

DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD
DASH.HARVARD.EDU

HARVARD LIBRARY


Energetic Costs of Reproductive Effort in Male Chimpanzees

Citation

Georgiev, Alexander. 2012. Energetic Costs of Reproductive Effort in Male Chimpanzees. Doctoral dissertation, Harvard University.

Link

http://nrs.harvard.edu/urn-3:HUL.InstRepos:9555923

Terms of use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material (LAA), as set forth at

https://harvardwiki.atlassian.net/wiki/external/NGY5NDE4ZjgzNTc5NDQzMGIzZWZhMGFlOWI2M2EwYTg

Accessibility

https://accessibility.huit.harvard.edu/digital-accessibility-policy

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. Submit a story

© 2012 – Alexander Ventsislavov Georgiev
All rights reserved.

Advisor: Richard W. Wrangham Alexander V. Georgiev

Energetic costs of reproductive effort in male chimpanzees

Abstract

Male reproductive success in many mammals depends on their ability to allocate sufficient energetic resources to mating competition. Such costs are particularly pronounced in species with high levels of sexual body dimorphism, intense polygyny and distinct breeding seasons. I tested the hypothesis that male reproductive effort incurs significant energetic costs in wild chimpanzees (*Pan troglodytes*), a species with moderate sexual dimorphism, promiscuous mating and lack of breeding seasonality. My field studies combined behavioral observations on male chimpanzee behavior with non-invasive sampling of urinary C-peptide (UCP). UCP is a biomarker of insulin production that indexes individual energy balance. This dissertation contributes to the understanding of UCP as an energy assay by (1) validating the application of UCP for assessing dietary quality in bonobos (*Pan paniscus*) at Kokolopori, DRC and (2) providing a detailed assessment of diurnal variation in UCP levels in relation to short-term changes in food intake in chimpanzees at Kanyawara, Kibale NP, Uganda. I used UCP measurements in conjunction with full-day focal observations of male chimpanzees to assess the energetic costs of male-male competition for status and mating opportunities. Data on feeding time and rates of aggression suggested that males experience a reduction in energy intake and an increase in energy expenditure when highly attractive parous females were in estrus. UCP

data supported these conclusions because males had lower UCP levels on mating days, and rates of aggression were negatively associated with UCP levels. Mean daily party size was also associated with low UCP levels, controlling for the presence of estrous females. Habitat-wide availability of preferred fruits was positively associated with male rates of aggression suggesting that energy availability mediates male investment towards energetically costly competitive behaviors. Contrary to expectations males who were most successful in obtaining copulations (high-ranking males) did not suffer higher energetic costs than lower-ranking males during periods of mating competition. Costs or reproductive effort include both direct competition for matings and long-term competition over social status. Maintenance of social rank over long periods appears to be particularly important in this slow-reproducing, long-lived and non-seasonally breeding primate.

Table of contents

	Abstract	iii
	Acknowledgements	vi
Chapter 1:	Introduction	1
Chapter 2:	Seed predation by bonobos (*Pan paniscus*) at Kokolopori, Democratic Republic of the Congo: diet and urinary C-peptide	17
Chapter 3:	Diurnal variation in urinary C-peptide production in wild chimpanzees: the effect of feeding schedules and ripe fruit consumption	31
Chapter 4:	Estrous females and male feeding behavior in chimpanzees: foraging constraints of mating effort in a promiscuous non-seasonally breeding primate	71
Chapter 5:	The energetic costs of sex and violence: urinary C-peptide analyses of mating effort and dominance competition in male chimpanzees	107
Chapter 6:	Conclusions	179
	Bibliography	186

Acknowledgements

A doctoral dissertation is written in the first person. I did this and I conclude that. If *I* have learned anything during the 7 years of graduate school it is that science at its best is a wonderfully collective enterprise. If you are lucky you will work with a team that makes you a better researcher, has your back and inspires you all at the same time.

Collaborators, thesis committee members and advisors

Even though the chapters that follow may leave you with the impression that I have produced all of this all on my own, such a claim cannot be further from the truth. First, I owe a huge debt of gratitude to my collaborators on the papers that will result from this work (Chapters 3-5) or have already been published (Chapter 2): Richard Wrangham, Melissa Emery Thompson, Martin Muller, Andy Russell, Emily Otali and Albert Lokasola. Second, I thank the people who read the drafts of this dissertation and took the time to give me much needed feedback, my thesis committee members: Richard Wrangham, Melissa Emery Thompson, Martin Muller, Andy Russell, Charlie Nunn and Peter Ellison. They all relentlessly tried to steer me away from erratic logic and cluttered writing. They were often successful in these efforts but no doubt there is room for improvement still. Any clutter in the final version of the dissertation is there because of my poor judgment and frantic deadline-chasing, rather than the lack of effort on the part of my committee. Melissa Emery Thompson also trained me in the lab at Harvard to analyze the bonobo samples and she later analyzed all the chimpanzee samples

at her lab in New Mexico. Several other people were very helpful during my early training in the program and the planning stages of this work: Cheryl Knott, Karen Kramer and Dan Lieberman. I thank them all sincerely.

Advisor

Richard Wrangham. You were the main reason I came to the US and you are there main reason I graduated from the program. I don't think I can properly describe how happy I am that you were my advisor. My English may likely possibly be better thanks to all the time you spent correcting my double negatives, double conditionals and the liberal use of commas. My understanding of chimpanzees, behavioral ecology and science in general is definitely much better. For that and for much more – thank you!

Kibale Chimpanzee Project and the Kanyawara Chimpanzees

Having access to a community of well-habituated chimpanzees that allow you to follow them all day long at close quarters is a privilege that cannot be underestimated. Working at Kanyawara has been exceptional and the quality of data I was able to collect there is a direct result of the efforts of numerous people who have followed chimpanzees in this part of Uganda for the past 25 years. When in 1987 Richard Wrangham began the long-term study you would have been lucky to see the apes for a few minutes at a time, apparently. Today you would be unlucky if you lose them for more than a few minutes at a time (it still happens often enough though, especially if you're on your own…). I thank all the people who have contributed to KCP over the years and in particular the people I worked with in the field on a regular basis, the current team of field assistants:

Francis Mugurusi, Solomon Musana, James Kyomuhendo, Wilberforce Tweheyo, Sunday John, Christopher Irumba & Friday Charles. They made tracking chimpanzees much easier and contributed daily observations to the large data-set of the KCP some of which I used in my analyses. Emily Otali is a wonderful field manager and a good friend. She was able to cheer me up even at the height of the wet season. Martin Muller, the co-director of KCP, produced some important findings during his own PhD study at Kanyawara. His work on aggression, hormones and dominance helped shape my interest in these topics and I thank him for encouraging me to develop my thesis project.

Human Evolutionary Biology

Meg Lynch is the most organized, patient and helpful person I know. HEB is lucky to have her! The Behavioral Ecology/Wrangham Lab at our Department is the best one. Our weekly meetings have been the highlight of my week. Among many other activities, in these meetings students, post-docs and faculty, past and present, have listened to, commented on and dismembered (in the good sense of the word) various talks that I have presented. I have learned a lot from all of these sessions. Thanks to Zarin Machanda, Ian Gilby, Meg Crofoot, Sonya Kahlenberg, Melissa Emery Thompson, Tory Wobber, Katie McAuliffe, Rachel Carmody, Luke Glowacki, Andy Cunningham, Alex Rosati, Stephanie Meredith, Cheryl Knott, Andy Russell, Karen Kramer, Charlie Nunn and Katie Hinde. I also thank my G1 cohort for a great start to graduate student life: Meredith Reiches, Neil Roach and Rachel Carmody.

