**
T + Rank + Season + Age + Rainfall + (1|Male) + (1|Month)	366.87	0.012
T + GC + Rank + Season + Age + Rainfall + (1|Male) + (1|Month)	367.14	0.012
T * GC + Rank + Season + Age + Rainfall + (1|Male) + (1|Month)	367.80	0.013
Rank + Season + Age + Rainfall + (1|Male) + (1|Month)	368.99	0.031
Null: (1|Male)	371.25	-
Model E. Strongyloid Prevalence		
Rank + Season + (1|Male) + (1|Month)	**429.70**	**0.067**
T + Rank + Season + (1|Male) + (1|Month)	430.24	0.071
GC + Rank + Season + (1|Male) + (1|Month)	430.41	0.076
Null: (1|Male) + (1|Month)	430.88	-
T + GC + Rank + Season + (1|Male) + (1|Month)	432.02	0.115
T * GC + Rank + Season + (1|Male) + (1|Month)	433.66	0.162
Model F. Trichuris Prevalence		
T * GC + Rank + Rainfall + Age + (1|Male) + (1|Month)	**358.08**	**0.036**

Rank + Rainfall + Age + (1Male) + (1|Month)	358.57	0.075
Null: (1|Male) + (1|Month)	358.59	-
T + Rank + Rainfall + Age + (1|Male) + (1|Month)	360.09	0.105
GC + Rank + Rainfall + Age + (1|Male) + (1|Month)	360.38	0.116
T + GC + Rank + Rainfall + Age + (1|Male) + (1|Month)	362.08	0.162
Model G. Strongylida Prevalence		
T * GC + Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	**273.57**	**0.003**
T + GC + Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	274.52	0.005
Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	277.62	0.020
GC + Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	277.79	0.022
T + Season + Rank + Rainfall + Age + (1|Male) + (1|Month)	279.23	0.041
Null: (1|Male) + (1|Month)	281.46	-

Notes: T = Androgens, GC = Glucocorticoids. All were fitted with a binomial distribution with a logit link in the glmmTMB() function in the "glmmTMB" package in R (n=366). Those with the lowest AIC value, which were also significantly better than the null models, were considered to be the best fitting model. Significant results after correcting for multiple testing of each variable (p=0.05/6 = 0.008 or p=0.05/5= 0.01) are in bold. If null model was the best fit, the least significant predictors were removed until a model was significantly better than the null.

Overall, there was not just one variable that was a significant predictor of all the parasites. Rainfall was a significant predictor for both Iodamoeba and Trichuris prevalence and in both cases was negatively correlated with prevalence (*Table 5.B & E*). Iodamoeba prevalence was also significantly negatively correlated with male rank (*Table 5.B*). GCs were a significant predictor of Chilomastix, Balantidium and Strongylida prevalence in which there was a negative correlation between GCs and prevalence of both parasites (*Table 5. C, D & G*). Strongylia prevalence was also significantly negatively correlated with age but was positively correlated with androgens (*Table 5. G*). Only Entamoeba prevalence was significantly by the interaction between androgens and GCs (*Table 5. A*). Androgens were positively correlated with Entamoeba prevalence when GCs were low but were negatively correlated with Entamoeba prevalence when GCs were high (*Figure 5.*) In the best fitting model for Strongyloides (although was not significantly better than the null), none of the predictors

were significantly correlated with prevalence (*Table 5. E*), so there could be other influential factors are missing from the model.

Table 5. *Summary of predictors for the best fitting models predicting prevalence of each parasite.*

Predictors	*Estimates*	*std. Error*	*Z-vlaue*	*P*
Model A. Entamoeba Prevalence ~ T*GC + Rank + Age + Rainfall + (1|Male) + (1|Month)				
MeanT	-0.01240	0.21869	-0.057	0.95480
MeanGC	0.18792	0.19244	0.976	0.32882
Season: MS	0.02633	0.75716	0.035	0.97226
OT	0.27419	0.71617	0.383	0.70183
Rank	-0.03634	0.17494	-0.208	0.83543
Age	0.04479	0.18382	0.244	0.80748
Rainfall	0.16953	0.37390	0.453	0.65024
T * GC	**-0.59782**	**0.21981**	**-2.720**	**0.00653**
Model B. Iodamoeba Prevalence ~ Season + Rank + Rainfall + Age + (1|Male)				
Season: MS	-0.5242	0.7204	-0.728	0.46687
OT	1.4630	1.3790	1.061	0.28873
Rank	**-0.9338**	**0.3554**	**-2.628**	**0.00860**
Age	0.0950	0.2732	0.348	0.72808
Rainfall	**-1.1945**	**0.3706**	**-3.223**	**0.00127**
Model C. Chilomastix Prevalence ~ MeanGC + (1|Male) + (1|Month)				
MeanGC	**-0.3972**	**0.1804**	**-2.202**	**0.0277**
Model D. Balantidium Prevalence ~ Season + Rank + Age + Rainfall + (1|Male) + (1|Month)				
MeanGC	**-0.38605**	**0.16391**	**-2.355**	**0.0185**
Season: MS	-1.04590	0.63074	-1.658	0.0973
OT	-0.44139	1.07608	-0.410	0.6817
Rank	0.31838	0.23490	1.355	0.1753
Age	-0.02326	0.23215	-0.100	0.9202

Rainfall	0.53163	0.27951	1.902	0.0572
Model E. Strongyloides Prevalence ~ Rank + Season + (1|Male)				
Rank	0.3951	0.2313	1.708	0.08759
Season: MS	0.8282	0.5023	1.649	0.09920
OT	-0.6144	0.7889	-0.779	0.43612
Model F. Trichuris Prevalence ~ T + Rank + Rainfall + Age + (1|Male)				
MeanT	0.243487	0.266467	0.914	0.360842
MeanGC	-0.001113	0.210818	-0.005	0.995788
Rank	-0.058861	0.268589	-0.219	0.826533
Age	-0.273575	0.345455	-0.792	0.428403
Rainfall	**-0.619906**	**0.169380**	**-3.660**	**0.000252**
T*GC	-0.467082	0.259707	-1.798	0.072098
Model G. Strongylida Prevalence ~ T * GC + Rank + Age + (1|Male) + (1|Month)				
MeanT	**0.90495**	**0.34675**	**2**	**0.00691**
MeanGC	**-0.68912**	**0.33588**	**-2.052**	**0.04020**
Rank	0.39528	0.32472	1.217	0.22349
Age	**-0.80251**	**0.37842**	**-2.121**	**0.03395**
T*GC	-0.32375	0.25401	-1.275	0.20247

Notes: All were fitted with a binomial distribution with a logit link in the glmmTMB() function in the "glmmTMB" package in R (n=366). Significant predictors are in bold. Rank: 1= Alpha, 15= lowest ranking male, T = Androgens, GC = Glucocorticoids, MS = Mating Season, OT= Overthrow period.

