Applying the Challenge Hypothesis to Wild, Adult Male Chacma Baboons (*Papio ursinus*)

by

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Abstract

In male birds and mammals, testosterone (T) is the primary hormonal regulator of reproductive physiology and behavior. Though it is necessary for reproduction, sustained levels impose social and physiological costs. As such, fluctuation of T across a male's lifetime should reflect efficiency in T allocation to relevant life-history stages; mainly, up-regulation should be associated with attaining mating opportunities followed by down-regulation when these periods are over. For example, because high levels of T are known to interfere with paternal behavior, T levels should decrease when males are required to care for offspring. The theoretical framework that explains this, the Challenge Hypothesis (CH; Wingfield, Hegner, Dufty & Balle, 1990), proposes that variation in male T across life-history stages reflects differential allocation to mating and parenting behavior. Successful for explaining T across breeding and non-breeding seasons in avian taxa, application of the CH to mammals (both seasonally and non-seasonally-breeding) has received mixed support. Here, I apply the CH to a non-seasonally breeding mammal, the chacma baboon (*Papio ursinus*). Chacma baboons are ideal for this study because they exhibit high mating skew (characterized by high competition for mates mediated through a dominance hierarchy) as well as true paternal care (in the form of "friendships" with lactating females). Results suggest that male behaviors related to acquiring mating opportunities (i.e., rank changes) and paternal care were the best predictors of male T levels after controlling for age and rank. Such results found across the life-span for chacma males mirror results found for seasonal species within each breeding season suggesting that the CH applies to non-seasonal primates with mate competition and paternal care.

Applying the Challenge Hypothesis to Wild, Adult Male Chacma Baboons (*Papio ursinus*)

Behavioral endocrinology, the study of how hormones and behavior exert a reciprocal effect on one another, presents a unique perspective for exploring how biology and the environment interact to produce behavior. Of particular relevance to behavior are steroid hormones because they exert a dualistic effect on the ontogenetic trajectory of an individual. First, they are important regulators of somatic and neural development, sexual differentiation, and early origins of adult phenotype. Second, they act in the brain and on body tissue throughout life as proximate mechanisms for a wide variety of behaviors. In male birds and mammals, testosterone (T) is critical for reproductive physiology and behavior, but poses social and physiological costs. As such, T levels of male birds and mammals should vary as a function of life-history stage. In other words, T should be up-regulated when it is needed, but down-regulated when it is not. To this end, the Challenge Hypothesis (CH; Wingfield et al., 1990) proposes that variation in male T across life-history stages reflects differential allocation to mating and parenting behavior. Although successful at explaining hormone-behavior relationships in birds, the CH has garnered inconsistent support across mammals. Here, my primary objective was to apply the CH to a novel model system for non-seasonally breeding, long-lived mammals, the chacma baboon (*Papio ursinus*). I combine over 5 years of longitudinal and cross-sectional data from a wild population of chacma baboons living in the Okavango Delta, Botswana. Using demographic, hormonal, and behavioral data, I test the CH as it applies to a non-seasonal species.

Testosterone and Behavior

Testosterone (T) is an androgenic steroid hormone. Derived from cholesterol, steroids are a class of chemical messengers that shares a similar 4-ring structure and method of action. This

structure is lipophilic, and allows steroids to readily diffuse across the plasma membrane of cells. After entering the cell, they may bind to an appropriate receptor (usually in the cytoplasm), and this complex moves into the nucleus where it may bind directly to DNA and affect transcription (Nelson, 2005). Steroids are considered slow-acting because their main mechanism of action is to regulate gene expression; although there are exceptions (see Duval, Durant & Homo-Delarche, 1983). Additionally, steroids may act on tissues in the periphery, as with the effect of androgens on secondary sex characteristics. Steroids are released from endocrine glands throughout the body in a pulsatile fashion, and regulate both physiology and behavior in most vertebrates. The androgen group of steroid hormones includes testosterone, and refers to any 19-carbon metabolite thereof that can bind to the androgen receptor. Additionally, some androgens can be further metabolized into an 18-carbon estrogen by the enzyme *aromatase*. Males have high levels of this enzyme in the brain and body, allowing estrogens to play a significant role in male development as well (i.e. the effect of aromatized testosterone on territoriality, Soma et al., 2000).

Of the androgens, T has the most profound effect on behavior. Although elevations in T are often associated with an increase in mating behaviors and aggression, T alone generally does not *cause* these behaviors. Rather, T and behavior interact in a feedback loop where one influences the other (Oyegbile & Marler, 2005). Not all aggression is mediated by androgenic activity, however. It is well understood that anti-predator or irritable aggression are not androgen-dependent (Moyer, 1968; Nelson, 2005). Thus, it is specifically aggression in a reproductive context that is associated with varying degrees of T (Nelson, 2005).

With respect to reproductive behavior, T is generally necessary for the normal development of secondary sex characteristics required for attracting and competing for mates. In

some cases, these secondary sex characteristics include "signals" that males can use to assess one

another prior to engaging in a physical contest (Maynard Smith, 2003). Winning such contests

can result in acquisition of a prime territory, immediate access to females (e.g., acquisition of a

harem of females), or an elevation in dominance rank. The original work done on the role of T in

dominance contests comes from red deer (*Cervus elaphus)* on the island of Rhum, Scotland.

During the breeding season, T levels in male red deer were associated with antler production,

male loud calls ("roars"), and an increase in male-male aggression. Testosterone (and not a

different growth factor) has been shown to be directly responsible for the growth of male antlers

(Bartoš, Schams, & Bubenik, 2009), and males that were castrated showed a decrease in

aggressive behaviors (Lincoln, Guinness, & Short, 1972). Additionally, males use the loudness

of other males' roars to assess a rival's condition and to determine whether or not to challenge

that rival. Because the loudness of a male's roar is directly correlated with his T levels (Clutton-

Brock & Albon, 1979), it has been proposed that roars are honest signals of a male's willingness

to fight. Signals such as roaring must be honest, that is they incur some cost to the individual

(Zahavi, 1975), so that low quality males (i.e., "cheaters") cannot attain the benefits of rising in

dominance. Because there are metabolic, energetic, and social costs to maintaining high levels of

T, only males who are of high quality can produce very load roars. This allows males to

accurately assess one another before fighting and engage only in evenly-matched fights. Rival

assessment, escalated aggression, and antlers themselves all interact to produce the dynamics of

male-male dominance determination. However T only modulates these factors and does not act

on dominance directly. For example, amputation of a gonadally intact stag's antlers produces an

immediate and sharp drop in social status without any change in T (Bouissou, 1983).

While T has often been demonstrated to modulate behavior, behavior is just as likely to modulate T. The "winner effect" describes this influence in the context of contest outcome. That is, through positive feedback of T on behavior (Oyegbile & Marler, 2005), the winner of an agonistic outcome is more likely to both (1) engage in another contest, and (2) win that subsequent interaction (Dugatkin, 1997). Because T regulates reproductive parameters, the winner effect has implications for dominance rank attainment. When males engage in contests for access to mates, the outcome of these interactions can affect future ability to win such contests.

Hormones and Life-History Strategies

Life-history theory (Gadgil & Bossert, 1970) describes the ontogenetic trajectory of an individual as comprised by identifiable events or periods. Life-history milestones can be discrete (e.g., adrenarche, rank attainment) or continuous (e.g., parenting, senescence) periods of time when an individual undergoes fundamental changes in their physiology or behavior. Transitions between these periods, however, may be associated with costs and benefits; otherwise known as life-history tradeoffs (Stearns, 1989). Such tradeoffs are manifested when an adaptive change in one trait is linked to a cost in another trait. From a mechanistic perspective, T often causes the expression of many traits, not all of which are adaptive during a given period (Ketterson & Nolan, 1992; reviewed in Zera & Harshman, 2001). For example, T potentiates the male-male encounters requisite across taxa in competition for mates. Assuming a true winner effect, males with higher T will win more male contests, and this in turn will further up-regulate these males' T. This will result in successful males with respect to male competition. However, sustained circulation of T at high levels can have deleterious consequences (reviewed in Kempenaers, Peters, & Foerster, 2008; McGlothlin, Jawor & Ketterson, 2007). As such, males are faced with

selective pressure to have sufficient T for mating success on the one hand, but avoid unnecessary elevation of T on the other. In sum, it is expected that lifetime profiles of T reflect efficiency in the timing and duration of elevated androgens.

The Challenge Hypothesis

Original Hypothesis

With the advent of new techniques in behavioral endocrinology (Wingfield & Farner, 1976), researchers began to integrate hormones into their behavioral studies of wild animals, especially in seasonally-breeding birds. This allowed researchers to compare endocrine profiles across closely-related species. When divergent patterns began to emerge regarding the role of T in the life-histories of males across different species, Wingfield and colleagues (1990) developed the CH to make sense of this variability. Specifically, the CH proposes three hypothetical T levels (Appendix 1) and utilizes information about a species' mating system and life-history stages to explain variation between them. The first level ("a") is the non-breeding baseline. It describes the average T level for adult males outside of the breeding season, and represents the lowest level of circulating T. The second level ("b") is the breeding baseline, and describes the minimum T level for adult males to produce sperm and mating behavior during the breeding season. It is also representative of the level necessary and sufficient for the development of secondary sexual characteristics. The third level ("c") is the physiological maximum for T. Given that baseline b is sufficient for mating behavior, elevations above this theoretical baseline require explanation.

The CH posits that variation in T levels above "b" reflect male-male agonism (challenges) in the context of reproduction. Because T has a minimal "necessary but sufficient" role in the expression of secondary sex characteristics and reproductive behavior, highly

fluctuating T levels (e.g., between b and c) are unlikely to be the result of mating itself. Rather, the *acquisition* of mates via mate-guarding, territory establishment, acquisition of high rank, and other mechanisms for gaining access to mates are more likely candidates for explaining this T variability. Although typically not discussed in the CH context, the winner effect actually corroborates the CH in that small elevations in T can be amplified based on the outcome of competition. Thus, the CH views mate acquisition and mating itself as separate processes with very different T profiles. Accordingly, a multitude of subsequent studies have explored the predictions of the CH together with the hormonal and immune parameters of life-history tradeoffs. As applied to avian taxa, the CH does a very good job at explaining the observed T variation in birds across a single breeding season (Goymann, Landys, & Wingfield, 1997).

In addition to explaining broad patterns of T within a species, the CH also seeks to explain the androgen responsiveness (AR) of a species, population, or (in some cases), an individual. AR refers to the potential for increases in T above baseline b, and is expressed by the following relationship: (c-a)/(b-a), where a, b, and c represent the three levels of T (minimum, breeding baseline, and maximum, respectively). Additionally, the CH proposes a *Rank Order Index*. This is a somewhat arbitrary measure calculated as the male-male aggression to parenting ratio. Male-male aggression is ranked from 1-3 (with 3 being the highest aggression) and paternal care is ranked as 1 or 2. Therefore, to use the Rank Order Index, each species/population/individual would be assigned a male-male aggression score (1-3), to be divided by their parenting score (1 or 2), to produce a Rank Order Index that will range between 0.5 (low aggression, high parenting) and 3.0 (high aggression, low parenting). When Wingfield and colleagues (1990) examined representative avian species with respect to Rank Order Index and AR, clusters indicative of similarities in breeding strategies emerged (Appendix 2). This was

explained by life-history strategies common to different breeding systems. In particular, polygynous species typically exhibit sustained high T levels during the breeding season to facilitate competition for mates and are likely to have little paternal care. Conversely, monogamous males typically exhibit low T during the breeding season and instead have high levels of paternal care. Underlying these predictions are two main assumptions: (1) individuals of a particular species, indeed of a particular mating system, employ life-history strategies comprised of similar behaviors, and (2) parenting and mate-acquisition are incompatible life-history stages and represent an absolute trade-off that is modulated by T. While these assumptions hold true for birds, they must be reexamined when applying the CH to mammals.