Data and statistics

Paco Bertolani created special software for me to use in the field. He also produced some wonderful GPS maps that changed the way students find their way in the forest. Andrew Russell was my stats-savior. I also got additional help on rather daunting statistical methods and concepts from Roger Mundry, Natalie Cooper, Charlie Nunn, Steven Worthington and Erik Otarola-Castillo. AnthroTree in Amherst was also quite nice! Zarin Machanda, and before her Ian Gilby, made chimpanzee data available at the click of a mouse. For their work on the KCP long-term data-base and their patience with my numerous Access queries (pun intended) I am much grateful. Edgar Mugenyi enters all the KCP data in the field, and Nick Brazeau, Daniel Carroll and Rob Tennyson helped with some data-entry of my own observations back in the US.

Research permits & logistics

The research described in this dissertation complied with the requirements of the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Arts and Sciences at Harvard University, as well as with relevant legislation of the host countries. I thank Sally Coxe and Michael Hurley from the Bonobo Conservation Initiative (BCI) for the invitation to work at Kokolopori and for their support in the field. The study on bonobos was made possible by research permission obtained by the BCI and with the cooperation of Dr. Mwanza Ndunda at the Centre Recherche de Ecologie et Forestrie at Mbandaka. In Uganda, research permissions were granted by the Uganda Wildlife Authority, the Ugandan National Council of Science and Technology and the Makerere

University Biological Field Station. Jerry Lwanga and his colleagues at MUBFS, in particular, often went beyond the call of duty to make sure the station was not just a research facility but also a home. Never mind the hot water!

Funding

Harvard University provided me with generous support for the entire course of my graduate studies. The Department of Human Evolutionary Biology provided further financial support for research, training and conference travel. Field-work in Uganda was possible thanks to research grants from the Wenner-Gren Foundation, The National Science Foundation and the International Primatological Society. The Cora du Bois Trust funded part of my write-up period and the Graduate Society at Harvard University provided me with a fellowship for the remainder of that intense period. The American Society of Primatologist awarded me with a travel grant to go to Austin, TX where I presented some of the results of my work. Earlier, a grant from the Arthur L Greene Fund helped with research costs in the DRC. A Bristol Myers Freedom to Discover Award to B. Hahn at University of Alabama at Birmingham also contributed to costs in the DRC. Without the support from these organizations my life over the past 7 years would have been very different and much less exciting.

Other

There are a few other people I should mention. Milen Marinov, Diana Zlatanova, Teodora Ivanova, Bjorn Siemers, John Atkins, Jeffery Boswall, James Stiller, Jean Hartley, Barbara Fruth, Gottfried Hohmann and Darina Penkova. In

various and significant ways they influenced my thinking and career choices at a critical stage (my undergraduate years). For that I thank them very much.

Arriving to the US for first time in 2005 was a big change. The transition was made much easier by Danese and Barbara Carey who as part of the International Student Host Program volunteered to show foreigners around upon their arrival at Harvard. I am sorry Barbara is not around anymore to see me graduate. I am also very glad that Danese is. She has been a great friend from day one. Travelling to African countries from the US usually involves a short layover in some European airport. For a variety of reasons I was often able to make the most of these layovers and spend some time in London. For their hospitality I am greatly indebted to Manos Agianniotakis, Maria Lambrianidou, Maria Petridou, Magda Kolokitha and Alex Lord. I also have to thank Manos for introducing me to G&Ts, as well as to bar-hopping in Soho. Good times. Sarah Papworth came up with the blue monkey joke back in Uganda and remained similarly inventive whenever we had time to catch up in the UK. When not travelling to or from Africa, I still moved around often. I was lucky to meet some amazing room-mates along the way: Christian Arnold and Tomek Proszynski in the US, and Pawel Fedurek in Uganda. All of them have been wonderfully cheerful, understanding and altogether brilliant even at the height of my grant/chapter writing crises or whatever else was going on. Pawel is also a great guy to have around when the chimpanzee you are following falls asleep. His company in the forest and at the Obligato made a real difference. Krista Milich and Jeff Bittner made my year in the field much less isolating than it could have

been. Sergei Ganchev took time out of his busy schedule and came to visit me in Uganda and in the US. I should return the gesture and visit him in Bulgaria sometime soon. Petar Nikolov, a real doctor, wrote me some prescriptions for anti-malarials during a short break from Uganda when I had to return to Sofia to get a new passport. I regret I could not afford his services as an 'expedition doctor' in the field. May be next time. In the US, two particular individuals transformed the final stages of my PhD life beyond recognition: Natalie Cooper and Hannah Koon. I thank them for their learned advice on all matter of things and for their ability to be cajoled to go out for a drink with barely a 5-minute notice. Even on a Monday. Luchezar Nikolov has always been an inspiration. I am running out of words to express how thankful I am. Nazdrave! Last but definitely not least I thank my mum. She encouraged and supported me without hesitation at every important stage in my life. I am sorry I have not been very good at keeping in touch these last few years!

CHAPTER 1

Introduction

Optimal foraging theory broadly defines males as time-minimizers and females as energy-maximizers (Schoener 1971). This dichotomy is rooted in the factors determining the differential reproductive success of males and females (Bateman 1948; Trivers 1972). Adequate food intake is key to female fecundity and reproduction (Wade and Schneider 1992), whereas for males the limiting factor is the number of mates they can acquire, and thus the time available for searching and competing over fertile females, as well as their ability to outcompete other males (Darwin 1871; Emlen and Oring 1977; Andersson 1994; Clutton-Brock 2004). Despite robust empirical support of this principle across taxa, such a 'foraging' dichotomy tends to underestimate the importance of energy acquisition for male fitness. Although, relative to females, energy can be less important for male reproductive outcomes (Gittleman and Thompson 1988), differences in energetic condition among males are expected and known to influence their access to females and mating success (Andersson 1994; Higham et al. 2011; Crocker et al. 2012).

In this thesis I examine the role of energy for male reproductive effort in chimpanzees, a species that offers three important lines of comparison for research in sexual selection and life history theory. First, as a promiscuous, non-seasonally breeding mammal with extensive male-male bonding this species offers a contrast to many studies on the costs of male reproduction, a significant proportion of which focus on extremely polygynous large mammals such as pinnipeds and ungulates, where

male-male bonds are non-existent and breeding is highly seasonal. Second, as a primate with a fission-fusion social system, male philopatry and weak female-female bonding, this species also provides some useful contrasts within the primate order that can help understand the relative importance of ecology, highly dynamic grouping patterns and male sociality in shaping male reproductive strategies. Finally, as one of our closest living relatives, studying male reproductive costs in this species can contribute to the lively debate concerning the unique life history of the genus *Homo* and its evolutionary origins.

Energetic costs of reproductive effort in male chimpanzees

In this dissertation I test the hypothesis that male reproductive effort is energetically costly in a promiscuous non-seasonally breeding primate, the chimpanzee, *Pan troglodytes schweinfurthii* at Kanyawara in Kibale National Park, Uganda. Chimpanzees present a fascinating system in which to examine the relationship between sexual selection, ecology and male behavior for several reasons.

First, most research that has identified considerable costs to male reproductive effort focuses on species that are characterized by high breeding seasonality and levels of sexual dimorphism (Galimberti et al. 2007) and are often capital breeders (i.e. males fast during the mating period using up previously stored energy reserves). Chimpanzees do not have a distinct mating season, do not cease feeding when mating opportunities are available, and have only moderate levels of sexual dimorphism (~33%; Plavcan and van Schaik 1992). In short, it is expected that energetic condition will not be as important for them as it is for males who rely on stored energy reserves to

maintain their reproductive effort during seasonal mating periods. Investigating the energetic correlates of male reproductive effort in chimpanzees would therefore evaluate the general applicability of the principle of energy allocation to competing demands in mammalian reproduction (Stearns 1992).