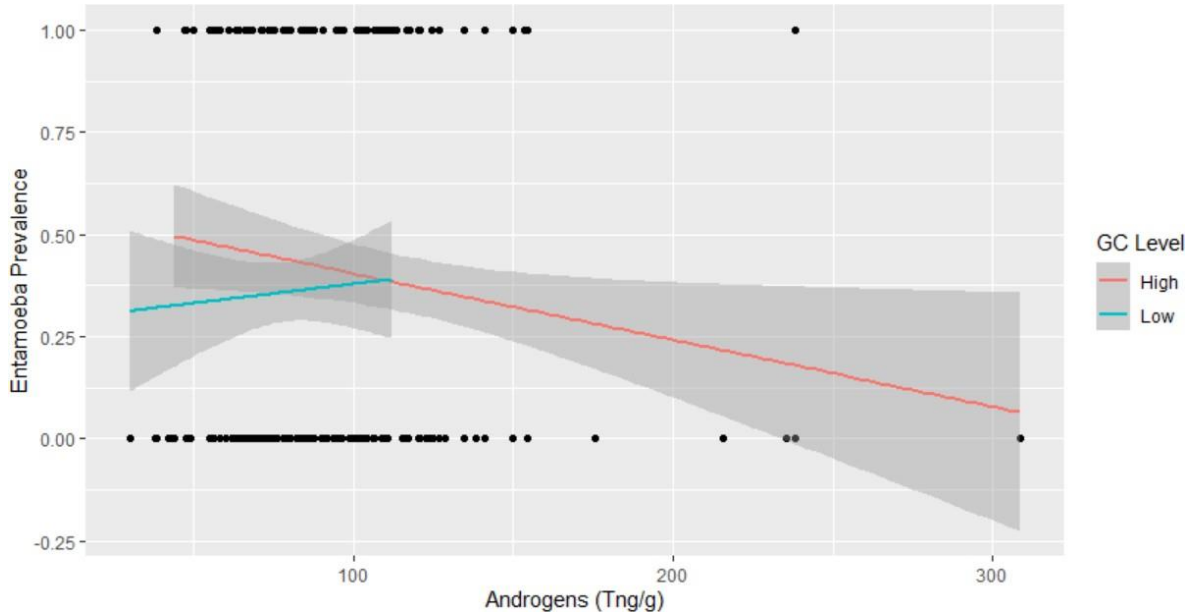

Figure 5*. The relationship between androgens and Entamoeba prevalence when glucocorticoids are high or low(N=366). Low glucocorticoids = <182.23 (median), High glucocorticoids = >182.23 (median).*

Parasitism Measure: Parasite EPG

Overall, the parasite EPG counts generally showed a negative relationship with both androgens and GCs however, none of these relationships were significant when using male averages for the whole study period. There was no significant relationship between Balantidium EPG and androgens (*rho= -0.250, n=15, p= 0.368) and GCs (rho= -0.125, n=15, p= 0.658*). Both Strongyloides and Strongylida EPG also showed no relationship with androgens *(rho=-0.132, n=15, p=0.639) (rho=0.036, n=15, p=0.899);* or GCs (*rho=-0.132, n=15, p=0.639), (rho=-0.104, n=15, p=0.713)* respectively. Similarly, Trichuris EPG showed no relationship with androgens (*rho=0.264, n=15,p=0.340*) or GCs (*rho=0.032, n=15, p=0.913*) using male means.

Graphically, similar patterns to the previous mixed models are seen in terms of the interaction between androgens and GCs and their relationship with EPG counts (*Figure 6*). All parasite EPG

counts show at least some negative relationship with androgens when GCs are high (although a very weak relationship for all but Balantidium). Both Trichuris and Strongylida EPG also show a positive relationship when GCs are low (*Figure 6. C&D*) but there appears little relationship between Balantidium and Strongyloides EPG with androgens when GCs are low (*Figure 7.A & B*).

When testing the relationships using male averages, when GCs are low, there was no relationship between androgens and Balantidium (*rho= -0.189, n=15, p=0.498*), Strongyloides (*rho=0.03, n=15, p=0.889*), Trichuris (*rho= 0.156, n=15, p=0.578*), or Strongylida (*rho=-0.179, n=15, p=0.524*). Similar results were found when GCs were high (using male averages) where androgens were unrelated to Balantidium (*rho= 0.039, n=15, p=0.893*), Strongyloides (*rho=0.038, n=15, p=0.894*), Trichuris (*rho= -0.357, n=15, p=0.192*), and Strongylida (*rho=-0.044, n=15, p=0.877).* A one sample T-test showed that the correlation coefficients for androgens were consistent across the parasites when GCs were both low (*t=-0.542, N=4, p=0.626*), and high (t=-0.861, N=4, p=0.452).

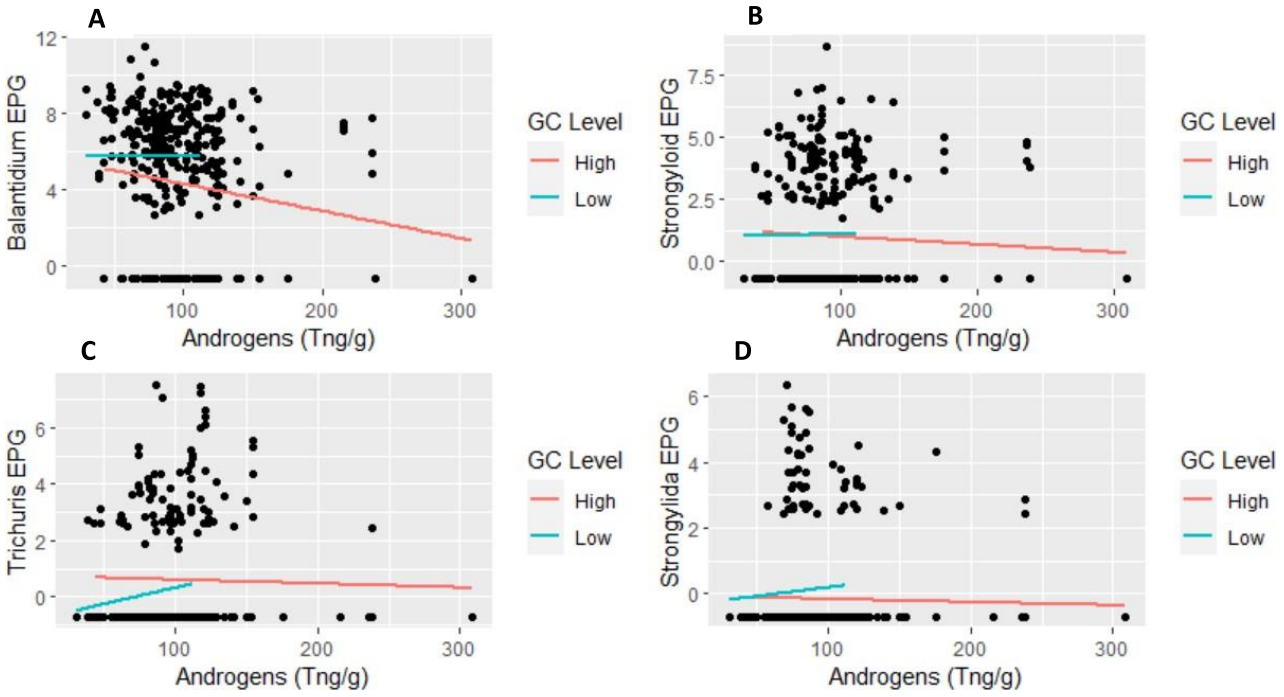

Figure 6. *The relationship between male parasite EPG (log transformed) in each faecal sample and monthly average androgen levels for each male. A) Balantidium EPG and androgens, B) Strongyloid EPG and androgens, C) Trichuris EPG and androgens, D) Strongylida EPG and androgens.*

For most parasite species, there was no relationship between their EPG counts and male dominance rank (*Figure 7*) as most had no significant correlation when using averages for each rank position (Balantidium: *rho=-0.05, n=15, p=0.863*, Strongyloides: *rho=0.332, n=15, p=0.226*, Trichuris: *rho=0.146, n=15, p=0.602*). However, Strongylida showed a positive correlation with rank (*Figure 7. D)* and this was significant using rank position averages (*rho= 0.616, n=15, p=0.041*), so the lower ranking males had the highest EPG for Strongylida. However, when accounting for Bonferroni correction this was no longer significant (p=0.05/5=0.01).