The Challenge Hypothesis in Mammals

The predominant mating system in birds is monogamy (Mock & Fujioka, 1990), though some species show polygyny due to socioecological constraints. While genetic evidence has shown that this monogamy is not strict (there is a high rate of extra-pair copulations, EPCs; Hughes, 1998), it is not necessary that divergence from strict monogamy implicate an increase in T. As predicted by the CH, only *challenges* by males or *competition* for mates should cause an increase in T above baseline b. It is possible that these EPCs are "sneaky" or circumvent the classical model of competition for mates. Especially if these EPCs are initiated by females, it would not be expected that they alter male endocrine profiles (despite having far-reaching genetic consequences). Indeed, Wingfield's findings (1990; Appendix 2) would support this notion.

By contrast, polygyny is the predominant mating system in mammals (Kleiman, 1977; Eberle & Kappeler, 2004). The CH predicts endocrine patterns for monogamy, polygyny, and promiscuity, and thus has the potential to be applicable across taxa. Because polygyny is so

widespread in mammals, the predictions of the CH are expected to be similarly upheld across mammalian species. On the contrary, however, such analyses have yielded little consensus (Hirschenhauser and Oliveira, 2006). This lack of consensus may be due to the high degree variability in mammalian life-histories. For example: (1) mammals show both seasonal and non-seasonal patterns of breeding, (2) mammalian lifespans vary widely, ranging from short-lived rodents to long-lived humans; (3) many 'polygynous' mammals are genetically promiscuous or monogamous (McEachern, 2009), and as opposed to birds this divergence from polygyny may be accompanied by an increase in T-dependent behaviors; (4) for species that do show "polygyny", significant variation in endocrine profiles exists (Beehner et al., 2009); and (5) paternal care is rare in mammals. In addition to this variability, other (related) obstacles have hindered our ability to apply the CH (as originally conceived for birds) to mammals. For example, for long-lived, non-seasonally breeding species, long-term demography records are necessary since it is more biologically relevant to study an individual's T profile across a lifetime to capture the entire period of maturation, breeding, and parenting (Bribiescas, 2006; Crawford, Harewood, & Handelsman, 1997; Ellison et al., 2002; Martin, Swenson & Collins, 1977). Additionally, paternal care is relatively rare in mammals. As such, testing this hypothesis at all is inherently constrained. Indeed, analyses of some non-human primates have generally supported that T and male-male aggression for mates are correlated, but none of these species exhibit paternal care (as reviewed in Huck, Löttker, Heymann, & Heistermann, 2005). Of the mammals that *do* show paternal care, however, T has an unclear response to the onset of paternal care, and (in contrast with birds) does not always decline for paternal males during the non-breeding season (reviewed in Nunes, Fite, Patera & French, 2001). Additionally, high T can *promote* paternal behavior in some species by conversion to E2 by aromatase (Trainor & Marler, 2002).

In fact, human males (Berg & Wynne-Edward, 2001; Muller et al., 2009), and black tufted-ear marmosets (*Callithrix kuhli;* Nunes et al., 2001), and prairie voles (*Michrotus ochrogaster;* Wang & DeVries, 1993) are the only mammals thus far to show a decline in T associated with the onset of paternal care.

Finally, determining the true mating system of a given species usually requires genetic data. This can be circumvented if the species in question is amenable to constant and highly transparent behavioral observations (such as baboons, *Papio* spp.). Under these conditions researchers are likely to see most copulations, and if patterns of copulations differ appreciably from expected patterns they are likely to be noticed. Unfortunately, many mammalian species have cryptic aspects of behavior that are not easily explored by humans (such as nocturnal or arboreal species).

Chacma Baboons as a Model System

It is becoming increasingly clear that a "strict-sense" CH does not apply broadly to mammals, but how to test whether it applies at all? First, it would perhaps be beneficial to divide mammals into three broad categories: short-lived seasonal breeders (such as rodents), long-lived seasonal breeders (such as elephants or horses), and long-lived non-seasonal breeders (such as humans). It is likely that the seasonal breeders will have more in common with each other than the long-lived species due to fundamental differences in life-histories. The relatively brief and punctuated cycles of breeding and parenting, with complete separation of these life-history stages, are likely to affect CH more than lifespan; the latter probably influences these predictions but in more subtle ways. Implicit in this framework is a critical departure from the original CH. Instead of stratifying lifetime T patterns based on mating system, I propose to stratify based on similarities in life-histories. This enables a behavior-based approach that is obscured when

invoking broad classifications such as "monogamy" or "polygyny". While these classifications are certainly useful for understanding social systems across taxa, they may not be as appropriate for studying behavioral endocrinology (as evidenced in Beehner et al. 2009).

At this point, the focus of discussion shifts from broad-scale considerations of the CH to how the CH may apply in non-seasonally breeding, long-lived mammals (NSLMs). (However, it should be noted that any of the fundamental discussions can be broadly applied with respect to testing the CH outside *Aves*). In order to analyze how all facets of the CH may be applied widely across NSLMs, a *model species* requires both existence of paternal care and longitudinal data. A lack of paternal care does not preclude a species from fitting the CH (Wingfield et al., 1990 accounted for these types of species using Rank Order Index), however paternal care is a useful quality in a model system because it enables a full exploration of the CH predictions. Similarly, longitudinal data need not be a requirement of researchers wishing to study the CH (cross-sectional data could be sufficient), but having this data provides information on individual trajectories, and these are critical to validating new theory. Furthermore, it would be useful if this system had observable, transparent behavior with a well-elucidated mating system.

A population of chacma baboons (*Papio ursinus*) living in the Moremi Game Reserve in the Okavango Delta, Botswana provide an appropriate model system for testing the CH. This population was been studied extensively from 1987 (Bulger & Hamilton, 1987) to 2007, with longitudinal hormonal collection from 2001-2006. The taxonomic status of chacma baboons is somewhat contested, so to avoid confusion they will simply be referred to here as a species (*Papio ursinus*). The discussion of *Papio*'s taxonomic divisions is not relevant to the topic at hand, as has no bearing on the results herein or their interpretation. Chacma baboons are a highly

polygynous species with a strict linear dominance hierarchy and are one of the few primate species to demonstrate true paternal care (Palombit, Seyfarth, & Cheney, 1997).

Paternal behavior in chacma baboons.

Across all Savannah baboons, paternal care involves an increase in male-female affiliative behaviors outside the mating context. Such associations (especially in baboons) are known as friendships, and occur for a number of proximate and ultimate reasons (Smuts, 1985). The length, strength, and reasons for these friendships vary across the *Papio* taxa, and in chacmas are known to be specific to relationships between adult males and lactating females (Cheney & Seyfarth, 2007; Palombit et al., 1997). Females and males both benefit from friendships (in the form of mating access, protection, or social reassurance (Smuts 1985), but infants are most widely referenced when explaining the functional significance of friendships. Infanticide is a major cause of mortality for baboon infants (Cheney et al., 2004), and a hallmark of chacma paternal behavior is protection of these infants from potentially infanticidal males (Palombit et al., 1997). Females work more than males to maintain these friendships, perhaps reflecting a greater lifetime benefit to females, though paternity, stress alleviation, and other unknown social factors may be enhancing the benefit to males (Cheney and Seyfarth, 2007, Smuts 1985). Indeed, putative differences in the benefits of friendships for young vs. old males or natal vs. immigrant males (in example) have not been well characterized. With respect to this study, paternal care will be quantified using both measurements of paternal behavior and affiliations with the infant's mother. This interchangeable usage of parenting and friendship behaviors are appropriate for chacma baboons, but may not be in other baboons (i.e. kinda baboons, *Papio cynocephalus kindae;* Weyher, personal communication).

Mating competition in chacma baboons.

The circumstances under which baboons may obtain mating benefits has been well-elucidated. The priority-of-access (POA; Alberts, Watts, & Altmann, 2003) model depicts the baboon male hierarchy as a queue, with mating access as the end reward. The POA predicts that mating opportunities are acquired only by acquiring rank in the top of the hierarchy (from 1 to n, where n is the number of estrus females available for mating). In practice, this means that the alpha male is first in line to mate with estrus females, and mating access is granted down the hierarchy in a linear fashion until there are no more estrus females available in the group. As such, acquiring rank in chacma baboon society is the single most important fitness-conferring endeavor for adult males. Accordingly, it is often characterized by intensely agonistic interactions, although rank changes occasionally occur with little aggression (Beehner, personal communication). In the context of the CH, male-male competition involved with attaining high rank is likely to be a better predictor of increases in T than mating itself. Preliminary work in this area has already identified T profiles which track broad life-history milestones in the following way: an increase in T shortly after sexual maturity is accompanied by competition for rank acquisition, and steady declines from this point forward advance at variable rates until old age (Beehner et al., 2009). Understanding of these shared life-history traits provides a framework for further investigation. It is expected that age and T will show some broad correlation with rank acquisition, mating, and parenting periods, however variation within these stages remains to be understood (Figure 1). Specifically, variations in life-history strategies (the ratio and temporal distribution of mating and parenting behaviors) are likely to produce vastly different endocrine profiles in males of similar rank and age.

The Moremi chacma baboons are an NSLM population that exhibit true paternal care, have well-elucidated mechanisms of mate acquisition, live in an open area amenable to constant and complete observation, and have a longitudinal dataset complemented by 5 years of hormone and behavior samples from known males. As such, they emerge as perhaps the best (and only) non-human NSLM population currently available for exploring in-depth predictions of the CH. This project capitalized upon this opportunity and sought to test the following predictions under the CH: (1) male T levels are not affected by mating itself, (2) the process of attaining high rank will be associated with elevated T; (3) after controlling for age and rank, significant and sustained drops in T will reflect the onset and termination of paternal care; and (4) the same variation in AR and mating/parenting ratio documented across bird species will be found within chacma baboons corresponding to different male reproductive strategies. This longitudinal and cross-sectional approach enables one of the first in-depth explorations of the CH in a non-avian species. It may help resolve the problem of why male mammals show little consensus in their T profiles, and whether it is possible to construct an adapted version of the CH with applicability to NSLMs and perhaps mammals in general.

Method

Study Site and Subjects

Behavior and fecal samples were collected from a population of free-living chacma baboons (*Papio ursinus*) at the Moremi Game Reserve, Botswana. The population has been under study since 1987 (Bulger and Hamilton, 1987), and under almost-daily observation since 1992 (Cheney et al., 2004). The group is fully habituated to humans on foot. The ages of all natal individuals are known, and estimated ages from immigrant males reflect identification of age-specific traits and appearance. The matrilineal relationships are also known for all natal

individuals. In addition, the following demographic data was recorded for the entirety of this

population's study period: birth, deaths, male and female rank changes, immigrations,

emigrations, female reproductive state (i.e. cycling, pregnancy, and lactation), and consortships.