Second, the socio-ecology of chimpanzees in their natural habitat allows investigating how changing levels of food availability affect the ability of males to allocate energy to agonistic competition in ways that are not possible in many other taxa. Chimpanzees in general and at Kanyawara (our study site) in particular experience a variable supply of ripe fruit (their preferred food) throughout the year (Conklin-Brittain et al. 1998; Wrangham et al. 1998). They respond to this variation by changing the size of their foraging parties (sub-groups) through the day so as to minimize the intensity of scramble feeding competition (Wrangham et al. 1996; Wrangham 2000). Changes in dietary quality affect male energetic status (Emery Thompson et al. 2009) and the abundance of high-quality non-fig fruit in particular can affect the tendency of males to engage in energetically costly activities such as monkey-hunting (Gilby and Wrangham 2007). We can thus expect current male energetic condition to also affect the amount of energy males allocate to aggressive competition even in the absence of seasonal breeding and cessation of feeding during mating periods.

Third, given their fission-fusion social organization (Nishida 1968), chimpanzee males may face special challenges in maintaining their position in the hierarchy. High social status is important for male reproductive success (Boesch et al. 2006; Inoue et al. 2008; Wroblewski et al. 2009; Newton-Fisher et al. 2010) and most male chimpanzees

allocate significant time and effort to improve their social standing (Goodall 1986; Nishida 2012). Because all males in the community are rarely in the same party (subgroup) for long, there is much opportunity for clandestine bonding and alliance building, away from the eyes of the current alpha male (Nishida 2012, p. 250). If this is indeed creates a social climate of instability, as suggested by Muller & Wrangham (2004a), alpha males may be under particular pressure to constantly allocate energy to dominance displays, regardless of current mating opportunities in order to maintain their rank.

Fourth, although male aggressive competition is very important in establishing dominance rank and thus negotiating access to estrous females (Bygott 1979; Tutin 1979; Goodall 1986), male chimpanzees develop highly cooperative relationships (Muller and Mitani 2005) that extend, in some cases, to mating tolerance and cooperative mate-guarding (Watts 1998; Duffy et al. 2007). Thus although high rates of aggression during periods of mating competition suggest that condition and fighting ability are important for male reproductive success, the existence of other mating strategies indicate a weaker relationship between energy and reproductive performance, relative to species that do not have extensive male-male bonding and cooperation.

Finally, because of intense and efficient sexual coercion exercised by males, female choice is unimportant in this species or at least in the study population at Kanyawara (Kahlenberg 2006; Muller et al. 2007; 2009; 2011). In species in which females exercise mate choice the relationship between male competitive ability and reproductive success can be less pronounced (Dubuc et al. 2011). In chimpanzees,

therefore we would expect sexual selection to favor intense male-male competition and thus energy to be a critical constraint. Alternatively the ability of males to exercise control over female sexuality may reduce the costs of mating competition with other males.

In summary, the behavioral ecology of chimpanzees leads to a number of contrasting expectations in regard to the importance of energy for male reproductive effort and fitness.

Relevance to the study of human evolutionary biology

Chimpanzees are a particularly useful study species for understanding the evolution of human behavior by the virtue of our close phylogenetic relationship. The lineage that led to extant bonobos and chimpanzees diverged from the human lineage about between 4.98 – 7.02 million years ago (Kumar et al. 2005; Patterson et al. 2006) and these two species are more closely related to humans than they are to gorillas (Ruvolo 1997; Chen and Li 2001; Bradley 2008). Because of a number of derived traits seen in bonobos relative to chimpanzees (Lieberman et al. 2007; Wobber et al. 2010a; 2010b), it is hypothesized that chimpanzees provide a more useful behavioral model for the last common ancestor that we shared with the genus *Pan* than bonobos do (Pilbeam 1996; Wrangham and Pilbeam 2001; Pilbeam and Young 2004). Although the role of chimpanzee/bonobo referential models for understanding human evolution has been questioned recently (Sayers and Lovejoy 2008; Lovejoy 2009; Lovejoy et al. 2010), there is still a consensus that research of living apes can provide rich comparative data on which to base inferences about the socio-ecological, life-history

and cognitive traits of the last common ancestor of *Homo* and *Pan* (Robson and Wood 2008; Foley and Gamble 2009; Jolly 2009; Hawkes 2010; McGrew 2010; Whiten et al. 2010; Hare 2011). Investigating the energetic costs of reproductive effort in male chimpanzees will therefore provide insight to the ancestral condition of the lineage leading to the genus *Homo* and thus allow a better understanding of the derived features of human male life history.

Energy availability, acquisition and allocation are central issues for the study of human evolution as well as for investigations of modern human reproductive biology and life history (Leonard and Robertson 1992; Aiello and Wheeler 1995; Leonard and Robertson 1997; Marlowe 1999a; Kaplan et al. 2000; Bribiescas 2001; Marlowe 2001; Aiello and Wells 2002; Aiello and Key 2002; Panter-Brick 2002; Ellison 2003; Kramer and Ellison 2010; Wrangham and Carmody 2010). The importance of energy for female reproduction is widely acknowledged (Ellison 2008). Conversely, male reproduction is typically viewed as being less dependent on energy. Male fecundity is much more resilient to changes in energy availability and testicular function is only impaired under severe food shortages (Bribiescas 2001; 2006). Even though energy still plays a role in somatic mating effort through the building and maintenance of muscle tissue (Bribiescas 2006), little attention has been paid to establishing how inter-male differences in energy intake can affect their fitness outcomes in the context of behavioral mating effort. This can be partially explained by the increased importance of male parental effort in the genus *Homo*, which has become a more central aspect of male reproductive effort than direct mating competition (Bribiescas et al. in press). Accordingly, much of anthropological theory is concerned with the social significance and indirect effects of

acquisition of food by men (usually through hunting) on fitness (Hawkes 1991; Marlowe 1999b; Bliege Bird et al. 2001; Marlowe 2001; Gurven and Hill 2009; Hawkes et al. 2010; Codding et al. 2011). Meat is provided, exchanged and shared for a variety of social rewards but the caloric significance of meat for the hunters' own body condition and the way this might enhance their reproductive success more directly than provisioning mates and offspring is not considered explicitly. Nevertheless, there is evidence from the ethnographic literature to suggest that men may actually feed better than women, e.g. they are subject to fewer food taboos than women, and thus, caloric gain in itself may be a strong influence on male foraging strategies (Collier and Rosaldo 1981; Spielmann 1989; Speth 1990; Kelly 1993; Pate 1997; 2006). Re-directing the focus of current anthropological research towards a more explicit consideration of the caloric importance of meat and other 'male' foods for the hunters themselves would contribute to a more complete understanding of male foraging and reproductive effort in extant foragers. Examining how energy constrains male reproductive effort in chimpanzees would inform models of the ancestral condition, against which the derived features of human male life history can be better appreciated.

C-peptide of insulin

A key element of this research project is the use of non-invasive collection of urine to assess individual energetic balance of male chimpanzees by measuring their levels of urinary C-peptide (UCP) of insulin.