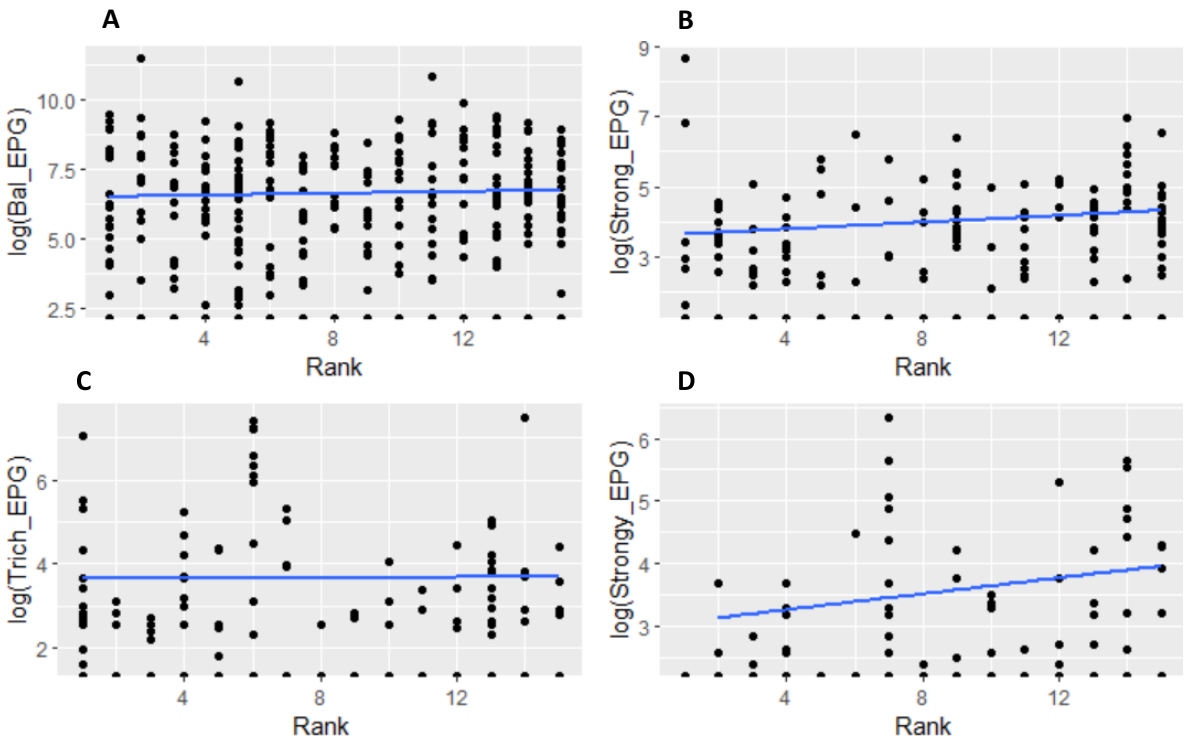

Figure 7*. The relationship between rank and parasite EPG's, n=366. A) Balantidium, B) Strongyloides, C)Trichuris, D) Strongylida*

Does male Parasitism influence the number of Copulations they achieve?

The model results (Table 6) show that male copulations generally decreased over the course of the MS (*rho=-0.464*, N=59, P = 0.003). The number of copulations occurring peaked in April during the

middle of the MS (mean = 1.52 *± 0.38*) then decreased in the following two months (supplementary *Figure 1.A*). Average parasite richness was higher in the last two months of the MS *(mean= 2.311 ± 0.715*) compared to the first two months *(mean = 1.914 ± 1.036).* There was no significant correlation between parasite richness and copulation rate in either the period before *(rho=0.043, N=15, p=0.879*) or after the peak *(r= 0.11, N=15, p0.693).*

There was no significant relationship between parasite richness and either androgens nor GCs using male averages in the MS (*Androgens: rho=0.046, n=15, p=0.869, GC: rho=0.306, n=15, p=0.268*). The alpha male during the MS (male 11Z) achieved the highest average copulation rate (*mean= 5.7 ± 1.11 copulations per month*) but also suffered thehighest average parasite richness (*mean = 2.9 ± 0.28 parasites per sample)* across the whole study (*Supplementary Table 1*), compared to the average copulation rate *(1.8 ± 0.23 copulations per month*) and parasite richness *(2.14 ± 0.07 parasites per sample)* across all the males.

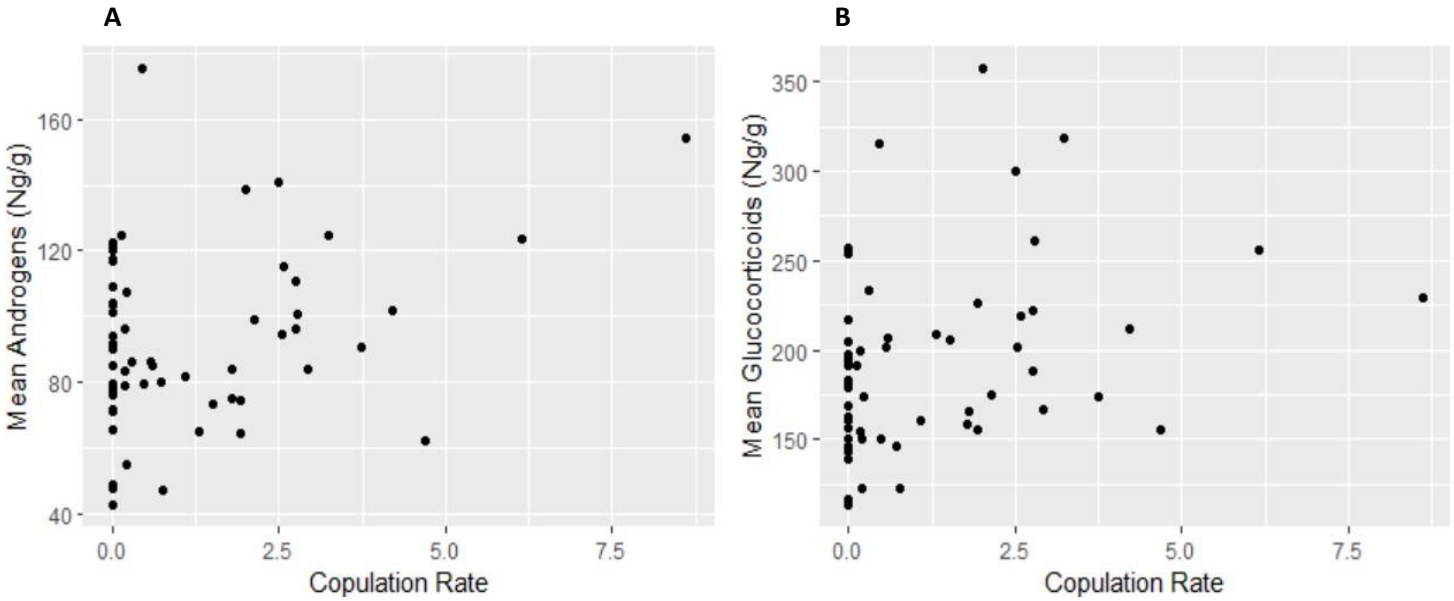

Figure 8. *Male-monthly average of A) Androgens and B) Glucocorticoids and their relationship with male-monthly average copulation rate (total copulation count each month divided by observation time) in the MS. However, the relationship between both hormones and copulations were not significant when using male averages (n=15).*

The best fitting model for male copulations did not include either endocrine measure but did include rank, age, observation time and month (*Table 6*). Observation time was included as a fixed effect to account for the amount of time the males were being observed (hrs).