This study used behavioral and hormonal data collected from 2001-2005, at which time adult

males in the group ranged from 4-16 males, all with variable tenures in the group (Table 1 for

demographic data). The data collected was for a number of different projects, and none were

designed specifically to test the CH. Once a longitudinal dataset was assembled, however, there

was sufficient data for addressing the predictions as outlined above.

Male Dominance Rank

Male dominance rank was scored by daily recordings of dyadic interactions (approach-

retreat interactions). Male rank changes are obvious, so upon observation they were noted in the

project demography. Absolute ranks (# rank/ n males in the group) were transformed to relative

rank for these analyses to control for fluctuation in the number of ranked, adult males present at

any given time. This is done by computing $(n - \#) / (n-1)$, where n are the number males in the

group and # is the absolute rank of the individual. All relative ranks were rounded to the nearest

whole number.

Behavior

Throughout the study period, 10-minute focal animal samples (Altmann, 1974) were

collected routinely for all males, whereby every individual was sampled before starting over

again (in an attempt to have an even distribution of sampling across individuals). A total of

19,690 minutes of behavior was recorded for 26 males over the study period, resulting in an

average of 757.31 minutes/male (ranging from 50-2,360 minutes/male). This wide range was due

to variation in the number of months each male was in the study. All social behavior was

recorded during focal animal sampling, but for this study I only analyzed the behaviors

summarized in Table 2 (description of analyses to come below). Behavior and hormone samples

were collected by the following postdoctoral researchers: Thore Bergman and Jacinta Beehner,

2001-2003; Anne Engh, Catherine Crockford, and Roman Wittig; 2003-2005.

Hormone Collection and Processing

When a positively identified fecal sample was observed, the fecal sample was collected in

a plastic cup and thoroughly mixed with a spatula. Next, 0.5 g of the sample was placed in 10 ml

of a methanol/acetone solution (100% methanol; 8:2) and the sample was immediately

homogenized (1 min) using a battery-powered homogenizer (BioVortexer, MidWest Scientific).

Following homogenization, samples were capped tightly and stored at ambient temperature until

processing. Ten hours after the sample was collected and homogenized, 4.0 ml of the sample

solution were filtered through a polytetrafluoroethylene (PTFE) syringeless filter (0.2 Am;

catalogue AV125EORG, Whatman, Clifton, NJ) to remove particulate matter. The filter was then

washed with 4.0 ml of methanol/acetone solution (100% methanol; 8:2). The filtrate was diluted

1:2 with distilled water and set aside while cartridges were primed. Sep-Pak Plus C18 cartridges

(catalogue WAT020515, Waters Associates, Milford, MA) were primed according to the

manufacturer's instructions using 2.0 ml of 100% methanol followed by 5.0 ml of distilled water.

The filtrate was loaded onto the primed cartridge at a steady rate (f0.2 ml/s) using a syringe. The

cartridge was washed with 2.0 ml of a sodium azide solution (0.1%) to further reduce

degradation, placed in a sterile Whirl-Pak bag with 1.0 g of silica beads (a desiccant), and stored

for up to 40 days at ambient temperature until shipped (via DHL) to the United States. On

arrival, all samples were immediately frozen at 10°C. After allowing the residual fecal matter to

dry completely (up to 2 weeks), undigested seeds were removed and the dry weight of the

residual fecal material was recorded. Samples were originally shipped to the laboratory of Dr.

Patricia Whitten (Emory University), and stored there until January 2009. At that time, all

samples were shipped to the University of Michigan for me to process.

When the samples arrived at the University of Michigan, I became the sole researcher on

this project (under the advising of Dr. Beehner). All samples were transferred into new tubes for

permanent storage at the University of Michigan, and checked for proper identification and

missing samples. From May-Sep 2009, I assayed all fecal samples (N=1,355) for T metabolites

using a modified protocol from a commercially-available T RIA kit (Diagnostics Systems

Laboratories, Beckman Coulter, Webster, TX). All assays used the standards, the primary

antibody, and the precipitant solution provided by the kit. Working buffer was a charcoal

adsorbed human serum similar to the buffer in which the standards were diluted (American

Biological Technologies, Inc., Seguin, TX). The primary antibody from the DSL T kit cross-

reacts 100% with testosterone, 6.6% with 5α-dihydrotestosterone, 2.2% with 5-androstane-3β,

17β-diol, 1.8% with 11-oxotestosterone, 0.9% with androstenedione, and 0.6% with 5β-

dihydrotestosterone. Cross reactivity of the antiserum with all other steroids is less than 0.5%.

All samples were run in duplicate, and the results are expressed as ng/g dry fecal matter. Inter-

assay coefficients of variation were: 15.85% for the low kit control, 11.91% for the high kit

control, 52.2% for the low volume fecal pool, 15.75% for the middle volume pool, and 16.18%

in the high volume pool.

Data analysis

All data analysis was run on SPSS (version 17.0 for PC), and the statistical threshold for

all tests was set to $p<0.05$. A General Linear Mixed Model (GLMM; see Muehlenbein, Watts &

Whitten, 2004) was used whenever appropriate to control for individual differences in baseline

T. In most cases, multiple models were tested for goodness of fit using Akaike's Information

Criterion (AIC), and models with the best fit were selected for use.

Testosterone Data

This non-invasive method for obtaining hormone information uses *metabolites* of T to

gain information about relative circulating T levels. Because of the time it takes for these

metabolites to become present in feces, T samples collected on any given day reflect circulating

T from the previous 24-48 hours. T values were non-normally distributed, and thus were log-

transformed to facilitate the use of parametric statistics. With one exception (the examination of

the effect of male consortships on T levels), a mean monthly rate of all other variables was

calculated for each male. This included average monthly LogT, age at the mid-point of the

month, rank during each month, future rank change (based on the following month), and other,

analysis-specific variables as described in the appropriate sections below. The number of data

points after this transformation (i.e. the total number of months for which there was data on

individual males) was *n*=412 male months. To account for individual variation in baseline T

levels (Figure 2), a monthly Z-score for each male's T was calculated using a standard Z-score

equation; ((monthly average T level)-(*total* average T)/(*total* standard deviation of T)). This "Z-

T" value was used mostly for heuristic purposes, and *was not used in any analyses or figures*

unless otherwise noted.

Rank and Age Analysis

First, I examined the extent to which relative age or rank could explain differences in T

for this dataset (as shown in Beehner, Bergman, Cheney, Seyfarth & Whitten, 2006; Beehner et

al., 2009). Based on previous results from a subset of this dataset, age was expected to be

associated with T levels (Beehner et al., 2009) but rank was not (Beehner et al., 2006). A quadratic regression was used to examine LogT as a function of age, and a linear regression was used to examine LogT as a function of relative rank.

Aggression Analysis

Aggression rates were calculated on a monthly basis for each individual male in the following way: (counts of total "aggressive" behaviors; Table 2)/ (total focal minutes). After monthly rates had been calculated for each male, it became apparent that there was not a sufficient amount of behavioral data on aggression to continue with this analysis. This is because focals are often aborted when males begin fighting as the move quickly out of the range of visibility (Beehner, personal communication). As aggression was only supplemental to rank changes with respect to identifying pertinent male-male competition, it was not analyzed further.

Mating and Mate-acquisition Analysis

The CH treats the act of mating and mate acquisition as two separate stages. To appropriately test the CH, and due to the social structure of chacma baboons, these two stages were analyzed separately in this study as well. Individual T samples collected 24-48 hours after a male had been in consort with a female were marked as a "consort" sample. All other samples for that male were marked as "non-consort" samples. For males with both consort and non-consort samples (n=22), I conducted a paired T-test to examine whether consort samples were higher in T than non-consort samples were.

To examine how T relates to mate-acquisition for chacma males, I reason that rank changes (rather than rank, per se) should serve as good proxy for male-male competition. Specifically, rising and falling in rank indicates acquisition or loss of mating access. This is especially true since overt aggression does not always accompany a rank change. Therefore,

restricting the definition of male-male competition to include only the presence of aggressive behaviors may be leaving out critical changes in life-history stages. Here, I explored two facets of rank change: the direction of rank change and whether all rank changes, or just those in the top of the hierarchy, effect T levels. In addition to monthly T values, monthly ranks were recorded for each male while he was in the study. The month before a rank change was marked as "unstable" (for that male), and the direction of change was recorded. It is important to identify the month before (and not the month of) rank changes since these changes are the *result* of male-male interactions. The behavior predicted by the CH to cause an increase in T are the male-male interactions themselves, and thus rank change is used only as a marker that such events occurred (especially in cases where there is an absence of actual aggression). Four separate GLMMs were used to test whether males (1) falling in rank ("falling"), stable in rank ("stable"), or rising in rank ("rising") showed any differences in their T levels, (2) whether falling, stable, or rising males in the top 1/3 of the hierarchy showed differences in T, (3) whether unstable males showed differences in T across all ranks, and (4) whether unstable males in the top 1/3 of the hierarchy showed differences in T. I used the top 1/3 of the hierarchy because based on the number of estrous females at any given time, these are the only males whose ranks result in mate acquisition. All GLMMs used logT as a dependent variable and rank change as the independent variable (where rank change was defined as one of the four ways described above). It is important to highlight that this study defined rank instability with reference to the individual, not the group. As it is being used as a proxy for mating access under the POA model (which is likely to have been maintained during the course of this study), individual T profiles are expected to respond only to a male's own trajectory and not that of the group.

Paternal care analysis

Each mother-infant dyad throughout the study (*n*=93) was coded as a single unit, and all "parenting" behaviors (Table 2) with the dyad (to or from the mother or to the infant) from adult males were analyzed. Rates of parenting with each dyad were calculated for each male *individually* (as described for aggression above): # parenting behaviors/# focal minutes, where the timeframe of interest was not each month but the whole period of paternal care. This period was defined as extending from the birth of the infant until the male stopped interacting with the dyad. In no case did this period extend to weaning, and there was wide variation in the duration of parenting "bouts" (from 0.3 to 15.3 months). Every male received a paternal behavior rate for each dyad (even if this rate was 0.00), except males who were not present during the period of lactation for each dyad (that is, if they had no focal minutes during the period of dyad formation). A histogram of these rates (Figure 3) revealed a nonzero drop-off point around 0.03 acts/min, indicating the threshold for rare rates of paternal behavior. A male whose paternal behavior rate exceeded 0.03 acts/min for a given dyad, and whose behavior persisted across >30% of the parenting period were designated as putative "fathers". This was a binary assignment of "yes" or "no". In this case it did not matter whether the male in question was indeed the genetic father, as our hypothesis was testing whether paternal behavior (not genetic parentage) was correlated with decreases in T levels. A GLMM with LogT as the dependent variable, male ID as a random variable, parenting as a factor, and age and relative rank as covariates was used to test whether paternal behavior had a measurable effect on T levels. In addition, a GLMM designed to specifically test the challenge hypothesis was run which included LogT as the dependent variable, male ID as a random variable, parenting as a factor, and rank changes within the top 1/3 of the hierarchy and age as independent variables.