C-peptide of insulin provides an accurate integrated measure of insulin secretion, which in turn reflects the energetic balance of the organism (Sherry and Ellison 2007;

Deschner et al. 2008; Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Higham et al. 2011). Thus in captive bonobos, within-individual variation of levels of C-peptide mirrored changes in body mass even over periods as short as several days (Deschner et al. 2008). In wild orangutans C-peptide was positively correlated with calculated estimates of individual caloric intake (Emery Thompson and Knott 2008). In wild chimpanzees C-peptide levels were likewise positively correlated with monthly indices of dietary quality (Emery Thompson et al. 2009). Furthermore male chimpanzees at Ngogo, which feed better than those at Kanyawara (Potts et al. 2011) had higher levels of C-peptide in their urine than males at Kanyawara (Emery Thompson et al. 2009). These field studies incorporated behavioral and physiological samples from periods of high and low food availability spanning at least one year, and indicate as expected that better feeding conditions were associated with higher C-peptide values. They therefore suggest that C-peptide values provide an effective index of energy balance in wild apes.

Food, energy expenditure their effects on insulin and C-peptide

A recent study of rural Gambian women shows a complex relationship between energy intake and expenditure, on the one hand, and urinary C-peptide excretion, on the other (Reiches 2011). Individuals had lower levels of fasting UCP production both during periods when their food intake was low and also when energy expenditure was high, even if no change in body mass occurred (Reiches 2011). This suggests UCP can be conceived of as a marker not only of stored energy reserves but also of energy throughput (flux). Surprisingly, however, no metric of body composition or changes in body composition between seasons predicted declines in UCP during that study.

Individuals who lost weight had similar UCP levels to individuals who gained weight within one sampling season (Reiches 2011) and weight gain was positively correlated with UCP levels in only one of the three sampling seasons. While some cultural dietary practices in the population during one of the study seasons may have confounded the outcomes of some of the analyses, what is clear is that UCP responds to different combinations of factors in ways that cannot always be easily explained.

The effect of diet quality on insulin production is also established in wild primates. Baboons that regularly foraged on human rubbish at safari lodges in Kenya had significantly higher insulin levels than adjacent baboon groups that fed only on naturally available foods (Kemnitz et al. 2002). Baboons that foraged for human food left-overs not only had higher energy intake but also reduced levels of activity (Altmann and Muruthi 1988).

The nutritional composition of meals ingested affects UCP production even under iso-caloric conditions with carbohydrate and protein content having generally the strongest effect (Hoogwerf et al. 1986; Wolever and Bolognesi 1996). Nevertheless studies of human subjects have shown that 24-h energy intake (kcal) is significantly positively associated with 24-h UCP production (Galgani et al. 2010).

Energy expenditure and C-peptide

Insulin production is not solely dependent on the amount and quality of food ingested. The first evidence that C-peptide levels may be used to assess energetic costs of activity in primates comes from a rhesus macaque study, conducted under controlled conditions in a captive setting (Wolden-Hanson et al. 1993). Physical activity and food intake of obese and non-obese macaques were monitored, along with

sampling of serum insulin and C-peptide. Even though food intake did not differ between the two experimental groups, non-obese monkeys were much more active. Their serum insulin and C-peptide levels were significantly lower than those of obese subjects (Wolden-Hanson et al. 1993), suggesting that even if two animals consume approximately the same amount of food, those that are more physically active will have lower insulin and C-peptide production. Ideally, such a study should be replicated with healthy non-obese subjects and examine whether physical activity, under identical food intake, has the same negative impact on C-peptide production. Nevertheless, these findings provide further indication that UCP may be sensitive to changes in energetic expenditure, all other things being equal.

The only study to date that has examined the relationship between behavioral activity (rather than just food intake) and UCP excretion in free-living, non-obese primates involved rhesus macaques on Cayo Santiago Island (Higham et al. 2011). Restlessness (the rate of changing behaviors) and traveling time were negatively correlated with male UCP levels during the non-breeding season, while copulation behavior affected UCP negatively during the breeding season (Higham et al. 2011). Even though these findings at first seem to suggest that UCP reflects the increase of energetic expenditure during the breeding season for males who engaged most in prolonged copulations, an alternative explanation is also plausible. Males that copulated most (generally, high-ranking males) also fed less than lower-ranking males during the breeding season. The reverse was true during the birth season – high-ranking males fed more on high-energy monkey chow than low-ranking males and had higher UCP levels. Considering the possible confounding effect of food intake on UCP production,

copulatory behavior (which was also negatively correlated with feeding time) may not be the main cause for low UCP levels during the breeding season. Thus while, undoubtedly this finding demonstrates significant cumulative energetic deficit in male macaques during their breeding period, whether UCP reflects energetic expenditure rather than loss of energy intake, or more likely a combination of the two, is difficult to say. In this respect, a multivariate statistical approach may help evaluate the relative contribution of different factors.

Sources of 'random' variation in UCP measurements

There are many factors that may contribute to inter-individual differences in insulin and UCP production, beyond variables of interest such as food intake and energetic expenditure on physical activity. I refer here to such factors as 'random' because they are associated with the characteristics of each individual and thus do not help to explain overall patterns in UCP production that we are interested in such as overall effects of dominance rank, of seasonal changes in food availability, or of exertion during periods of intense mating competition.

Individuals differ from one another in their resistance to the action of insulin. A study of human subjects who were healthy and non-diabetic showed that insulin-resistant individuals had higher UCP production per unit of energy ingested, relative to insulin-sensitive individuals (Galgani et al. 2010). Differences in individual sensitivity to insulin are particularly pronounced between endurance trained and untrained subjects (even after controlling for body mass and percentage of body fat). Endurance trained subjects had lower fasting insulin levels and they also secreted significantly less insulin in response to a standard insulin sensitivity test (hyperinsulinemic euglycemic clamp

procedure) (King et al. 1987). The effects of training and physical exercise on insulin sensitivity are well known (Henriksson 1995) and even moderate differences in levels of physical activity in non-laboratory conditions can contribute to lower levels of fasting insulin production in human subjects who reported to be more physically active (Regensteiner et al. 1991; Kriska et al. 2001).

Additional variation in measurements arise from the inter-individual differences in renal uptake and clearance of C-peptide. Approximately 25% of circulating C-peptide is filtered in the kidneys (Zavaroni et al. 1987). After most of that amount is converted to amino-acids, only about 15% is excreted in urine with inter-individual variation 6 - 24% (Zavaroni et al. 1987). This means that two individuals secreting the same amount of insulin may differ in the amount of C-peptide excreted and measured in the urine.

All these issues are difficult to address completely in a field study of wild subjects. As one possible approach for accounting for some of the variation in UCP measures I applied linear mixed model analysis, which specify the identity of the chimpanzees in our sample as a random effect.

Thesis aims

The overall aim of this thesis is to test the hypothesis that energy matters for male reproductive success in chimpanzees. My approach is to investigate the effect of behaviors associated with male competition for dominance status and for access to females on male energetic condition. Specifically, I address the following questions:

1. Do males incur foraging costs during periods of mating competition?
2. Does aggressive behavior restrict the aggressor's feeding activity?

3. Does aggressive behavior have a negative impact on the aggressor's energy balance?
4. Do high-ranking males incur greater costs during periods of mating competition than lower-ranking individuals?
5. What explains the previously reported negative association between high dominance rank and UCP levels?

To assess the reliability of using UCP measures to describe male energetic balance on a daily scale I also examine diurnal variation in UCP levels and carry out analysis relating to the questions above at several different temporal scales.

Overview of thesis content

Before addressing the central hypothesis of my study, in the next two chapters, I discuss some methodological issues related to the use of urinary C-peptide (UCP) as a measure of energy balance in wild primates. My first study of UCP was conducted on wild bonobos (Ch. 2) and the second deals with the data collected during my research on male chimpanzees (Ch. 3).