***Table 6**. Comparison of mixed models predicting number of male copulations during the mating season, using monthly averages for each male.*

Comparison of Parasite Richness	***AIC***	***P-Value***
Model 1. Parasite Richness		
a) Null: (1|Male) + (1|Month)	1225.822	-
b) Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	1232.831	0.7014
c) T + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	1230.276	0.2733
d) GC + Season + Rank + Rainfall + (1|Male) + (1|Month)	1227.865	0.1752
d) T + GC + Season + Rank + Age + Rainfall + (1|Male) (1Month)	1229.578	0.1752
e) T*GC + Season + Rank + Age + Rainfall + (1|Male) + (1|Month)	**1222.083**	**0.01137**

Notes: modelled using glmmTMB() function in the "glmmTMB" package with a negative binomial distribution (n=59), models were compared to the null model using anova. The best fitting model was determined using a backwards stepwise method, the best fitting model is in bold. CopCount = Copulation count, ObvTime = Observatin time (hrs), AvParaRich = Average parasite richness., T = Androgens, GC = Glucocorticoids.

The model summary shows that all of the predictors in the best fitting model, apart from male age, were significant predictors of male copulation rate (*Table 7).* Rank was negatively correlated with copulation count, so the higher ranked males generally achieved a higher number of copulations (Figure7). The highest- ranking male had a much higher copulation rate (*mean= 5.67 copulation per hour of observation*) than any other male (next highest male had a *mean= 3.12 copulations per hour of observation*). Month was also negatively correlated with copulation count and so more copulations tended to occur during the start of the mating season.

Table 7. *Summary of predictors in the best fitting model to predict number of copulations.*

Summary: Model 1. CopCount ~ Rank + Age + ObvTime + Month + (1|Male)				
Predictors	*Estimates*	*std. Error*	*Z-vlaue*	*P*
Rank	-0.16478	0.08127	-2.028	**0.04260**
Age	-0.01818	0.14110	-0.129	0.89751
ObvTime	0.27144	0.08580	3.164	**0.00156**
Month	-0.46386	0.15836	-2.929	**0.00340**

Notes: n=59, Significant predictors in bold. Rank: 1= Alpha, 15= lowest ranking male. CopCount = copulation count, ObvTime = observatin time (hrs), AvParaRich = average parasite richness.

Table 8*. A summary of the most results and if they support the ICHH, SL-ICHH or neither*

Prediction	**Support ICHH**	**Support SL-ICHH**
Parasitism will be highest when androgens are highest	• No – there was no significant difference in parasite richness across the three periods	-
Parasitism will be highest in males with higher androgens	• Some – Androgens had a significant effect on prevalence of Strongylida parasites • No- Androgens had no significant effect on any other parasite measure • No -Male with the highest average androgen level did not experience high average parasite richness	
Parasitism will be higher during times of competition when androgens are higher, in individuals also experiencing high GCs	-	• No – there was no significant difference in parasite richness across the three periods

The positive relationship between androgens and parasitism will only be present when GCs are high	-	• No – The interaction of androgens and GCs had a significant effect on parasite richness and entamoeba prevalence but in the opposite direction to the prediction (positive relationship between androgens and entamoeba prevalence but only when GCs are low and negative relationship when GCs are high) • No- The interaction between the two hormones did not significant affect any other parasite measure
High ranking males will display lower levels of parasitism	• No – Rank only had a significant effect on Iodamoeba prevalence, but higher-ranking males exhibited higher prevalence of Iodamoeba parasites (opposite to prediction) • No – Rank had no significant effect on any other parasite measure	
Males who are better at defending against parasites will be more attractive to females.	• No – Parasite richness did not significantly affect the number of male copulationsachieved • No – The alpha male (11Z) had on average the highest level of parasite richness andhighest copulation rate (supp….)	

Discussion

Overall, across all the males, there was no significant relationship between androgen and GCs and there appeared little difference in hormone levels between high and low ranked males (*supplementary Figure 3. A&B.*). This is possibly influenced by the period of high social instability (OT period), which was characterised by intense agonistic competition among many of the adult males (Georgiev et al. 2016).

Despite every faecal sample containing at least one parasite species and the majority with multiple species present, none of the males exhibited any serious illness during the study period apart from some minor cases of diarrhoea (A. Georgiev, pers. Comm.). This suggests none of them were suffering severe pathology from their parasite infection. However, 11Z (who experienced highest average parasite richness) was observed to have lost a lot of weight after he was overthrown by the other males. This could be a result of parasite infection (although unlikely likely as no other male exhibited illness) or due to the overthrow event and the consequences of dropping to the bottom of the hierarchy This included experiencing targeted aggression from the other males and no longer having priority access to the feeding corrals (Georgiev et al. 2016), both of which could have

impacted his physical health.

Seasonal Variation

There was no significant difference between parasite richness in the different periods; however, there was also no significant difference in either endocrine measure across the three periods so we cannot say for sure, that had there been significant variation of androgens and GCs, parasite richness would not reflect that. Furthermore, the results show that parasite richness was highest during the MS when androgens were also highest, which could suggest that had androgens been significantly higher, parasite richness may have shown the same. According to the ICHH, parasite richness would be highest during times of high androgens due to the immune costs of maintaining elevated androgens. Copulation rates peaked at the start of the MS and decreased throughout the season (supplementary *Figure 1.A*), whereas parasite richness appeared to peak towards the end of the MS (*supplementary Figure 1.B*). This suggests that perhaps parasite infections increase as the energetic costs of endurance rivalries during the mating season begin to affect their energy balance (Higham et al. 2011). Analysis did not show any significant relationships between parasite richness and copulation rate when data was split into before and after the peak of copulation rate. However, the small sample size using means for the 15 males over only 2 months makes it hard to draw conclusions.

Unlike androgens, GC levels were highest during the OT period, likely due to the increased competition for rank caused by the disruption to the hierarchy. However, as this period only lasted one week, it may not have as much of an influence on male parasitism as a whole competitive season (i.e., MS) during which levels of parasite richness were higher. Overall, the majority of the parasite measures were highest in the MS. However, as this was only significant for Trichuris parasites (prevalence and EPG) and Strongylida EPG, the results only provide some limited support for the prediction that parasitism is highest during times of competition and energetic stress. The competition may be limited to reproductive effort/competition for mates rather than direct competition for rank as few parasites were highest during the OT. However, due to the short length

of the OT period this cannot be said for certain. Having data from multiple years would be beneficial for future studies as there is more chance of having more than just one week of competition, which would aid comparisons between competitive and non-competitive periods. There is some research that suggests during mating seasons, individuals rely less on Th-1 (innate) and more on the less costly Th-2 (adaptive) due to the extra demands in that period (Lee 2006) which could affect the seasonal patterns of parasitism depending what branch of the immune system a parasite species triggers.