Rank-order index

The original CH proposed an arbitrary index calculated as the male-male aggression to parenting ratio. While this was applied to the species-level in birds (Wingfield et al., 1990), I tested whether rank-order indexes for individual chacma males could predict T levels. Because there was not enough data to run this analysis within every male, I pooled all males by their monthly scores (as described above in *Testosterone data).* Every month, a male was assigned a male-male conflict score of 1, 2 or 3 (1 = males ranked in the lower 2/3 of the hierarchy, 2 = males with a steady rank in the top 1/3 of the hierarchy, and 3 = males with changing ranks in the top 1/3 of the hierarchy. This was divided by their parenting score (1 for all non-parenting months, and 2 for all parenting months). This yielded a range of scores from 0.5-3 (Table 3). I then used a one-way ANOVA to test for differences in T across the 5 groups (0.5, 1, 1.5, 2, and 3).

Androgen responsiveness during parenting vs. non-parenting periods.

It was not possible to test androgen responsiveness (AR) in this study as described in the original CH for two reasons: (1) the non-breeding (juvenile) T baseline was unknown for all individuals, and (2) the breeding baseline was only theoretically existent in this species, and not empirically observable. While the lower 2/3 of the hierarchy likely did not face much male-male competition, it would be inappropriate to say that they faced *no* male-male competition. Seasonal breeders have more discrete periods of male-male competition, potentiating a reliable measure of baseline levles. However, this reliability is obscured by relatively ambiguous life-history states in non-seasonal breeders.

To test a modified version of AR, I defined new, discrete categories based on previous results from this study. Using only males who exhibited some degree of parenting during the

study (*n*=12 males across 333 male-months of data), I calculated mean T levels across the

following 4 categories of males: stable parent, unstable parent, stable non-parent, and unstable

non-parent. 'Unstable' referred to rank changes in the top 1/3 of the hierarchy, representing a

'challenge' (mating competition). By subtracting the "stable" from the "unstable" mean T values,

it was possible to compare AR to 'challenges' across parenting and non-parenting males (Table

4). Additionally, I used a paired T-test to compare mean T levels for parenting males one month

after rank instability to that male's mean parenting T (*n*=18 instances of rank instability during

parenting across *n*=8 males). The purpose of this test was to see if T levels returned to parenting

baselines after the "challenge".

Data analysis was carried out as described above in 3 stages, with each successive stage

building upon results from the previous one. First, I examined the effect of rank, age, and consort

status on T levels. Second, I examined the effect of rank changes both across the hierarchy and

within the top 1/3 of the hierarchy on T levels, and the effect of parenting on T levels. Third, I

applied the Rank Order Index (Wingfield, 1990) at the individual level and tested whether

parenting and non-parenting males had a difference in AR.

Results

Stage 1

A quadratic regression for age and T (p=.019, r^2=.014), and a linear regression for rank

and T (p=.009, r^2=.016) revealed a significant, but loosely associated effect for both factors with

respect to T (Figure 4). There was no difference in average T levels for consort (M=1.05,

SD=.13) versus non-consort (M=1.04, SD=0.15) samples collected from the same male (pairwise

t-test: t(21)=0.024, p=0.796; Figure 5).

Stage 2

There was no relationship between T and the direction of rank change when all males were included in the analysis (GLMM: $F(2, 409)=0.31$), $p=0.730$), or when only the top 1/3 of the hierarchy was included (GLMM: $F(2, 151)=0.17$, $p=0.84$). Furthermore, T was unrelated to rank instability (regardless of direction of change) when all males were included in the analysis (GLMM: $F(1, 410)=0.63$, $p=0.43$) all returned non-significant results (Figure 6). By contrast, T was significantly higher for periods of rank instability when only the top 1/3 of the hierarchy was included in the analysis ($F(1, 410)=5.39$, $p=0.021$; Figure 7). From this point forward "rank instability" will be used to refer to a male in the top 1/3 of the hierarchy who has risen or fallen in rank.

T levels were significantly related to parenting, regardless of age (GLMM: $F(1,388)=0.28$, $p=0.60$) and rank (GLMM: $F(1,388)=3.64$, $p=0.06$), with males exhibiting lower T during parenting periods than non-parenting periods (GLMM: $F(1, 388)=9.60$, $p=0.002$; Figure 8), Additionally, T was also significantly related to rank instability (GLMM: $F(1, 388)=4.28$, $p=0.039$) and parenting (GLMM: $F(1, 388)=9.60$, $p=0.002$), regardless of age ($F(1, 388)=1.01$), $p=0.32$) with males exhibiting higher T during periods of rank instability.

Stage 3

T levels were significantly different across Rank Order Indexes (ANOVA: $F(4, 394)=7.12$, $p<.001$; see Figure 9 for Tukey's post-hoc results). Additionally, the modified AR score was 0.084 for parenting males and 0.037 for non-parenting males (Figure 10). Finally, there was no difference (T-test: $t(7)=.38$, $p=.72$) in parenting T levels before or after periods of rank instability.

Discussion

Stage 1

Dominance rank itself has been previously shown to be a less powerful predictor of T than the direction of rank change (Beehner et al., 2006), and these results corroborate previous findings. Additionally, age has been previously shown to be broadly associated with T production in this species and to have a reverse U-shaped curve (Beehner et al., 2009). There is substantial evidence for a generally negative relationship between adult male age and T, but variability exists within broad age classes. Explaining this variation was one of my goals here. Finally, mating (i.e., males in consort) itself did not appear to affect T levels. This has two implications for the CH. First, this result is the first piece of evidence in that supports the CH for chacma baboons. Because of high reproductive skew in this species, and under the POA model, chacmas obtain mates by fighting for high rank. However, once rank is attained mating is relatively assured. As such, the period of a male's life history where he is expected to have an increase in T is during rank acquisition, not during mating.

By contrast, chimpanzees (Muller & Wrangham, 2004; Muhlenbein et al., 2004) show an increase in T in the presence of estrous females. Because they have a fission-fusion society and relatively low contact with estrous females, competition for mates occurs very close to the act of mating. As such, T levels measured from this period are simultaneously reflective of two discrete life-history stages (mate-acquisition and mating itself). Such results do not refute the CH (as the authors note), rather they illustrate the difficulty of applying a strict-sense CH to LNSMs. This difficulty arises partly due to such confounds as those seen in chimpanzees, but also from the vast and subtle variation in the architecture of life-histories both across and within these types of species.

Stage 2.

Male T exhibited an anticipatory rise to changes in rank (whether rises or falls), but only for males in the top 1/3 of the hierarchy (Figures 6 and 7). This may be related to the male-male competition that precedes changes in rank (Alberts et al., 2003). Superficially, this contradicts previous findings that T predicts where a male is *going* in the dominance hierarchy (such that higher T predicts an increase in rank, and lower T predicts a fall in rank) rather than where he *is* (Beehner et al., 2006). However, this previous study tracked broad changes across the year rather than monthly changes. It is possible that this previous study picked up on larger life-history patterns across a male's reproductive lifespan while the current study has captured more short-term changes. According to both the CH and the winner effect, males engaging in competition for mates (which stands as a proxy for mating success in this case) should show an initial increase in T, followed by either positive or negative feedback in accordance with winning or losing, respectively. As such, males who win are likely to sustain neutral or continued increases in T, while males who have lost should show a decrease in T during subsequent months. If this decrease in T is correlated with a drop from the top 1/3 of the hierarchy, then males are expected to show a significant decrease in T during subsequent months; however this fall from the top of the hierarchy is not a requisite characteristic of losing a fight. Further analysis on this dataset may support such predictions, and would be an informative avenue for future research.

Paternal behavior was negatively related to T levels for all males regardless of age or rank. Interestingly, this result has also been shown in the black tufted-ear marmoset (*Callithrix kuhli;* Nunes et al., 2001), but not for the closely related moustached tamarins (*Saguinus mystax,* Huck, Löttker, Heymann, & Heistermann, 2005). Callitrichid primates are unique among the primate order in that they show highly variable mating systems across species and populations, and show varying degrees of breeding seasonality (reviewed in Huck et al., 2005). Indeed, Huck

and colleagues refer to this ambiguity of breeding patterns when explaining their findings.

Chacmas provide a contrast to the results of callitrichid and non-paternal species in that they are

unambiguously recognized as non-seasonal breeders and show true paternal care. Taken

together, these results suggest that physiological patterns related to phylogeny or mating system

interact with socioecological conditions to produce variation across species in their hormonal

responses to paternal care.

Relationships between age, mate acquisition, parenting and T.

Although T varies in a predictable way across male age (i.e., the reverse U-shaped

relationship, Beehner et al., 2009), results from the present study can explain much of the

variation within age groups based on whether a male is fighting for high rank or in a friendship

with a lactating female. In this population, however, the average age of non-parents is

10.88 ± 2.94, and the average age of parents is 12.45 ± 2.22. Additionally, 9 males were observed

to parent while occupying ranks .7-1 (the top 1/3 of the hierarchy). This suggests that while

"mating" and "parenting" may be broadly discrete categories for the extreme ages (young,

recently mature males and very old males), there exists substantial variation for the intermediate

ranges (Figure 1). In sum, the specific behavior of any individual is the best predictor of their

endocrine profiles, regardless of species-wide and taxon-wide trends. Furthermore, the *context* in

which behaviors occur is of critical importance. Here, rank changes were used only as a proxy

for "mating competition", but were found to be a valid proxy only for a specific subset of the

hierarchy. This underscores the usefulness of combining endocrine and behavioral measures

when empirically testing evolutionary theories.

Stage 3

In this analysis, I use information specific to chacma baboons to test applicability of the CH across similar taxa (NSLMs). The Rank Order Index (Wingfield et al., 1990) is an arbitrary scale for separating high aggression, low parenting males from low aggression, high parenting males. Its application in birds was useful for showing that endocrine patterns were correlated to mating system (Wingfield et al., 1990), however mammals show much greater behavioral and endocrine variability within mating system classifications and even across populations (as in callitrichids; Huck et al., 2005). In fact, individual variation among chacma baboons seems to be as much or greater than that seen across avian taxa. Males at the two extreme ends of the Rank Order Index show the expected result with higher T among males with high aggression and low parenting and lower T among males with low aggression and high parenting. These results suggest that this index is generally a good way to measure mating to parenting ratio across individuals within a species, as well as across species (Figure 9). As there has been great difficulty in identifying how the predictions of the CH may be applied broadly, having a standardized unit such as this will enable more reliable comparisons across diverse species. Indeed, for mammalian taxa that show little paternal care and/or long lifespans, cross-sectional data may be fitted to this index to produce interpretable measures of the CH's applicability *without* the presence of paternal care or longitudinal data.

For example, individuals in an NSLM species without paternal care could be scored for differences in male-male competition, but since they do not exhibit paternal care, the denominator would be the same for each male (though the numerator could theoretically be different). This would functionally be the same as testing the effect of male-male competition on T. If, however, a closely-related species *did* show paternal care, it would be possible to standardize male-male competition score across both species by using the same criterion to

generate the numerator, and then stratify the two species using the denominator. If the two species showed T differences in accordance with Rank Order Index measurements, a standardized comparison across males of the two species will have been realized. Furthermore, this index allows comparisons of males within a species who vary in their male-male competition and/or paternal care (different life-history strategies).