In Chapter 2, I present a study of bonobo diet and energetics at Kokolopori in the Democratic Republic of Congo that documents previously unrecognized monthly variation ripe fruit consumption among wild unprovisioned bonobos. I show that UCP was higher during a period of high ripe fruit consumption, relative to a period of low fruit consumption. Conversely, cortisol levels were higher during the period of low ripe fruit consumption. This result extends previous findings on the relationship between diet and UCP levels in wild chimpanzees and orangutans (Sherry and Ellison 2007; Emery

Thompson and Knott 2008; Emery Thompson et al. 2009) and captive bonobos (Deschner et al. 2008). It also supports the negative association between dietary quality and morning cortisol levels previously reported for wild chimpanzees (Muller and Wrangham 2004b).

In Chapter 3 I use a data-set of 690 male chimpanzee urine samples, collected over an 11-month period from 12 different males to examine diurnal variation in UCP production in relation to feeding effort and ripe fruit intake. Although UCP production does not show the circadian rhythms, which are typical of testosterone and cortisol production in this species (Muller and Lipson 2003), extensive research on the dynamics of insulin and C-peptide production in humans suggests that it is sensitive to transient changes in energy intake. Given that in studies of wild primates urine samples are collected opportunistically and it is not always feasible to sample individuals multiple times over a day, it is important to examine how diurnal variation in UCP levels may affect assessment of individual energy balance via singe spot-samples of urine. The analyses showed that when multiple urine samples were obtained per day, diurnal patterns in UCP levels can be discerned. UCP levels peak in late morning to around noon, reflecting the high intake or ripe fruit by chimpanzees early in the morning. Awareness of the effect that variation in feeding activity across the day has on UCP levels should encourage a more careful use of UCP data in primatology, particularly when relatively few urine samples are available per individual per unit of time.

Chapter 4 examines foraging constraint of mating effort in male chimpanzees. I used detailed behavioral data on male feeding activity to assess the impact of the presence of mating opportunities on male energy intake. Consistent with predictions,

males spent less time feeding when parous (highly attractive) estrous females were available. The reduction in feeding time was a result of shorter rather than fewer feeding bouts suggesting that males may need to interrupt their feeding more frequently during periods when mating competition is occurring. Aggression rate of focal males and their copulation rate had a significant negative effect on male feeding time providing further support for the claim that mating competition constrains male feeding.

Chapter 5 focuses on using UCP to measure the energetic costs of male aggressive behavior in the context of status and mating competition. While the analysis in Chapter 4 confirmed that males fed less when parous females were in estrous, the data did not reveal any significant differences in amount of ripe fruit during the same periods. Although total feeding time has previously been shown to correlate well with estimated caloric intake of males at our study site (Pokempner 2009), I wanted to test if the reduction in male feeding during periods of mating competition resulted in lower levels of UCP. We found that several factors were responsible for reduced UCP levels in males during periods of mating competition. Males were more aggressive and they formed large parties. Both factors had a negative impact on male UCP and this explained why in matched comparisons males had lower energetic balance on days with parous females in estrus. I also found that independently of the presence of mating opportunities, non-fig fruit (high quality foods at Kanyawara) abundance had a positive effect on male aggression rates. Taken together with data showing that males had lower UCP on days when they were more aggressive, these results support the hypothesis that male chimpanzee aggressive behavior can have a significant impact on energy balance and that habitat-wide food availability can constrain aggressive competition.

These findings thus explain why high-ranking male chimpanzees who are also consistently the most aggressive individuals have lower UCP levels overall. Maintenance of rank in this species incurs significant energetic costs because of the increased demand of agonistic competition.

Finally, in Chapter 6 I draw together the results from this study and discuss their significance for research on male reproductive strategies in primates.

CHAPTER 2

Seed predation by bonobos (*Pan paniscus*) at Kokolopori, Democratic Republic of the Congo: diet and urinary C-peptide

Alexander V. Georgiev, Melissa Emery Thompson, Albert Lotana Lokasola & Richard W. Wrangham

Published in *Primates*, 52: 309-314

DOI: 10.1007/s10329-011-0256-4

© *Japan Monkey Centre and Springer 2011*

ABSTRACT

We compared the feeding ecology of the Hali–Hali community of bonobos (*Pan paniscus*) at Kokolopori, a new field site in the Democratic Republic of the Congo, between two periods 5 months apart. During the first study period (SP1), bonobos relied heavily on the dry seeds of *Guibourtia* (Caesalpiniaceae), mostly eaten from the ground. The second period (SP2) was characterized by high consumption of ripe tree fruit. Terrestrial herbaceous vegetation (THV) contributed little to the diet in either study period. The low amount of ripe fruit and the high reliance on seeds in the diet during SP1 were associated with high cortisol production and low levels of urinary C-peptide in females, suggesting nutritional stress. However, female gregariousness was not constrained during the fruit-poor period, probably because high seed abundance on the ground ameliorated scramble feeding competition. This is the first description of extensive seed predation by bonobos. It suggests that bonobo feeding ecology may be more similar to that of chimpanzees than previously recognized.

INTRODUCTION

Primates have two major types of relationship with tree seeds. First, they disperse them (Idani 1986; Tsuji et al. 2010; Lambert 2011). Second, they prey on them (Norconk et al. 1998). Seed predation by great apes is little studied. While its nutritional significance is not well known, some evidence suggests that among primates, tree seeds are principally eaten as part of a broadening of a mainly frugivorous diet during periods of fruit scarcity (e.g. Ateles: Wallace 2005). Despite similar patterns having been observed for some chimpanzee (*Pan troglodytes*) populations (Suzuki 1969), tree seeds need not necessarily be regarded as fallback foods (Harrison and Marshall 2011), as their nutritional value may be high (Suzuki 1969; Rogers et al. 1990). For example, in Budongo, Uganda, during periods when chimpanzees fed on *Cynometra alexandrii* seeds, females tended to have high levels of ovarian hormones, suggestive of a positive energetic balance (Emery Thompson 2005). The significance of seed eating may therefore depend on the particular tree species and the ecological context of the period during which they are eaten. Here we report that bonobos at a new field site, at Kokolopori, Democratic Republic of the Congo, ate tree seeds extensively during a period when they ate few ripe fruits. Since bonobos have not been previously observed to rely on seeds for a major portion of their seasonal diet, we consider why Kokolopori bonobos ate seeds, and the socio-ecological consequences of seed eating.

We rely on direct observations to quantify dietary composition and gregariousness. To obtain preliminary data on whether bonobos at Kokolopori experienced energetic stress as a result of changes in diet composition, we use urinary C-peptide of insulin. The level of this molecule, produced during the activation of pro-

insulin, has been shown to correlate significantly with dietary quality and energy intake in wild chimpanzees, orangutans (*Pongo pygmaeus*), black-and-white colobus monkeys (*Colobus guereza*) , and free-ranging rhesus macaques (*Macaca mulatta*), and with changes in body weight in captive bonobos and two macaque species (*M. mulatta and M. fascicularis*) (Sherry and Ellison 2007; Deschner et al. 2008; Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Harris et al. 2010; Girard-Buttoz et al. 2011; Higham et al. 2011). It is therefore a promising new tool for assessing energetic condition in wild primates that fits the aims of our study. Since cortisol has often been used in primate field studies to evaluate levels of physiological stress brought about by ecological factors, social factors, or a combination of the two (Emery Thompson et al. 2010), we also use glucocorticoid production to determine whether physiological stress increased or decreased during a period of seed predation compared to a period of ripe fruit eating.

METHODS

Study site and subjects

A.V.G. conducted field work at Kokolopori, Democratic Republic of the Congo, during two study periods, SP1 and SP2. SP1 was 8th November to 20th December 2006; SP2 was 18th June to 26th July 2007. Observations were based at Nsondo Camp (0°12'N, 22°51'E), which was set up by A.L.L. and lies within Kokolopori Bonobo Reserve approximately 30 km to the east of the bonobo study site at Wamba. The study subjects were members of the Hali–Hali bonobo community. We observed and identified a total of eleven adult and adolescent members of the Hali–Hali community (3 males, 5 mothers, and 2 nulliparous females in both periods, plus one further

nulliparous female in 2007). Additionally ca. 5 juveniles and 5 infants were seen on a regular basis although they were not fully identified. Long term observations by A.L.L. suggest that the ca. 21 individuals seen in this study represent the entire Hali–Hali community, but this awaits confirmation.