In contrast to previous research *(*Chapman et al. 2010; Habig et al. 2019*),* rainfall was lowest during the period of highest average parasite richness (MS) but was highest during July, encompassing some of the BS and the whole OT period. Due to the study population being provisioned, rainfall may have less of an effect on the Cayo Santiago rhesus macaques as there would be little effect on food availability and thus nutritional condition of the males. Rainfall was also only significantly negatively correlated with the prevalence of two of the parasites (Trichuris & Iodamoeba). However, similarly to the relationship between rainfall and parasite richness, rainfall was negatively correlated with the prevalence of these parasites suggesting during periods of low rainfall Trichuris and Iodamoeba parasites were higher. This could be due to differences between the parasites and their optimum conditions for development/transmission. Previous research has found that, for Trichuris, an inverse relationship with rainfall could be due to the rainfall being above the optimum for their egg development (Manz et al. 2017). More details on each parasite's prime conditions for transmission and survival would need to be known to investigate this further. Another explanation for the differences across the parasite species could be due to a lag time for the maturation of certain parasite species. Parasite species differ in their development and prepatent periods, for example protozoans have a shorter prepatent period, whereas helminths are thought to have a longer prepatent period due to developing in the external environment (Friant et al. 2016). So, if rainfall was high in the months leading up to the MS, and did aid parasite transmission, for some species this would not be reflected in their faecal samples until the MS of this study period.

There are other factors that could also influence parasitism however, as this study consisted of a small sample size (15 males), other possible confounding variables could not be included in the models due to overfitting issues. Parasite transmission is known to be affected by many environmental and behavioural and factors such as social contact (Freeland 1976; Altizer et al. 2003; Habig et al. 2019), nutritional status of the individual (Chapman et al. 2005; Chapman et al. 2006; Prall & Muehlenbein 2014; Habig et al. 2018; Defolie et al. 2020), and grooming (Akinyi et al 2013; MacIntosh et al 2012). Ideally these factors should all be accounted for in ICHH research so that we can be certain the results are interpreted correctly.

Parasitism and Hormones

The results generally did not support the predictions for a positive relationship between androgens and parasitism or that the glucocorticoids modulate the effect of androgens on immune function such that a trade-off between high androgens and compromised immunity (increased parasitism) would only be seen when GCs are also high.

Those with higher androgens and those with low GCs appeared more likely to suffer from multiple co-infections as they had higher levels of parasite richness. These relationships were not significant when accounting for all confounding variables, but richness was significantly related to the interaction between these hormones, although not in the way proposed by the hypotheses. The interaction suggests that those with high androgens suffer less parasite infections simultaneously when their GCs are high, but when their glucocorticoids are low, they suffer highest levels of parasite richness when androgens are high (*Figure 4*). This suggests that those with high parasitism also had high androgens, but this only occurred when glucocorticoids were low, despite research suggesting immune costs of elevated GCs. The same pattern was also found for Entamoeba (*Figure 5*), where the interaction between androgens and GCs was significantly associated with their prevalence. The same pattern was also found (in varying strengths) for the relationship between the interaction of the hormones and all the parasite EPG's but appeared strongest for Balantidium parasites. This reoccurring pattern is in contrast to the predictions of the SL-ICHH and suggests that

elevated androgens may impact the males' defence against parasites but only when glucocorticoids are low. This gives the impression that GCs may impede the immunosuppressive effects of androgens for defending against parasites; however, the mechanism that could be behind this is unclear. This would need further research into the effects of increased production of GCs and activation of the HPA axis, on the relationship between androgens and immunocompetence and/or parasite defence. As the interaction was only a significantly related to parasite richness and the prevalence of Entamoeba, the idea of GCs moderating the relationship between androgens and immunocompetence cannot be applied to the other parasite measures in this study. However, visually a similar pattern was also seen for the relationship between Balantidium EPG and the interaction between androgens and GCs. Generally, EPG for the other parasite species tended to also show a negative relationship between androgens and EPG when GCs were high, however this appeared weaker. When GCs were low, half of the parasite species had a negative relationship between androgens and EPG suggesting those with low androgens suffered higher parasitism than those with higher androgen levels when their GCs are low *(Figure 6*). However, the other half of the species showed no relationship between androgens and EPG counts, so providing no support for either hypothesis (ICHH or SL-ICHH).

Overall, there was little support for the ICHH as androgens were only significantly correlated with one parasite measure overall. Androgens were positively correlated with Strongylida prevalence, but this was also accompanied by a significant negative correlation with GCs. The results show that GCs alone and GCs interaction with androgens were more often part of the best fitting model for predicting parasite prevalence than androgens, implying that GCs may have more of an effect on parasitism than androgens on their own. It could be possible that due to the limited direct competition for mates in rhesus macaques and low level of sexual dimorphism, even during the mating season, androgens do not increase to a level at which they may compromise immunity. In vervet monkeys (*Chlorocebus pygerythrus*), males display bright colouration on their scrotum which is thought to act as a secondary sexual characteristic, and this colouration has been found to

negatively correlate to parasite prevalence in the high-ranking males (Snyder 2021).Also, (despite limited comparability to primates), in Iberian red deer (*Cervus elaphus hispanicus*) there was only a positive relationship between testosterone and their secondary sexual characteristic (their dark ventral patch during rutting season) size in a population with high mate competition (de la Peña et al. 2020). Suggesting that the level of competition in a population, and the degree of sexual dimorphism in a species may impact the relationship between androgens, secondary sexual characteristics and parasitism in males. In contrast, in male mandrills (*Mandrillus sphinx*) there was little variation in parasitism between individuals despite different facial colourations (a testosterone-dependant secondary sexual characteristic (Setchell et al. 2008)), suggesting their colouration did not reflect their ability to defend against parasites, despite being a highly sexually dimorphic primate species (Setchell et al. 2009).

Another explanation for the lack of support for either hypothesis could relate to the high density of monkeys on Cayo Santiago. It could be that their baseline levels of parasitism are already high so that not much effect can be seen from these social and endocrinological factors. Future studies should compare baseline parasite levels with wild, captive or free-ranging populations depending on their study population to help make inferences from the results. It must also be noted that, as in other primate studies (e.g., Higham et al. 2013 Snyder 202), here we use a measure of androgens rather than specifically testing for testosterone levels. Therefore, we cannot say for certain that results would be the same if only using a measure of testosterone.