Finally, using the Rank Order Index helped identify an interesting source of within-species variation. The index classification "1.5" refers to males who are both parenting and experiencing rank instability, and their T is on par with that of non-parenting males experiencing rank instability. Because males who are parenting experience depressed T, a T increase of this magnitude may represent a greater AR than for the same male when he is not parenting. Indeed, a modified version of AR score showed a larger AR for parenting males. This disproportionate T response, coupled with a return to parenting T levels after the challenge is over, supports the possibility of within-individual variation in AR that is dependent upon life-history stage (see Figure 10). In other words, a reproductive strategy involving a breeding season imposes exogenous constraints on the time-period when T is necessary (and beneficial). When a species lacks such exogenous organization, the flexibility of life-history strategies becomes quite high. Additionally, this potentiates the likelihood that mating and parenting life-history stages become less mutually exclusive than for seasonally breeding species. As an adaptation to overlapping mating and parenting opportunities, it may be expected that NSLMs have evolved plasticity in AR, such that an individual may show "monogamous"-like AR during parenting periods and "polygynous"-like AR during non-parenting periods in order to balance mating and parenting efforts. This ides is further supported by observations that males in this population do not have an absolute trade-off between mating and parenting (8 males were observed to parent while

occupying ranks in the top 1/3 of the hierarchy). The results of this analysis should be interpreted with caution, however, as there was insufficient data to test each male individually for differences in AR. Rank instability during times of parenting were relatively rare (only 18 months across 8 males, 2.25 times per male, were observed), and even fewer males had periods of rank instability for both parenting and non-parenting periods (n=2). Nevertheless, this pooled data suggests that AR may be plastic across a male's lifetime, and future studies specifically designed to test this hypothesis would be highly informative.

Maynard Smith (1995) identified patterns associated with major transitions in evolution; one of them being that small autonomous entities come together to form a new unit of organization. I have shown here that some of the patterns found across seasonally breeding species are found within the lifetime of non-seasonally breeding individuals. This finding underscores the principles discussed by Maynard Smith, and provides insight into the evolution of non-seasonal patterns of reproduction.

Conclusions

This research has provided an in-depth analysis of the CH, and found support for all of its predictions in wild, adult male chacma baboons (*Papio ursinus*). Specifically, results have used T to further validate the POA model of mate acquisition in baboons, and quantify behavioral patterns that are specifically relevant to mating competition. It was found that both acquiring mates and paternal care have powerful and opposing effects on T, further supporting a model of hormonally mediated life-history tradeoffs (Ketterson & Nolan, 1992). Furthermore, it may be possible that an adaptation to non-mutually exclusive periods of mating and parenting in NSLMs (due to a lack of breeding season) has evolved in the form of AR plasticity. Finally, a method for

producing standardized results across studies when testing the CH has been proposed and

validated using the chacma baboon as a model system.

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"It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living". *-David Attenborough*

Table 1

Demographic information

	Demographic Variables			**Mean Values**			**Rank and Parenting**	
Name	# Fecal Samples	Years	# Months	LOGT	Age	Rank	Start/End Rank	# Times Parenting
AP	78	2001-3	19	0.81	15.55	0.2	0.5 / 0	1
AU	76	2001-3	23	0.98	16.47	0.2	0.3 / 0.3	1
BG	68	2002-4	24	1.05	10.33	0.9	1 / 0.8	1
BJ	89	2001-3	25	0.97	11.85	0.3	0.9 / 0.3	4
BR	25	2002-4	6	1.03	8.69	0.5	0.2 / 0.9	--
CC	19	2004-5	5	0.97	9.44	0.4	0.1 / 0.4	--
CY	8	2003-4	4	1.41	9.54	0.7	0.7 / 0.6	--
EL	91	2003-5	29	1.01	10.52	0.6	0.9 / 0	8
FG	33	2001-2	10	0.85	11.57	0.5	0.8 / 0.1	--
FT	47	2004-5	15	1.20	9.97	0.4	0 / 0.6	--
GM	11	2002	4	0.99	9.44	0.9	0.9 / 0.9	--
HA	18	2005	5	1.32	9.46	1.0	1 / 1	--
LO	70	2003-5	25	1.03	10.67	0.3	0.5 / 0.2	3
MG	87	2001-4	27	1.14	10.15	0.7	0.1 / 0.8	3
MU	19	2003-4	9	1.11	9.07	1.0	1 / 1	--
NA	67	2004-5	17	1.17	9.26	0.7	0 / 0.8	1
NK	87	2001-4	32	0.93	8.58	0.4	0 / 0.3	1
PO	83	2001-3	21	0.82	12.21	0.7	1 / 0.6	5
RK	20	2002-3	8	0.78	7.43	0.3	0.3 / 0.3	--
RS	5	2004	3	1.30	9.42	0.0	0/0	--
RY	157	2001-5	48	1.04	14.79	0.4	0.8 / 0.5	11
SOa	15	2001-3	7	1.00	--	0.5	0.7 / 0.3	--
SOb	50	2002	12	1.03	9.85	0.7	0 / 1	1
TH	78	2003	21	0.99	13.12	0.7	0.8 / 0.7	13
WA	32	2002	8	1.00	18.98	0.3	0.4 / 0.1	--
WO	15	2004-5	5	1.28	9.46	0.6	0.6 / 0.5	--
TOTALS	**1348**	**2001-2005**	**52**	**1.05**	**11.03**	**0.5**	**--**	**3.79**

Table 2

Description of behaviors

Focal Behaviors Analyzed

Aggression

*1) **Physical**: any physical aggression or chasing.*

*2) **Visual:** eye threat, ground slap, head bob.*

Mating

Consortships only

Paternal Care

1) Approach or is approached within 2 m.

2) Groom or is groomed by a lactating female.

3) Handling a baby.

4) Baby grunt directed at an infant or the infant's mother.

Table 3

Rank Order Index

Competition for mates		**Paternal Care**	
1	bottom 2/3 of hierarchy (LC)	**1**	no (NP)
2	top 1/3 of hierarchy (MC)	**2**	yes (P)
3	rank instability (HC)		

Categories		**Abbrevitions**	
0.5	*LC, P*	**LC**	Low Competition
1	*MC, P; LC, NP*	**MC**	Mid Competition
1.5	*HC, P*	**HC**	High Competition
2	*MC, NP*	**NP**	No Parent
3	*HC, NP*	**P**	Parent

Table 4

Modified Androgen Responsiveness (AR) Calculations

	Stable	Unstable	
Parent	A	B	**B-A**
Non-Parent	C	D	**D-C**

	Stable	Unstable	
Parent	0.958	1.043	**0.084**
Non-Parent	1.025	1.062	**0.037**
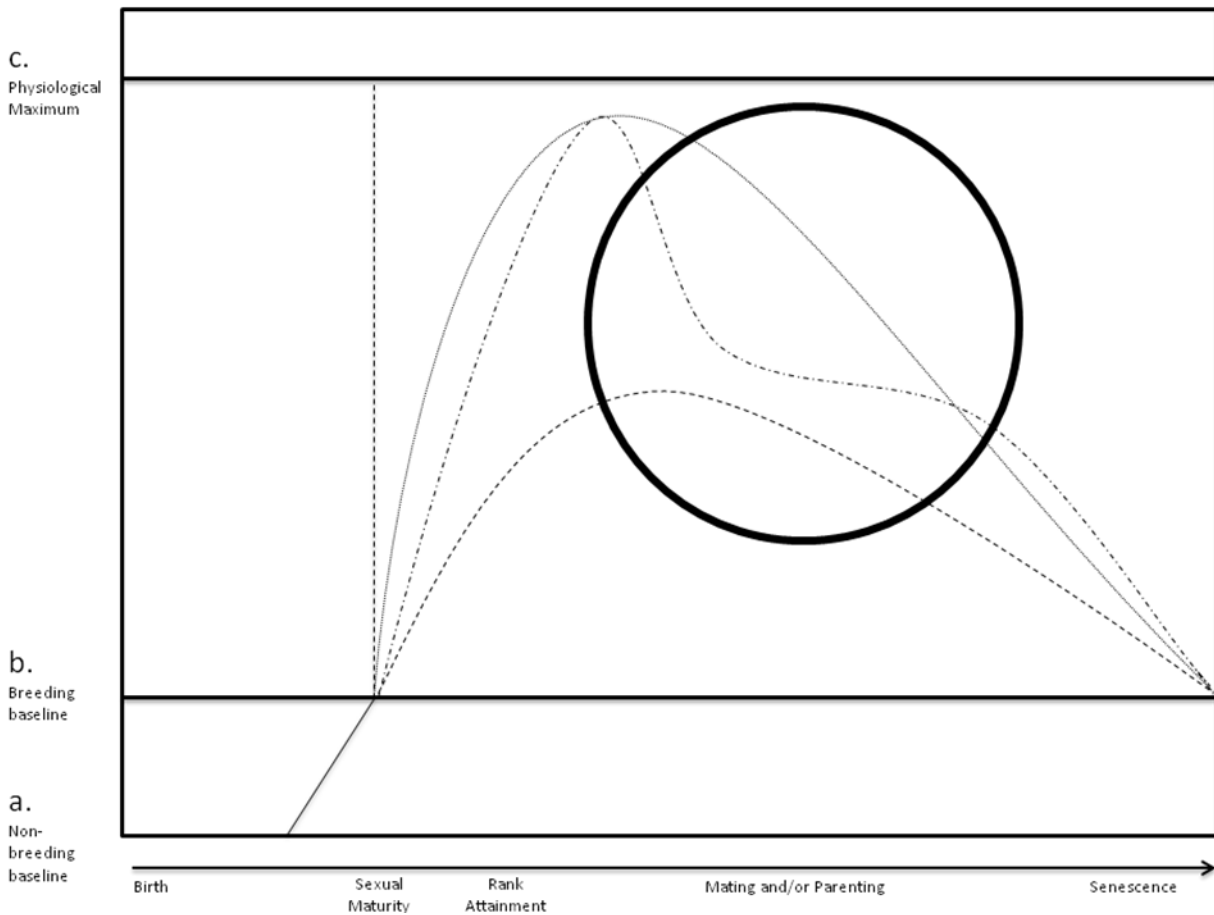
Figure 1. A modified depiction of the CH.

This heuristic model depicts variability in T across a male's lifetime, and identifies the region of

maximum variability in life-history strategies (circle). Males in the circle may be high-ranking

but parenting, moderately ranked but attempting to mate, etc. (Adapted from Figures 1 and 4,

Wingfield et al., 1990).
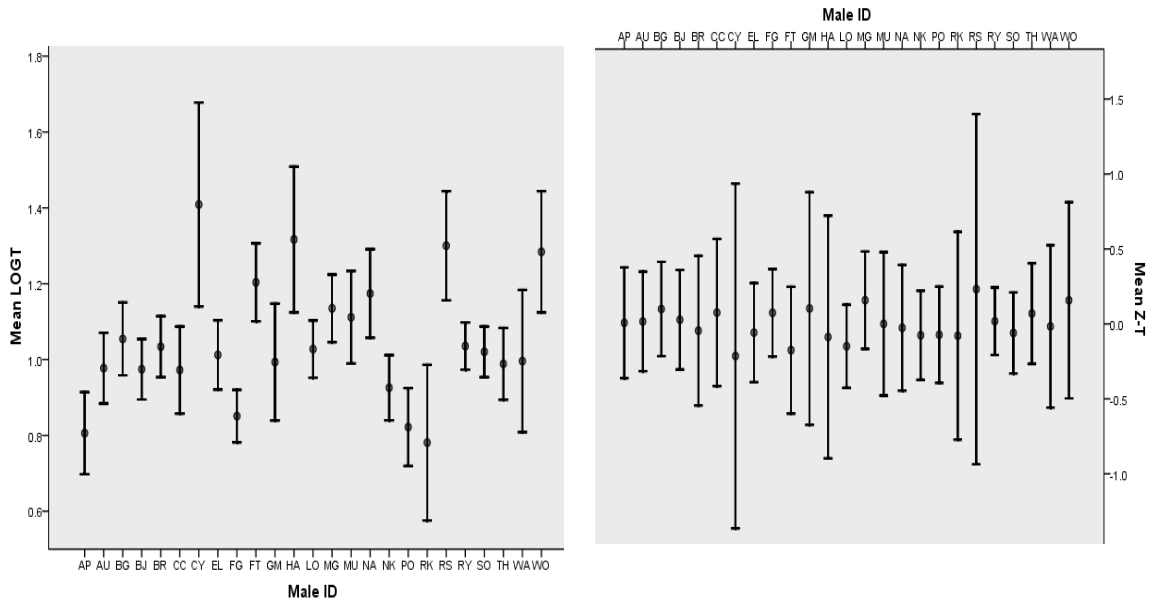
Figure 2. LogT vs. Z-T.