Data collection

Feeding behavior

We recorded the feeding of bonobos using instantaneous party scan sampling at 15-min intervals(Altmann 1974). If any food was eaten by any of the bonobos in sight on the sampling point, we noted the name of the food and the vegetative part eaten. For each feeding scan, we also noted whether party members fed on the ground, in the tree canopy, or both. We identified plants with the help of local field assistants and information from Wamba (Idani et al. 1994), and verified identities of the main food species against herbarium specimens at Royal Botanic Gardens, Kew and the Harvard University Herbaria. Daily diet composition was calculated as the percent of scans in which bonobos in the party fed on a particular food item from the total number of feeding scans recorded on that day. Dietary scores produced with this method were highly correlated with data on individual food intake obtained by the focal sampling of chimpanzee behavior at Kanyawara (Gilby et al. 2010). To maximize the sample size of observations lasting for at least half of the daylight hours, only observations ≥ 6 h in duration were used in this analysis (range 6–12.75 h). In SP1 we collected 16 observation days that satisfied this criterion (mean ± SEM hours per day: 10.2 ± 0.5 h/day). SP2 yielded 39 observation days (11.4 ± 0.2 h/day). The data-set on dietary intake thus comprises 608 observational hours spread over 55 days.

Party composition

We noted party composition continuously, including all independent individuals leaving or joining parties. Party size refers to the number of adults and adolescents travelling together within 50 m of each other, or arriving at the same feeding location within 15 min. We defined feeding locations in the following manner. An arboreal feeding location was a single tree; two or more trees with adjacent crowns allowing continuous feeding; or a grove of trees each of which was within 50 m of each other and in which bonobos were feeding concurrently. A terrestrial feeding location was the area under a tree crown; under adjacent crowns; or under the crowns of trees within the same grove (for items such as seeds that fell from trees); or as a patch of herbs separated by less than 50 meters from neighbouring patches. We calculated mean daily party size using party counts made following all new fission or fusion events on days when we were confident that the entire party had been observed. There were 58 parties recorded in this way on 34 days (24 in SP1, 10 in SP2). Observation times on the days for which we report mean party size varied between 50 min and 12 h (mean ± SEM hours per day: 5.6 ± 0.6 h/day). We refer to the proportion of individuals in a given party from the total community as the relative party size (Boesch 1996).

Urine sampling and analysis

We collected first-morning urine voids on disposable plastic bags or pipetted urine off vegetation when it was clear that the urine came from only one individual. We collected urine samples at dawn when bonobos urinated from their nests. We sampled from midstream using a 1-2 m long stick with an attached disposable plastic bag at the end whenever possible, and when not, we pipetted urine off vegetation if it was certain

that only one individual urinated in that place. Collecting chimpanzee urine from the forest foliage in this manner does not affect results of hormonal analyses (Muller and Wrangham 2004b). We stored urine samples on filter paper within 24 h of collection (usually within 12 h), following the protocol described by Knott (1997). We kept urine samples in airtight containers with silica gel for up to 3 months in the field, and then in a freezer (-20°C) at the Primate Reproductive Ecology Laboratory at Harvard University. A.V.G. and M.E.T. carried out the subsequent hormonal analysis at the same facility, with each set of samples assayed approximately 6 months after collection. We assayed urinary C-peptide with commercially-available radioimmunoassay kits (DSL Labs, Webster, TX) following previously-described protocols (Sherry and Ellison 2007; Emery Thompson and Knott 2008; Emery Thompson et al. 2009). Intra-assay coefficients of variation (CV) for replicated filter paper samples were 11.8% and 6.9% for low and high samples, respectively, and inter-assay CVs were 4.1% and 11.1%. C. Munro at the University of California, Davis provided enzyme-immunoassay reagents for cortisol assays. Cortisol protocols followed Muller et al. (2007) and Kahlenberg et al. (2008). Intra-assay CVs were 5.4 and 9.5% for low and high samples, respectively, and inter-assay CVs were 13.3% and 9.6%. Assay sensitivities were approximately 10 pg/ml for C-peptide and 300 pg/ml for cortisol. We standardized all hormonal values for concentration by using creatinine measurements. While cortisol shows circadian variation in secretion (Muller and Lipson 2003; Anestis and Bribiescas 2004), there was no need to time-adjust the cortisol values because all urine samples for this study were first-morning voids collected within a narrow time-window (5:00 – 6:20 am).

Data analysis

We analyzed data on dietary composition and party size with Mann–Whitney U-tests using each observation day as a unit of analysis. We log-transformed all endocrine data and tested them for differences between study periods with a two-sample t-test assuming unequal variance. Since we collected urine very early in the morning while visibility was poor, we could not normally record individual identity, despite being able to assign to age–sex class. We therefore present our results for adults by sex. We conducted all tests using two-tailed probabilities in PASW Statistics 18 with a significance level of 0.05.

RESULTS

Feeding ecology

Ripe fruit accounted for over half of all feeding observations (53.6%, Table 2.1). Terrestrial herbaceous vegetation (THV) and young arboreal leaves comprised only minor portions of the diet. Tree seeds were an important component of the diet, particularly during SP1 (57.3%; Tables 2.1, 2.2). Bonobos at Hali–Hali were also seen to feed on meliponine honey, dead wood, truffles, colonial spiders, and the tissue of a holoparasitic plant (*Chlamydophytum aphyllum*). They once attempted to catch a scaly-tailed flying squirrel (*Anomaluridae*), but we did not see meat consumption. To our knowledge, holoparasitic plants (Georgiev et al. 2010) and colonial spiders are new dietary records for bonobos.

Bonobos spent less time eating ripe fruit pulp in SP1 (28.5%) than in SP2 (71.3%, Mann–Whitney, $z = 4.9$, $N_1 = 16$, $N_2 = 39$ days, $P < 0.001$, Table 2.2). They also consumed a smaller number of different fruit species in SP1 (6) than in SP2 (24; Tables 2.1, 2.2). By contrast, the contribution of dry seeds to the diet was higher in SP1

(57.3%) than SP2 (18.5%; z = - 3.9, P < 0.001). There were no significant differences between study periods in the dietary contribution of either THV (z = - 1.6, P = 0.12) or young arboreal leaves (z = - 1.7, P = 0.09).

Across all observation days, the daily dietary proportions of fruit and dry seeds were inversely correlated (Spearman ρ = - 0.8; N = 55 days; P < 0.001). A weaker but similar correlation occurred between the daily dietary proportions of ripe fruit and THV (Spearman ρ = - 0.41; N = 55; P = 0.002), whereas there was no correlation between the consumption of THV and young arboreal leaves (Spearman ρ = 0.12; P = 0.4) or between ripe fruit and young arboreal leaves (Spearman ρ = - 0.07; P = 0.6).