In contrast to previous research showing negative immune effects of chronic elevations of GCs (McNamara & Buchanan 2005; Sapolsky 2005; Defolie et al. 2020), parasite richness and prevalence of Chilomastix and Balantidium, and EPG of Chilomastix, Balantidium, and Strongylida all showed a negative relationship with GCs (although only significant for Chilomastix and Balantidium prevalence). This suggests that levels of these parasite species increased when GCs are low. A recent meta-analysis showed parasites are generally linked to an increase in glucocorticoids, however

41.2% of the studies analysed (7 out of 17 studies) found a negative relationship between parasitism and GCs (Defolie et al. 2020). There has been some attempt to explain the cause of this, however, it is still largely not known why this occurs (Defolie et al. 2020). There is evidence that acute elevations of GCs can be beneficial in promoting immune responses against parasites. It is known that a short-term elevation can trigger the diversion of energy to areas essential to survival (Defolie et al. 2020; Sapolsky et al. 2000), however they may also promote some innate immune responses due to the anti-inflammatory responses of GCs (Koprivnikar et al. 2019). However, this is dependent on the hostas well as the species of parasite, so more would need to be known about the specific parasite species, and what branches of the immune system they activate to be able to investigate this line of research.

Some of the parasitism measures appear to show little or no relationship with male GC levels. One explanation for this could be due to the males' familiarity with the parasites. Generally, those in a population have a baseline for parasite infection for which they have already built up an effective immune response to and so, in this case, the parasites would not trigger a stress response (Defolie et al. 2020). Research shows that chronic infection by a parasite may lead to habituation of that species by the host (Defolie et al. 2020). As several of the parasites (Balantidium, Strongyloides and Trichuris) appear to have been present in our study population for a long period (File & Kessler 1989), the males may have habituated to some of these parasites. The results here may not reflect relationships that could occur between novel parasites, which are more likely to be pathogenic, and the variables tested in this study. It is also difficult to accurately identify elevations above their baseline level of infection in a study of this duration; a long-term study period may better allow this differentiation. As in this population, where a parasite and host have been coexisting for a long period, host-parasite coevolution could occur. This may result in the parasite species having little effect on the host and thus may not trigger substantial changes in GC levels (Defolie 2020).

The fact that majority of these parasites are generally not considered highly pathogenic (unlikely to trigger a severe immune response), and previous studies on the Cayo Santiago population show that

they have been exposed to several of these parasites for a long period, could mean that both androgen and GC levels have little effect on their ability to defend against these parasites. Using an unfamiliar or more pathogenic parasite to trigger immune responses may be better to understand how these hormones could affect their ability to defend against potentially pathogenic infections. For example, here *Balantidium coli* was by far the most prevalence species (*supplementary Figure 2.)* but is generally not considered pathogenic for this species; therefore, an immune response may not be triggered to an impactful level, so there would be no detectable relationships with either hormone.

The results here, which show that there is no consistent pattern across the parasites in terms of their relationships with the hormones and other factors, highlight the importance of testing each species of parasites individually. The species all have different life stages, pathologies and influencing factors etc. which cannot be accounted for if they grouped together as a single parasitism measure. It is possible that the different parasites affect a host's immune system differently, therefore, future studies should aim to analyse them separately so that the results reported are accurate.

Rank differences in Parasitism & Mating Success

Depending on the parasite species/measure, rank did appear to have some impact on male parasitism and copulation rate in the study population with varying strength. The alpha male for the mating season (11Z) had highest levels of the hormones (androgens and GCs) but also had the highest overall average parasite richness and copulation rate. This suggests that high rank and reproductive effort may have some immune associated costs which may also relate to the high levels of androgens and glucocorticoids also displayed. As the males did not seem to be suffering externally as a result of their parasite infection, it is possible that the "costs" of reproductive effort (i.e., a reduction in ability to defend against parasites) could be minimal compared to the benefits they gain from investing in reproductive effort. However, as speculated in Georgiev et al. (2016), the accumulating costs during the mating season (possibly including the higher level of parasitism during

this period), may have left the alpha male more vulnerable and resulted in his overthrow and beatings from the other males. This possibly provides some support for the cost of reproductive effort on the males' immunocompetence and their overall physical condition; although, this is only speculation. When accounting for all variables, rank was not significantly correlated with parasite richness (*Table 1),* suggesting there is no difference in the number of different parasite species infecting across the ranks. Using male averages, rank was only significantly negatively corelated to parasite richness during the OT period, but this was in an opposite direction to the prediction, as higher ranked males appeared to suffer higher levels of parasite richness during this period of competition. Suggesting that during the overthrow period higher ranked males suffered higher levels of parasitism. However,the small sample size means this pattern may not be an accurate representation.

Although only significant for Iodamoeba, generally the higher-ranking males suffered more occurrence of Protozoans (apart from Balantidium) and Trichuris parasites compared to lower-ranking males. However, the model output for Iodamoeba prevalence shows that that is unlikely to be due to differences in hormone levels as they were not variables in the best fitting model (*Table 4.B*). Rank also appeared to show at least some positive relationship with the prevalence of Balantidium, Strongyliodes and Strongylid EPG, meaning that generally, the lower ranks were more likely to be infected with these parasites which is in line with the predictions of the ICHH. The relationship between rank and EPG appeared strongest with Strongylid parasites. Strongylids are considered to be pathogenic in primate species (e.g., in Chimpanzees (*Pan trogylodytes schweinfurthii*): Terio et al. 2011), so the higher-ranking males may be experiencing a higher parasitic load of a potentially pathogenic parasites. This is opposite to the predictions of the ICHH which proposes higher-ranking (high-quality) males would be better at defending against parasites, due to being able withstand the immunosuppressive effects of high reproductive effort. The variation in results here, again, highlight the importance of analysing parasitism data at the species level.

The lack of consistent significant relationships between rank and male parasitism (only significant

for Iodamoeba prevalence) and how the males were showing serious symptoms of infection, may suggests that the more modern view of “costly signalling” (Higham 2014; Penn & Számadó 2020) could be more relevant here than the handicap principle. This view posits that males balance their investment in reproductive effort so that they do not incur any immunosuppressive effects. Males that are of a higher quality are able to invest more in reproductive effort compared to lower quality males, who would not be able to invest the same without incurring the costs. In this study, althoughhigh-ranking males are likely of higher quality, overall, they did not appear to suffer more/less than lower rank/quality males, possibly due to this optimum balance between reproductive effort and immunocompetence.

Parasite richness appeared to have no effect on male copulations but instead social status (rank) best predicted mating success (*Table 1),* whereby those of a higher rank appeared to achieve more copulations. A different explanation for this result could relate to the priority of access model, which proposes that higher-ranking males have better access to mates and may display mate guarding to try to prevent other males copulating (Dubuc et al. 2014).

The physical condition of the males may also play a role in the relationship between rank and copulation rate; in a previous study in rhesus macaques, high-ranking males had better physical condition and achieved higher copulation rates than lower-ranking males (Georgiev et al. 2015). This could result from the high-ranking individuals having better access to food (Habig et al. 2018), meaning they can maintain a higher nutritional and physical condition possible making them appear more attractive to females.

Male Copulations

The alpha male, during the mating season (11Z), had significantly higher mating success than any other male, while also having the highest average of average parasite richness in the mating season alone, as well as across the whole study period, suggesting that there could be some immune related costs associated with high mating effort. However, it could be possible these costs may also be

attributed to the overthrow of 11Z where he became the lowest ranking male during the OT and so may have impacted behaviour and hormone levels and thus parasitism/hormones. Visually those with high average parasite richness appeared to achieve higher average copulation rate, suggesting female choice was, therefore, not driven by low parasitism. However, parasites richness did not significantly correlate with male copulations when accounting for the effects of rank and age so overall, parasite richness had little effect on male copulation rate.