LogT values reflect variation in individual male's baselines. Z-T can be used to visualize relative

deviations from baseline values as they may pertain to mate acquisition or parenting effort.
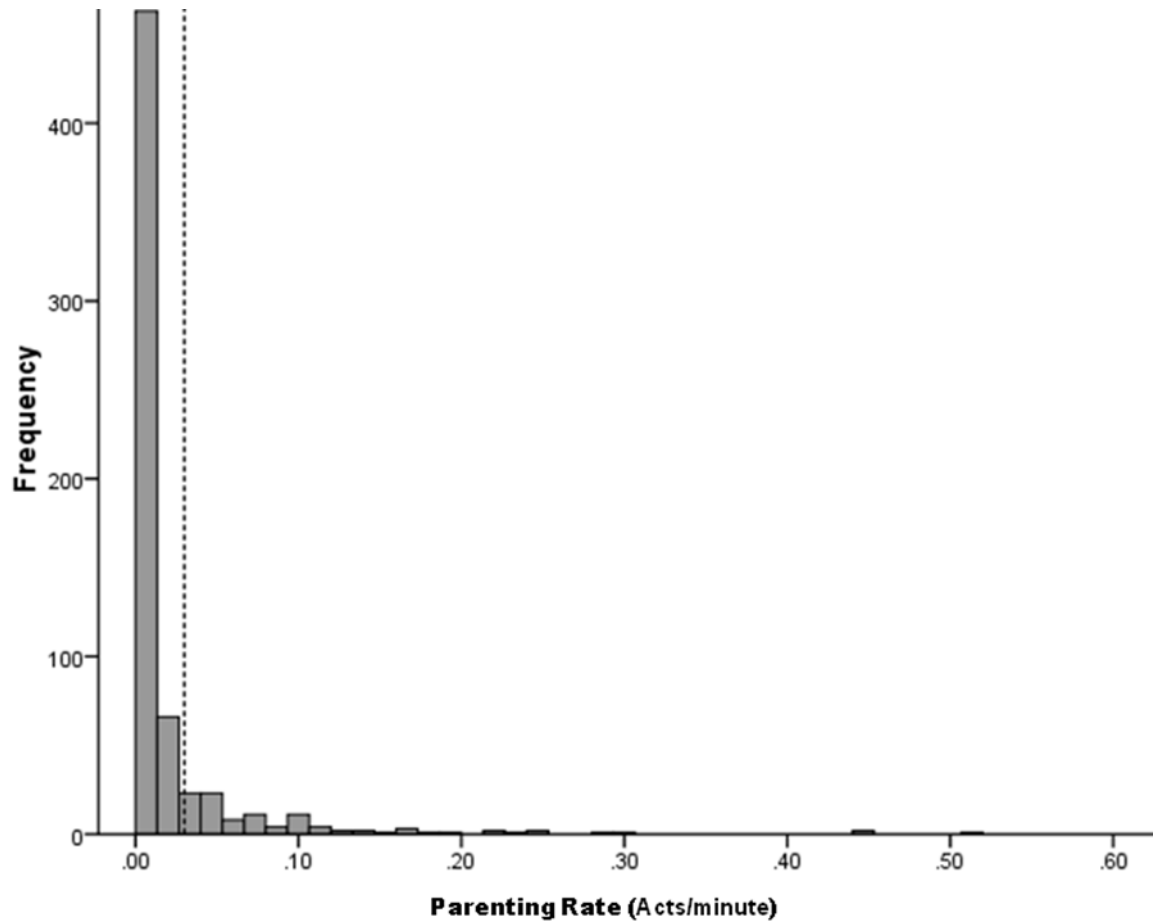
Figure 3. Histogram of parenting rates.

Histogram of parenting rates that any male gave to a specific mother-infant dyad during any given month. The dotted line represents a non-zero "drop-off" point, where paternal behaviors become much rarer. A cut-off rate of 0.03 acts/min was used in assigning a male as a "parent" towards a dyad.
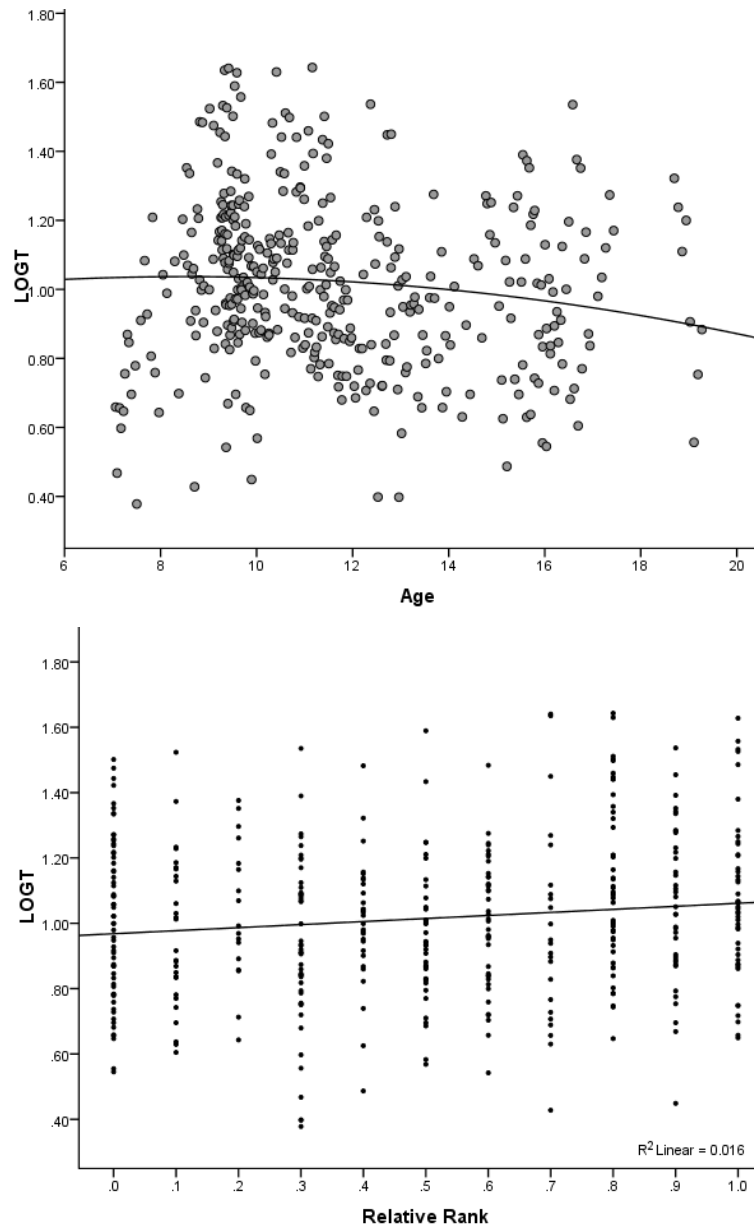
Figure 4. The effect of age and rank on T.

A quadratic regression for age revealed a significant, but loosely correlated effect for age

(p=.045, r^2=.015). A linear regression for rank (p=.009, r^2=.016) showed a slight positive

relationship between high T and high rank.
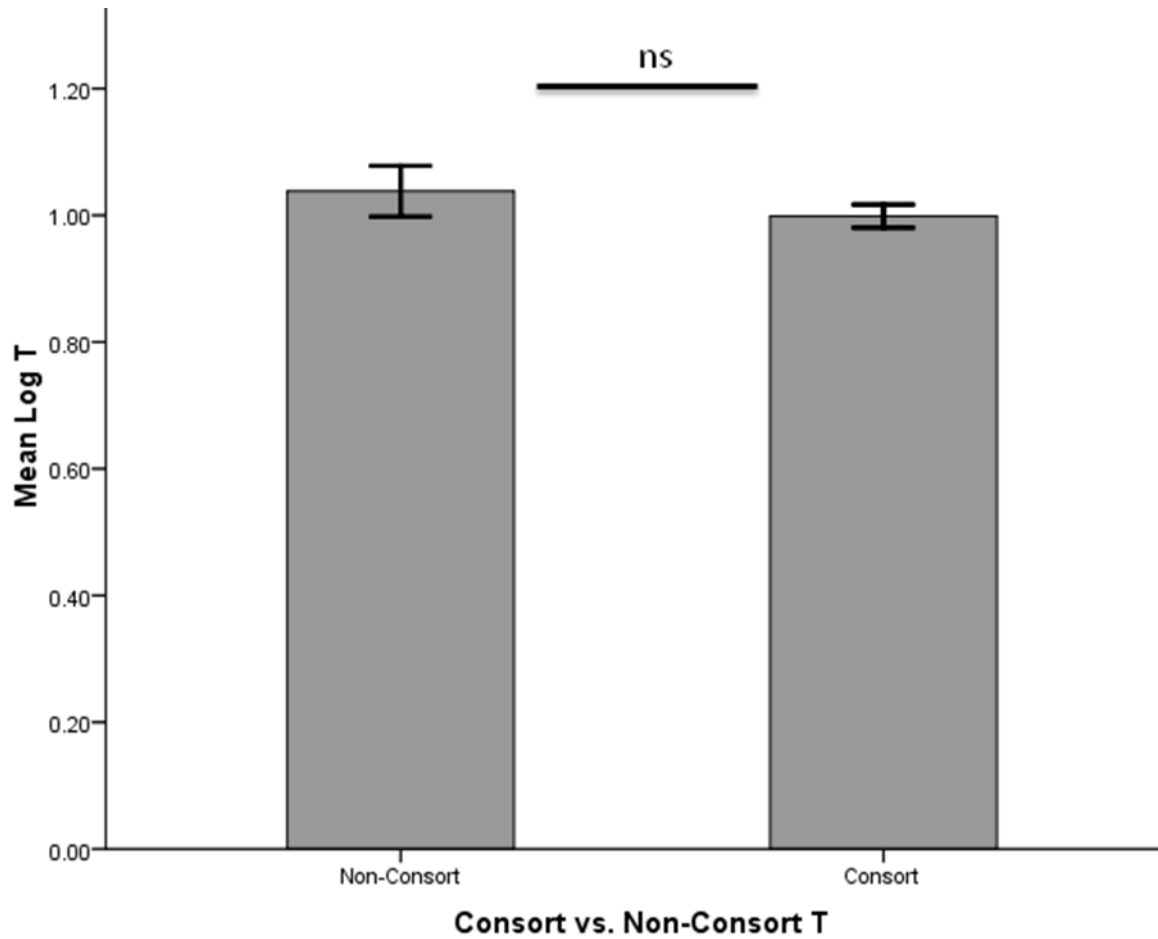
Figure 5. No effect of consorting on male T levels.