Table 2.1. Diet of the Hali–Hali bonobos during the four study months (Nov–Dec 2006 and June–July 2007). Only days with ≥6 observation hours are included. All named plants are trees unless noted otherwise

Food type/species	Family	Monthly dietary contribution (% of feeding observations)				Total (%)
		Nov-06	Dec-06	Jun-07	Jul-07	
Ripe fruit		**27.4**	**30.1**	**55.7**	**76.5**	**53.6**
Uapaca sp.	Euphorbiaceae	0	0	17	13.3	9.4
Sanitria trimera	Burseraceae	0	0	16.6	13.3	9.3
Pycnanthus marchalianus	Myristicaceae	0	0	12.6	11.3	7.6
Dialium sp. 1	Caesalpiniaceae	24.4	15.3	0	0.3	7.2
Annonidium mannii	Annonaceae	0	0	1.6	13	5.2
*Oxyanthus unilocularis (*vine*)*	Rubiaceae	0	0	0.4	5.9	2.3
Dialium sp. 2	Caesalpiniaceae	1.8	8.2	0	0	1.9
Garcinia punctata (?)	Guttiferae	0	0	0.8	3.1	1.4
Ficus spp. (2 spp.)	Moraceae	1.2	4.4	0.8	0	1.3
Other 19 spp.		0	2.2	5.9	16.1	8
Dry seeds		**67.7**	**56.8**	**28.5**	**15.9**	**36**
Guibourtia demeusei	Caesalpiniaceae	64.6	55.2	0	0	21.7
Leonardoxa romii	Caesalpiniaceae	0	0	26.1	7.6	9.8
Brachystegia laurentii	Caesalpiniaceae	2.4	1.1	1.2	3.1	2.1
Gilbertiodendron dewevrei	Caesalpiniaceae	0	0	0.4	3.1	1.3
Parkia bicolor	Mimosaceae	0	0.5	0.8	2	1
Acacia sp.	Mimosaceae	0.6	0	0	0	0.1
Terrestrial herbaceous vegetation (5 spp. of herbs, 1 tree sapling; petioles and pith)		**2.4**	**5.5**	**10.3**	**0.6**	**4.4**
Young arboreal leaves		**1.2**	**7.7**	**1.2**	**3.1**	**3.2**
Leonardoxa romii	Caesalpiniaceae	0	2.7	1.2	2	1.6
Scorodophloeus zenkeri	Caesalpiniaceae	0	4.9	0	0.6	1.2
Other 3 spp.		1.2	0	0	0.6	0.4
Fungi (truffles)		**0**	**0**	**2.4**	**2**	**1.4**
Dead wood (vine)		**0.6**	**0**	**0.8**	**1.7**	**0.9**
Unknown food item		**0**	**0**	**1.2**	**0**	**0.3**
Honey (meliponine)		**0**	**0**	**0**	**0.3**	**0.1**
Plant parasitic tissue (*Chlamydophytum aphyllum)*	**Balanophoraceae**	**0.6**	**0**	**0**	**0**	**0.1**
***N* 15-min feeding scans observed**		**164**	**183**	**253**	**353**	**953**

Table 2.2 Dietary quality, gregariousness and endocrine profiles of the Hali–Hali bonobos during the two study periods. N_1 and N_2 refer to the sample size for SP1 and SP2, respectively. Significant differences between study periods are indicated with an asterisk (*). See text for details on statistics

Measures	SP1 (Nov–Dec 2006) Mean (SEM)	SP2 (June–July 2007) Mean (SEM)
Dietary characteristics (N_1 = 16; N_2 = 39 days with more than 6 direct observational hours)		
Number of ripe fruit species eaten	6	24
Mean daily ripe fruit consumption (%)*	28.5 (5.1)	71.3 (3.3)
Mean daily dry seed consumption (%)*	57.3 (7.9)	18.5 (2.3)
Mean daily THV consumption (%)	5.9 (2.9)	3.9 (1.5)
Mean daily arboreal leaf consumption (%)	7.9 (3.0)	2.3 (0.8)
Gregariousness measures (N_1 = 24; N_2 = 10 days with mean party size)		
Mean adult party size	5.9 (0.3)	5.8 (0.6)
Mean female party size	4.2 (0.2)	4.4 (0.6)
Mean male party size	1.7 (0.1)	1.5 (0.1)
Mean relative adult party size (%)	59.3 (2.8)	53 (6.2)
Mean relative female party size (%)	60.1 (3.1)	54.7 (7.3)
Mean relative male party size (%)	57.4 (3.8)	48.3 (4.4)
Endocrine measures		
Urinary C-peptide levels: males, females, unknown samples (pg/mg Cr), N_1 = 43; N_2 = 44	1049.0 (177.0)	1603.6 (328.2)
Mean female C-peptide levels (pg/mg Cr)*, N_1 = 17; N_2 = 26	624.2 (84)	1306.2 (254.2)
Mean male C-peptide levels (pg/mg Cr), N_1 = 4; N_2 = 6	594.7 (165.1)	1657.1 (542.3)
Cortisol levels: males, females, juveniles and unknown samples (ng/mg Cr)*, N_1 = 23; N_2 = 41	196.2 (32.2)	75.5 (6.5)
Mean adult female cortisol levels (ng/mg Cr)*, N_1 = 16; N_2 = 24	213.3 (41.1)	69.8 (8.7)
Mean adult male cortisol levels (ng/mg Cr), N_1 = 4; N_2 = 6	157.2 (49.4)	87.2 (10.7)

One species of seed dominated the diet during SP1. *Guibourtia demeusei* is a swamp-forest upper-canopy tree that produces seeds in dry dehiscent single-seed pods. In early November of SP1, *Guibourtia* seeds were available in trees, but few had fallen to the ground. At this time, bonobos ate *Guibourtia* seeds in the tree crowns. During November the seeds matured and fell in large numbers to the ground, where bonobos continued to eat them. As a result, ground foraging was much more common overall during SP1 than arboreal foraging for bonobos eating *Guibourtia* seeds (79.7% of 207 feeding observations with ground foraging vs. 20.3% for arboreal or mixed ground/arboreal). During SP1, *Guibourtia* alone comprised 57% of all feeding observations. By contrast, during SP2, no *Guibourtia* seeds were recorded in the diet, though dry seeds of four other tree species still comprised 18.5% of the diet (Table 2.1).

Gregariousness of Hali–Hali bonobos

The number of adults per party averaged 5.8–5.9 in both study periods (Table 2.2). There was no significant difference between study periods in mean party size (Mann–Whitney, $z = -0.4$, $N_1 = 24$, $N_2 = 10$ days, $P = 0.7$), relative party size ($z = -1.1$; $P = 0.3$), female party size ($z = 0.153$; $P = 0.9$), relative female party size ($z = -0.9$; $P = 0.4$), male party size ($z = -1.3$; $P = 0.2$) or relative male party size ($z = -1.4$; $P = 0.2$).

Physiological status

Data from females indicated substantial differences in both C-peptide and cortisol status between study periods. In SP1, during the period of heavy seed consumption, C-peptide levels (624.2 pg/mg Cr) were relatively low, averaging 47.8% of those in SP2 (1306.2 pg/mg Cr, $t = -2.5$, $df = 36$, $N_1 = 17$, $N_2 = 26$ samples, $P = 0.02$, Table 2.2), indicating decreased energy balance in SP1. By contrast, cortisol levels in SP1 (213.3

ng/mg Cr) were relatively high, averaging 305.6% of those in SP2 (69.8 ng/mg Cr; t = 3.9, df = 26, N_1 = 16, N_2 = 24, P = 0.0006). Similar absolute differences occurred in male urines, but these were not statistically significant, presumably due to the small sample size (Table 2.2).