Surprisingly, although the model without endocrine measures was the best fit, the models containing GCs, without androgens, were generally a better fit with the copulation data, in comparison to the models containing androgens alone. This suggests that GCs may have more of an influence on male reproductive effort than predicted. Previous research has found evidence of GCs' role in reproductive effort due to their role in decreasing gonadal steroid production, which are used in the promotion of secondary sexual characteristics (Leary & Baugh 2020). However, this is not the case here as GCs and androgens appeared positively correlated. It would be interesting to investigate relationships between male facial colouration and GCs in rhesus macaques and how this would relate to their reproductive effort and parasitism. In rhesus macaques, facial colouration is thought to act as a sexual signal; those with darker redder faces may achiever higher mating success (Dubuc et al. 2014), so including this in future SL-ICHH studies may prove useful.

Unlike previous studies (Milich et al. 2020), age did not significantly impact male copulation rate. However, in this study the majority of the males (apart from three who were sub-adults) were only just passed or stillin their prime, so there may not have a been enough males from a variety of age groups to see the effect of age here.

The ICHH posits that females choose less parasitised males to prevent offspring suffering from high parasite loads which could affect their survival. As none of the parasites in this study appeared to be highly pathogenic, there may be less need for the females to have preference over less parasitised individuals as it would be unlikely their offspring would suffer severe illness from these. This could offer some explanation as to why parasite richness appeared to have little effect on male copulation

rate.

Conclusion

Overall, there appears little support for ICHH, or SL-ICHH (*Table 8*). The results show little effect of androgens on male parasitism. Where there is a significant relationship between the interaction of androgens and GCs, and parasitism, it is opposite to the predictions of the SL-ICHH. In these cases, there was evidence for a negative impact of elevated androgens on male parasitism but only when GCs are low, more suggestive of GCs impeding the immunosuppressive costs of elevated androgens. When GCs were high, those with high androgens experienced lower parasitism (although this was only detected for two parasitism measures). There was more evidence for a relationship between GCs and parasitism than androgens and parasitism, but results showed that this again was opposite to previous research where GCs were often negatively correlated with parasitism. This could possibly be due to benefits of acute elevations or issues of co-evolution and pre-exposure to the parasites present.

Male parasitism did not seem to impact male copulation rate, which was better predicted by male rank position where, as expected high-ranking males achieved higher copulation rates. There was evidence for immune costs of reproductive effort as the alpha male, on average, suffered highest levels of parasite richness as well as the highest copulation rate. However, results show this may not be due to endocrine-immune relationships but more likely the energetic costs of reproductive effort. Overall, there appears to be more evidence for the influence of male rank, rather than hormones, for the predictions of the ICHH and SL-ICHH, due to significantly affecting both, two parasite measures (Iodamoeba prevalence and Strongylida EPG (using male averages)), and male copulations. This study also shows the importance of investigating parasite species individually as the variation in their life history and development can affect how their transmission is influenced by biological and social factors.

Implications for the Reproductive Effort and Immunocompetence Trade-off

Results here show little evidence for an endocrine-parasite relationship suggesting there may not be a trade-off between reproductive effort and immunocompetence (in the form of male parasitism) in this study population. It is possible that other environmental, behavioural, and social factors could be more likely responsible for the difference in parasitism across the males. Therefore, future research should ensure a large sample size to allow all possible confounding variables to be included in analysis. Overall, neither androgens nor GCs alone appeared to show significant relationships with the parasite measures. Instead, there was some evidence for the role of an interaction between androgens and GCs in predicting some of the parasite measures included (even if this was not in the way predicted), therefore GCs and an interaction term with androgens should be included in further ICHH research. However, more work is still needed to understand how GCs and androgens could be interacting, and the mechanisms behind GCs moderating a relationship between androgens and immunocompetence. As mating success was not influenced by male parasitism, female mate choice is not driven by the ability of males to defend against parasites in these rhesus macaques. Therefore, future studies would benefit from using other measures of immunocompetence to identify any relationships between reproductive effort and immunocompetence.