A paired-sample T-test which controlled for differences in male T baseline revealed no effect of mating on average T levels (t(21)=0.02, p=0.80).
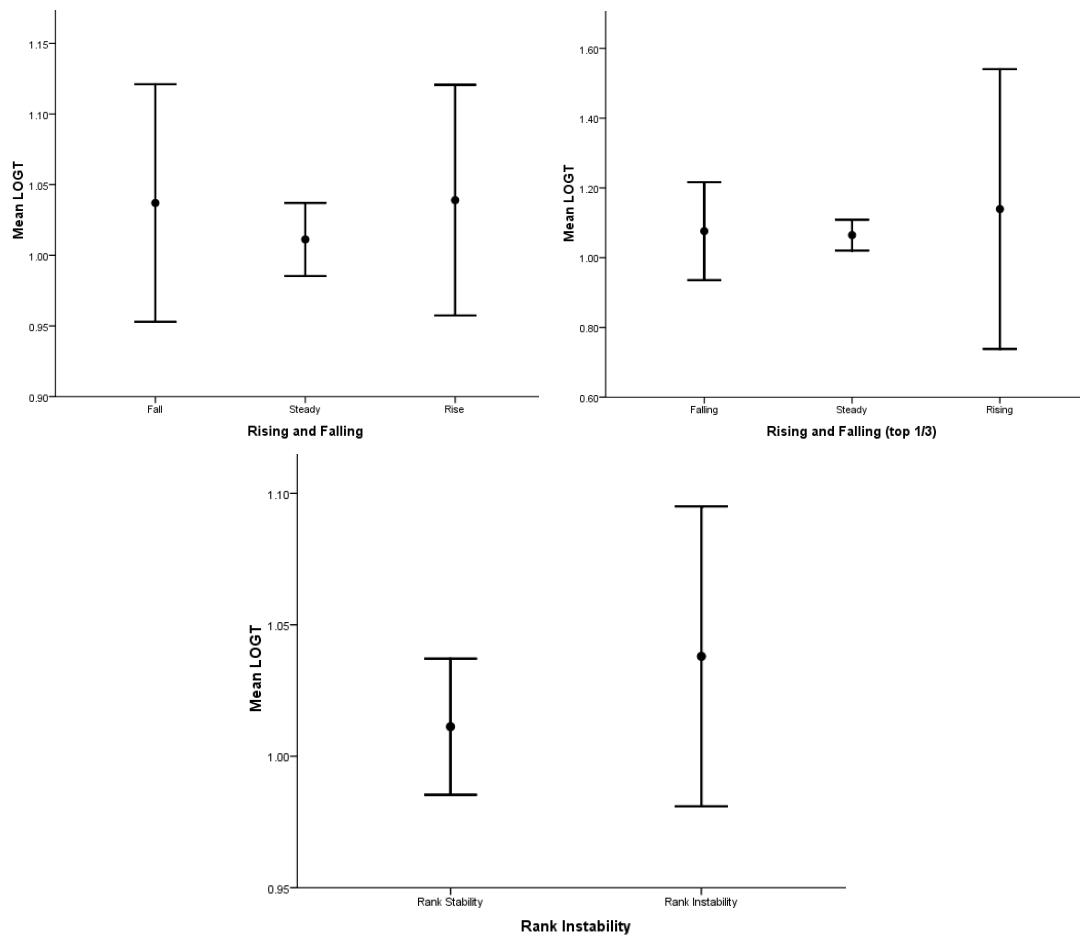
Figure 6. Non-significant permutations of the direction and location of rank change.

Direction of rank change throughout the hierarchy (GLMM: $F_{(2, 409)}=.32$), $p=.73$), within the

top 1/3 of the hierarchy (GLMM: $F_{(2, 151)}=.17$, $p=.84$), and rank instability (regardless of

direction of change) throughout the hierarchy (GLMM: $F_{(1, 410)}=.63$, $p=.43$) all returned non-

significant results.
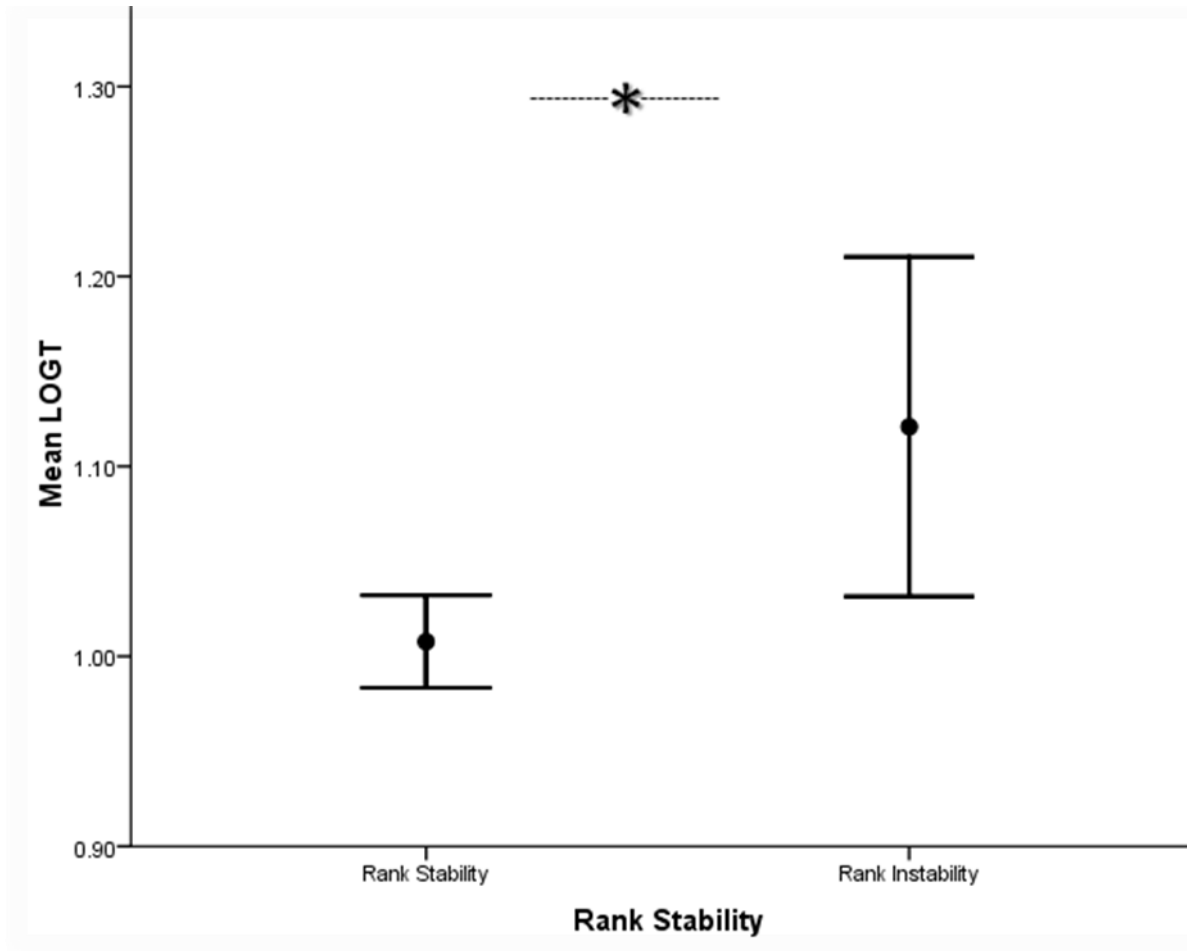
Figure 7. Differences in T levels during times of rank instability in the top 1/3 of the hierarchy.

The only parameter of mate acquisition showing a significant difference in T levels was rank

instability (as perceived by an individual male) within the top 1/3 of the hierarchy (GLMM: $F_{(1,\ 410)}=5.40$), p=.021).
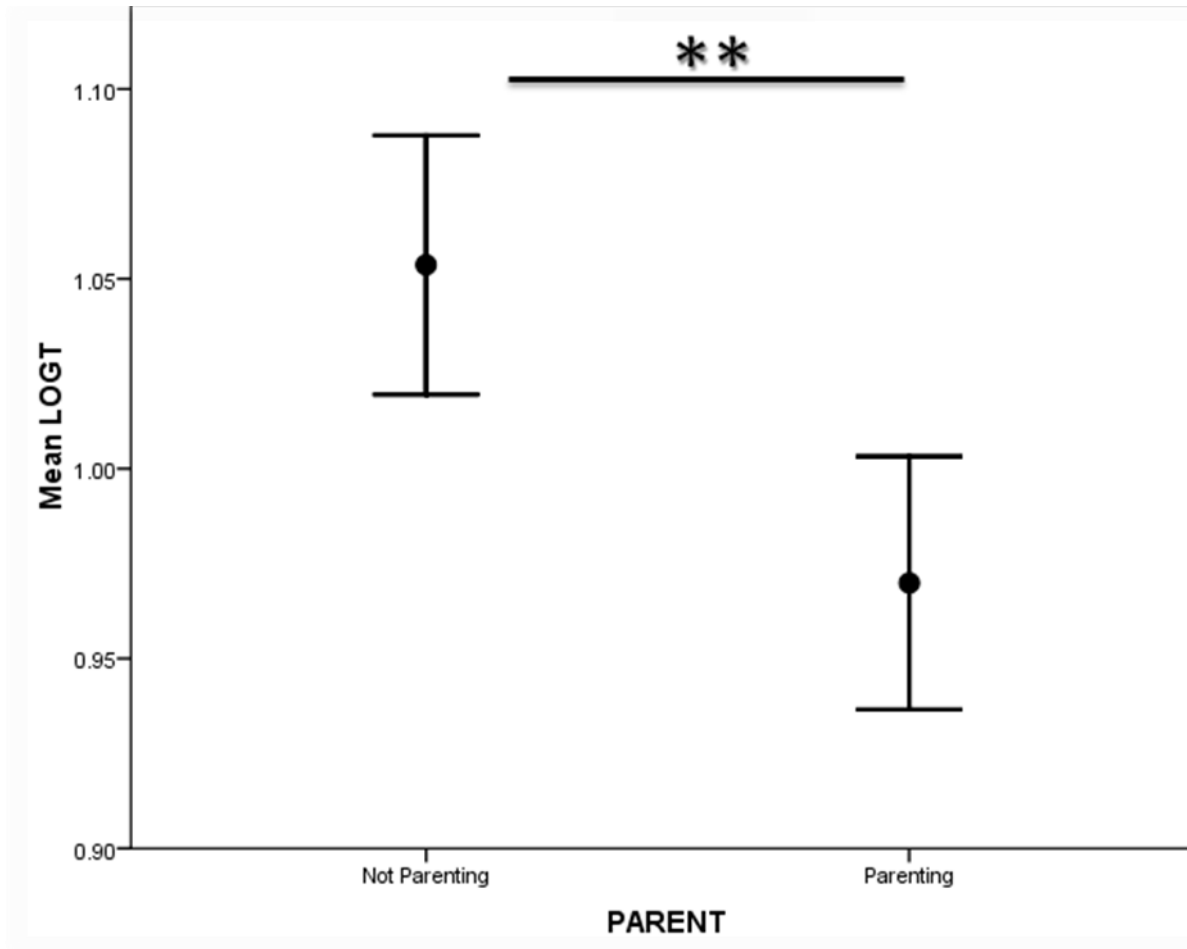
Figure 8. The effect of paternal behavior on T.