Declarations

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

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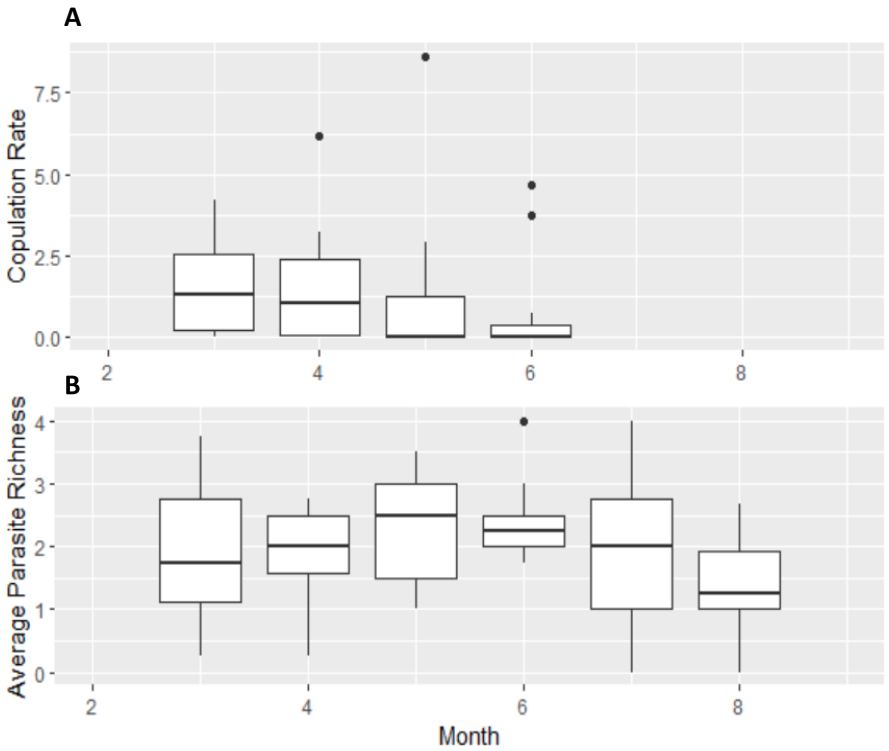
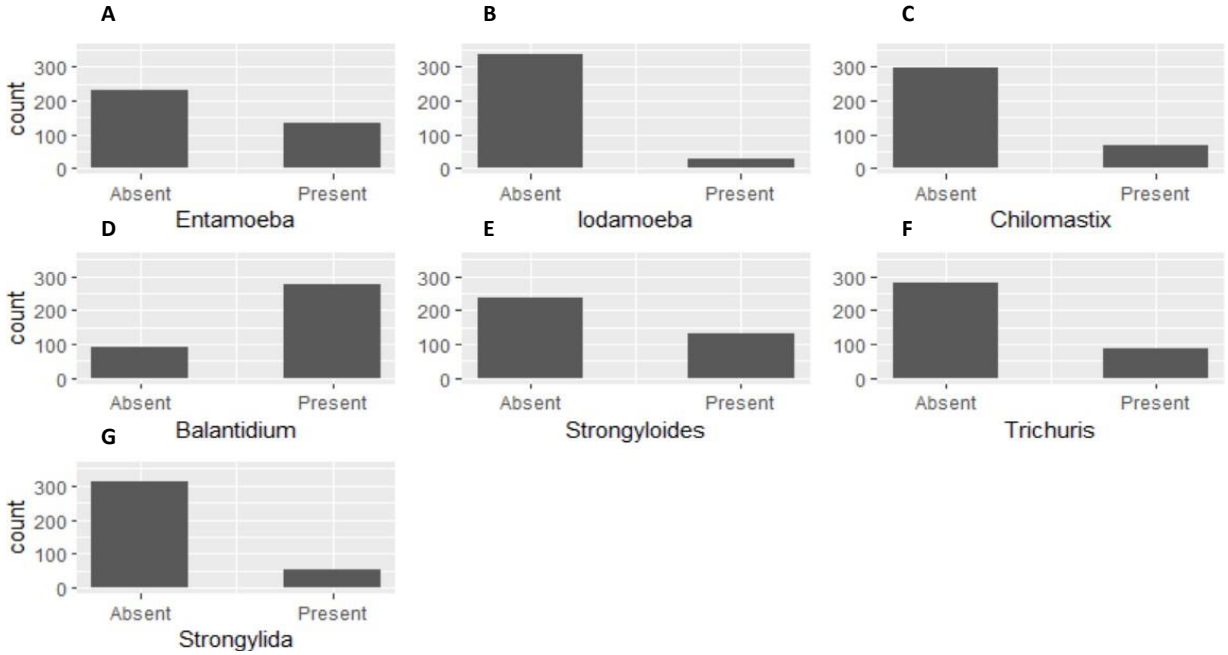
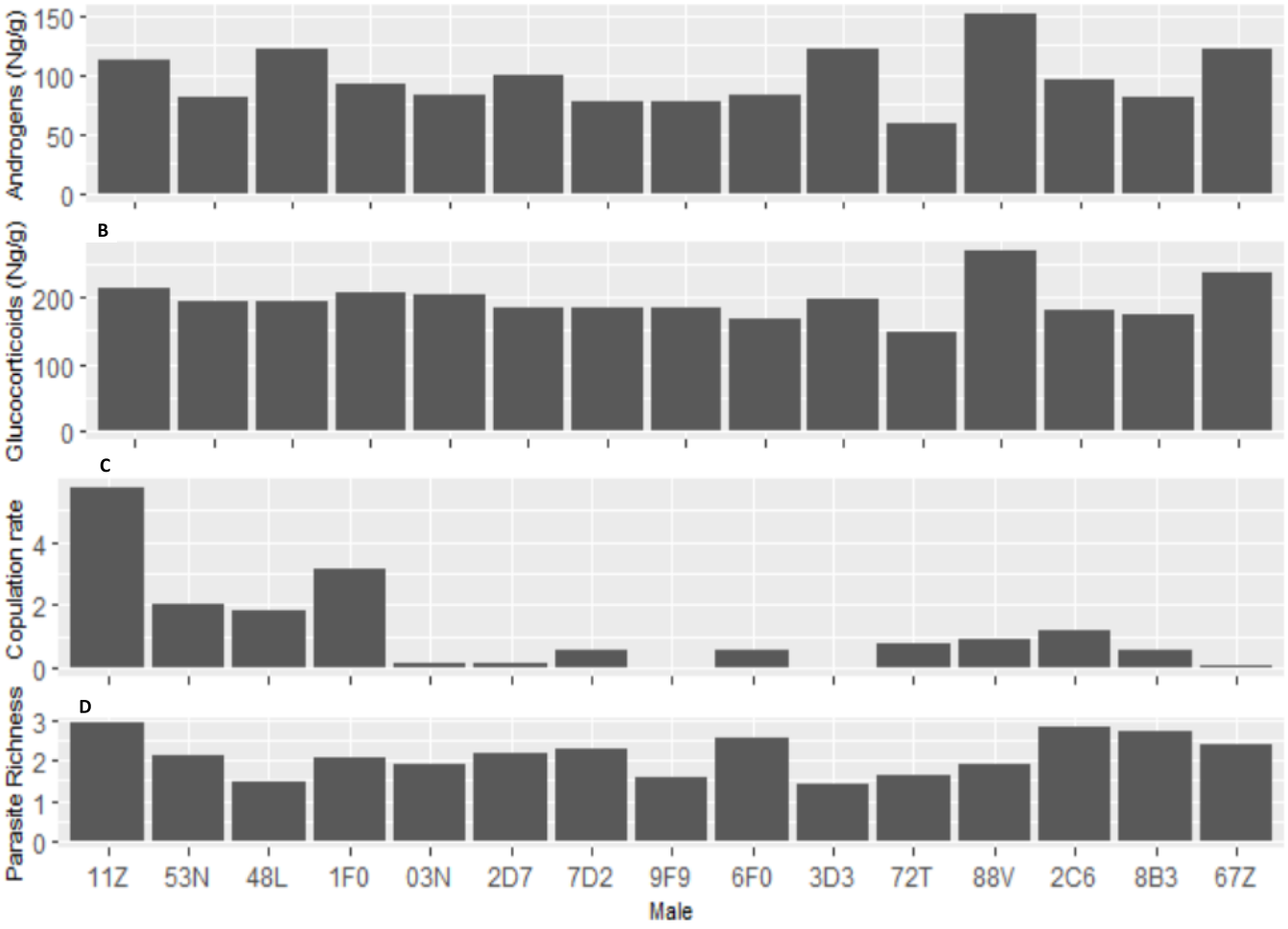
Supplementary Material



Supplementary Figure 1.A) Average number of copulations for each male each month B) Average number of unique parasites in each faecal sample for each male each month. N=108, line shows median of mean male-monthly values; box = upper and lower of the interquartile range; whisker extended to the highest value within 1.5 interquartile range of the box.



Supplementary Figure 2. The number of samples where A) Entamoeba B) Iodamoeba C) Chilomastix, D) Balantidium, E) Stronygloides, F) Trichuris, G) Strongylida parasites were present or absent (N=366).



***Supplementary Figure 3*.** Male averages across the whole study period in order of their rank in the MS (11Z= alpha, 67Z=lowest rank) of A) Androgens, B) Glucocorticoids, C)Copulation rate (only in the MS) and D) Parasite richness.

Supplementary Table: 1 Male averages across the whole study period in order of their rank during the mating season (MS)

Male	**Rank** (MS)	**Androgens**	**Glucocorticoids**	**Parasite Richness**	**Copulation Rate**
11Z	1	113.02449	**213.5501**	**2.916667**	**5.6717767**
53N	2	80.96385	194.7221	2.142857	**1.9926334**
48L	3	121.28217	192.7641	1.481481	1.8411182
1F0	4	92.30536	205.9590	2.040000	**3.1159240**
03N	5	82.96810	**202.7063**	1.892857	0.1552329
2D7	6	99.80132	183.7744	2.181818	0.1406250
7D2	7	76.54029	185.8322	2.272727	0.6021429
9F9	8	77.61756	184.4792	1.562500	0.0000000
6F0	9	82.85758	168.4737	2.578947	0.5508475
3D3	10	**122.39027**	197.3536	1.440000	0.0000000
72T	11	59.14353	149.2862	1.619048	0.8082298
88V	12	**151.58203**	**269.6748**	1.916667	0.9047619
2C6	13	96.48673	181.3272	**2.840000**	1.1896552
8B3	14	80.80514	173.6952	**2.695652**	0.5662208
67Z	15	**121.83739**	237.7137	2.384615	0.1125000

Notes: Highest three values are in bold

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