Mean parenting T levels were significantly lower than non-parenting T levels (GLMM: $F_{(1, 388)}=9.6$, $p=.002$), regardless of age ($F_{(1,388)}=0.283$, $p=0.60$) and rank ($F_{(1,388)}=3.64$, $p=.06$).
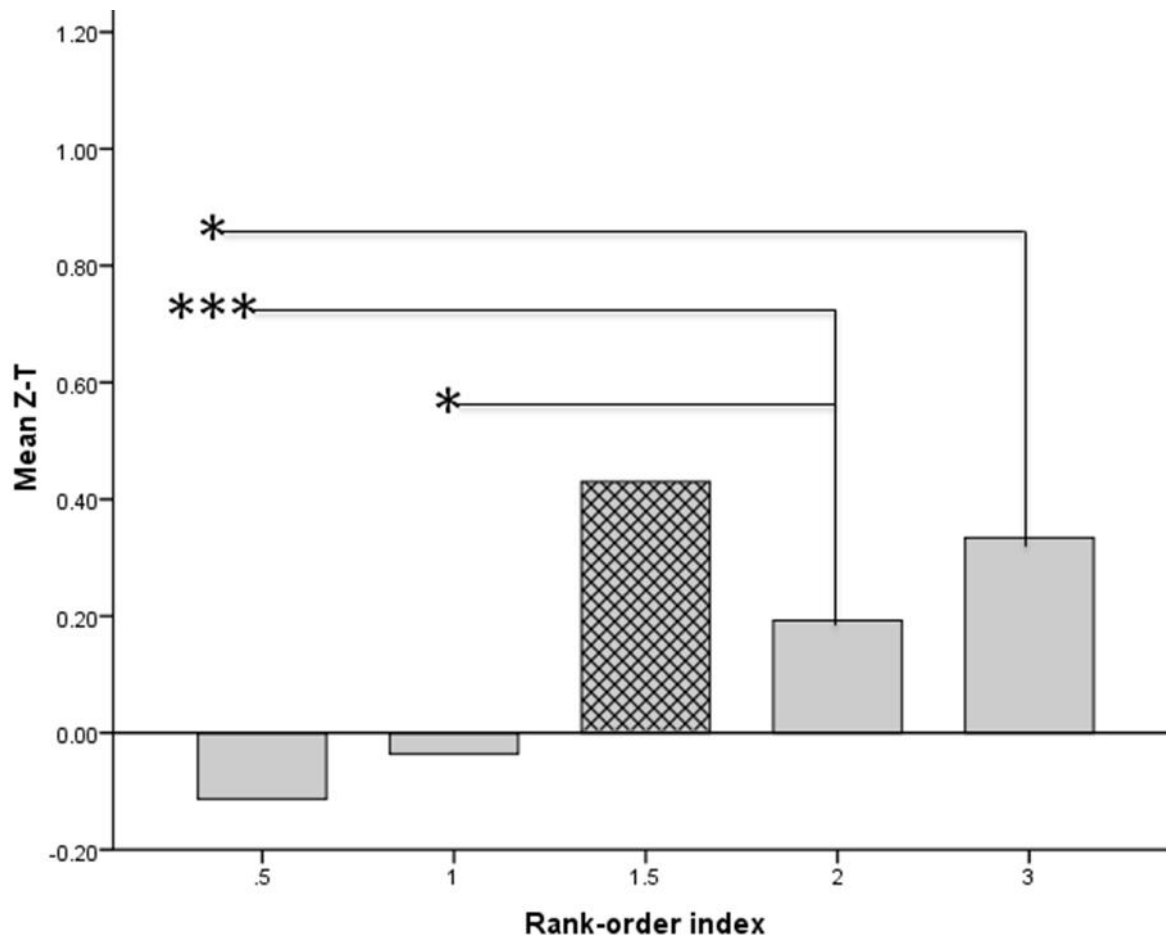
Figure 9. Mean Z-T and Rank Order Index.

Rank Order Index, an arbitrary measure of mate competition to parenting ratio, shows potential

for being applicable within and across species to predict T profiles. Increasing levels of male-

male competition and decreasing levels of paternal care (as represented by values .5-3) show

increasing average levels of T. The hatched bar (1.5) represents parenting males during mate

competition (as identified through rank instability). [('.5' and '.2', p<.001); ('1' and '2",
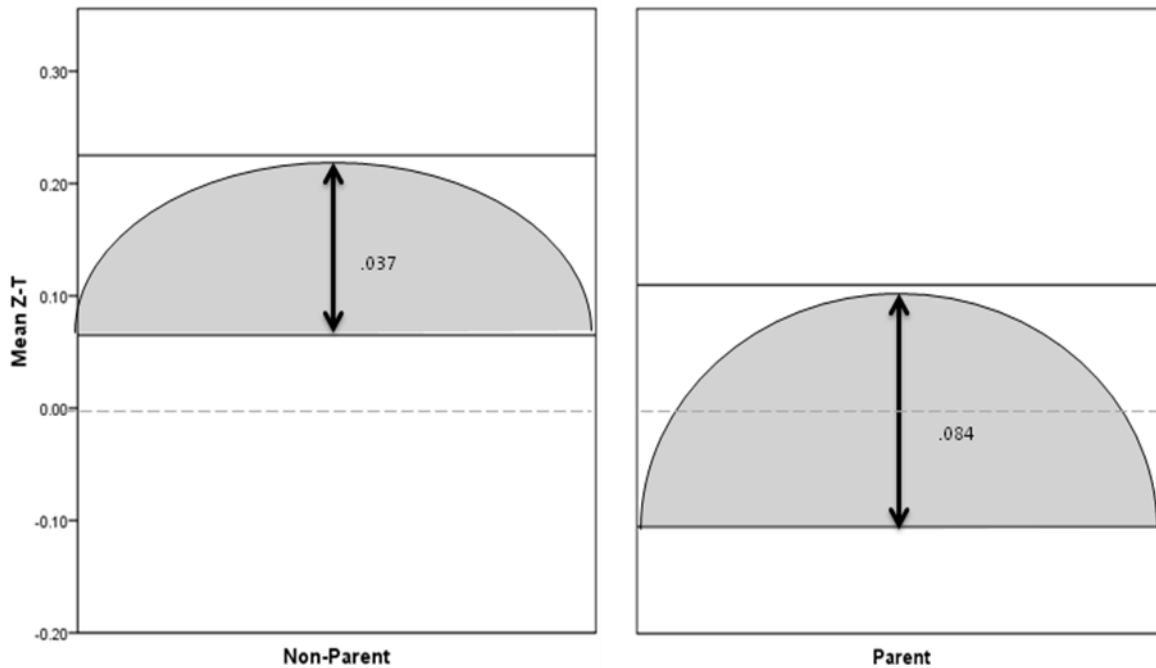
p=.047); ('.5' and '3', p=.047)].

Figure 10. Androgen Responsiveness (AR) differences for parenting and non-parenting males.

Mean parenting and non-parenting baselines (bottom line) versus mean 'challenge' T levels (top

line) represents a modified version of AR (adapted from *Figure 1*; Wingfield et al., 1990). Z-T

was used to demonstrate where parenting and non-parenting males fall with respect to the mean

adult T level in the population (dashed grey line). AR scores (0.037 and 0.084) were not Z-

transformed accordingly, but are shown on this graph for heuristic purposes.

Appendix A

Three Levels of Testosterone (Figure 1 from Wingfield et al., 1990).
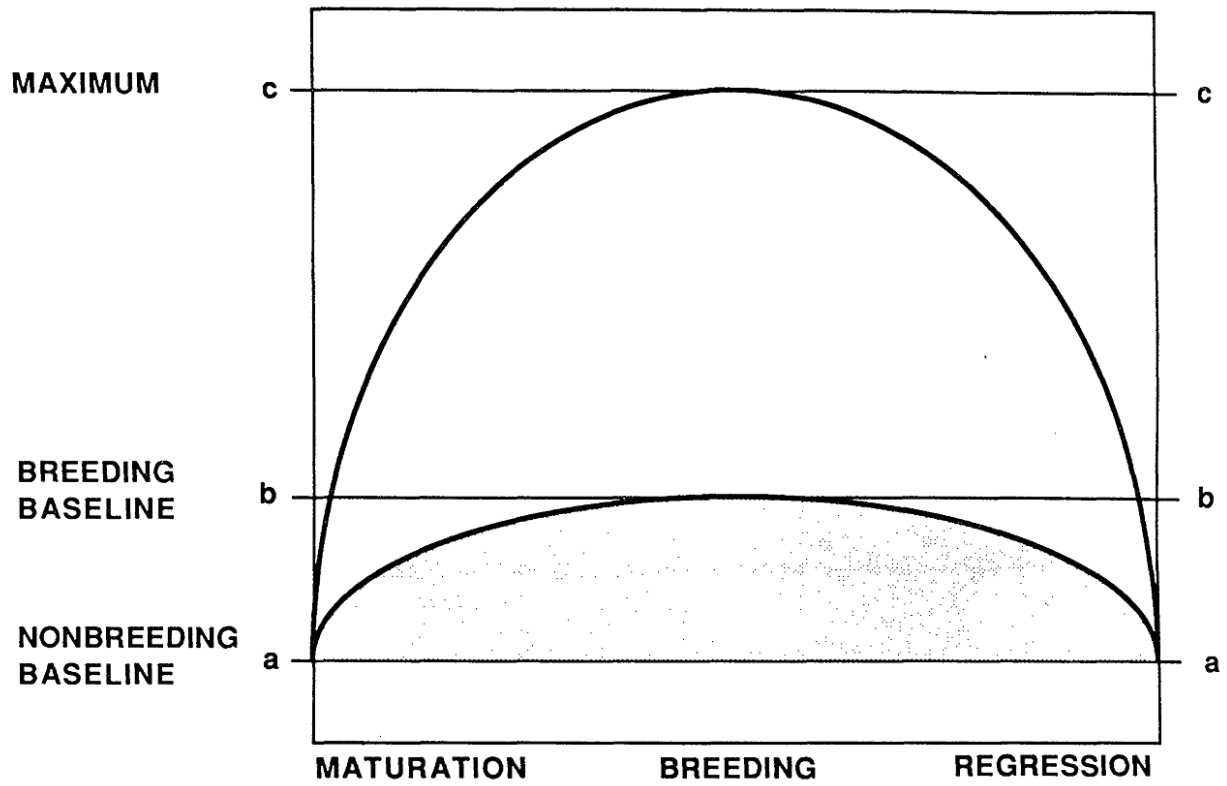
Appendix B

Mating Systems and the CH (Figure 7 from Wingfield et al., 1997).