DISCUSSION

We observed bonobo behavior and physiology during two 2-month periods characterized by markedly distinct dietary composition. Yet, in both periods, tree seeds (mostly from Caesalpiniaceae) were an important part of the diet, occurring in between 15.9 and 67.7% of monthly scans. Tree seeds have not been reported to occupy such a large part of bonobo diets. Our study therefore extends the range of diet types known for bonobos. Chimpanzees have been previously recorded eating tree seeds (especially Caesalpiniaceae) at high concentrations in some months (Wrangham 1975; Goodall 1986; Reynolds 2005). At Budongo, the seeds of *Cynometra alexandrii* are described as being one of the ‘‘important foods’’ during a dry season lasting between December and March, a period characterized by a scarcity of fruiting figs (Sugiyama 1968). Sugiyama (1968) argued that the pattern of use of *C. alexandrii* seeds shown by the Budongo chimpanzees was similar to the reliance of savannah chimpanzees on the hard seeds of other Caesalpiniaceae trees during dry months (Suzuki 1969), and could thus be an important fallback food that buffers them against food shortages in some years. This interpretation fits well with our observations of seed eating by the bonobos during the months of November and December at Kokolopori, and should be explored further when long-term data on feeding ecology at Hali–Hali become available. Another unexpected finding was that the consumption of tree fruit by the bonobos varied twofold

between the two study periods. This raised the question of whether seasonal fruit shortages occur regularly at Hali–Hali, a topic that requires future phonological observations. In summary, our observations on seed eating and variance in ripe fruit intake between different months indicate a closer similarity in the plant diets of bonobos and chimpanzees then previously recognized.

If either ripe fruit or seeds are preferred to the other, we expect that the preferred items should be more beneficial and associated with a more positive energy balance and less physiological stress. Our finding that ripe fruit diets were associated with high C-peptide and low cortisol therefore suggest that fruits were a more beneficial food item than tree seeds. The fact that our urine samples could not be identified to individual means the physiological data are preliminary, but our results clearly suggest that ripe fruits are preferred and that tree seeds are eaten when ripe fruits are scarce.

Bonobo females tend to be more gregarious than chimpanzee females (Furuichi 2009). One of the explanations proposed for this species difference is that bonobos have more seasonally stable and abundant fruit supplies than chimpanzees do (White and Wrangham 1988; Chapman et al. 1994; Hohmann et al. 2006). Another is that bonobos have greater access to THV than chimpanzees, allowing them to forage in a regime of low scramble competition (Wrangham 2000). The herbs available to bonobos have also been shown to be low in fiber and high in protein relative to similar plant foods in chimpanzee habitats (Sommer et al. 2010). Our data, however, show that even though ripe fruit was not eaten at uniformly high levels all year, and even though THV contributed little to the diet, gregariousness in the study community did not change. None of the prior explanations is therefore easily applicable to Kokolopori bonobos.

Nevertheless, because bonobos often fed on tree seeds collected from the ground, scramble competition was apparently low, allowing female gregariousness to remain high. Our observations thus suggest that the mechanisms allowing bonobos to forage in relatively stable parties are more variable than previously considered.

Despite the short duration of this investigation, the contrast in dietary composition between our two study periods, together with the fact that extensive seed eating has not yet been recorded at other sites, suggests that much ecological variation in bonobos remains to be described and explained. Establishing multiple conservation areas across the DRC where bonobos are protected by involving the local community, as is the case at Kokolopori, can be expected to improve not just the survival prospects for bonobos but also our ability to understand the range of their ecological and behavioral adaptations.

CHAPTER 3

Diurnal variation in urinary C-peptide production in wild chimpanzees: the effect of feeding schedules and ripe fruit consumption

ABSTRACT

Urinary C-peptide (UCP) of insulin is being increasingly used in studies of wild non-human primates for non-invasive assessment of individual energetic status, nutritional condition or energy balance. Clinical research on human subjects fed on standard modern diets shows a clear relationship between food intake and increase in UCP levels that can be measured on the scale of hours. However, the extent to which such short-term variation in UCP production applies to primates subsisting on wild foods is unknown. Because carbohydrate and protein consumption causes an immediate rise in C-peptide production in humans, I hypothesized that changes in the hourly proportion of feeding time and dietary composition across the day should result in changes in UCP in wild primates, too. Analyses of 690 urine samples collected from 12 male chimpanzees during an 11-month study period in Kibale National Park, Uganda showed systematic trends in hourly variation of UCP levels. Using observation days on which at least 4 urine samples were collected per male (an average of 5 urine samples per male per day), I found significant differences between fasting and non-fasting UCP levels. The choice of sampling window therefore affects estimates of 'average' daily energetic status. Diurnal variation in UCP levels was best explained by the hourly changes in ripe fruit intake: ripe-fruit feeding time

(but not total feeding time) was most strongly associated with UCP levels at a 2 hour time lag. These findings highlight the importance of repeated sampling of UCP levels across the day. The results show that UCP is a sensitive marker of energetic balance not just on a long-term (i.e. months, seasons) but also on a short-term scale (i.e. hours and days).

INTRODUCTION

C-peptide of insulin has been widely used in human medical studies to assess pancreatic function and insulin production (Brandenburg 2008). Developments in its non-invasive assessment from urine have been an important advance in clinical practice and research (Galgani et al. 2010; Besser et al. 2011; Bowman et al. 2011). Studies of obesity in animal models such as the rhesus macaque, *Macaca mulatta* (Wolden-Hanson et al. 1993), also contributed to the recognition that urinary C-peptide (UCP) can be a biomarker with applications beyond the clinical setting. Following its use in anthropological studies on human subjects outside the laboratory (Ellison and Valeggia 2003), this technique was extended to studies of wild and free-ranging primates. In such settings, UCP offers important advantages for measuring individual condition and physiology under variable ecological and social circumstances, particularly when invasive monitoring such as capture for obtaining body weight data and blood is not appropriate (Sherry and Ellison 2007; Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Harris et al. 2010; Georgiev et al. 2011; Higham et al. 2011).

The key benefit of UCP sampling is that it offers a measure of insulin production – the hormone responsible for glucose uptake from the bloodstream and the creation of energy reserves in the body (Havel 2001). Field studies of great apes have shown that when multiple urine samples are collected from individuals over relatively long periods of time (e.g. weeks and months), average UCP levels are higher during times when high-energy food (e.g. ripe fruit) dominates the diet of the study subjects (Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Georgiev et al. 2011). The same pattern has also been shown among folivorous monkeys even though their high-quality food is not fruit but young tree leaves (Harris et al. 2010). UCP measurement has also been proven useful in inter-group comparisons with chimpanzees living in a more productive habitat maintaining higher average UCP levels than those living in forests that are poorer in food trees (Emery Thompson et al. 2009). Experiments in captivity have demonstrated that UCP levels track changes in body mass, decreasing during periods of dietary restriction and increasing when food supply is restored (Wolden-Hanson et al. 1993; Deschner et al. 2008; Girard-Buttoz et al. 2011). UCP has also been used to monitor the deteriorating condition of male rhesus macaques engaging in endurance rivalry during the mating season: males who spent most time in ejaculatory copulation had the lowest levels of UCP (Higham et al. 2011). UCP levels were also negatively correlated with individual travel time and with restlessness, measured as the rate of change in behavioral activities (Higham et al. 2011).

Despite these recent advances in the application of UCP to field studies of primate behavior and ecology, an outstanding methodological problem is the lack of knowledge about the effect that diurnal patterns of feeding behavior may have on UCP levels measured in spot-samples (i.e. one-off measurements). This is an important issue because different sampling regimes may lead to divergent conclusions regarding an individual's 'average' energetic status and nutritional condition. In studies of wild primates, where it is often unfeasible to obtain multiple samples from the same individual on the same day, mapping the relationship between UCP production and diurnal changes in feeding behavior is essential for the interpretation of such data.

Insulin production and a subsequent rise in UCP are tightly related to food intake and digestion in humans. Insulin (and C-peptide) production begins even before any food has been ingested (Woods et al. 1977). This 'cephalic phase' of insulin secretion is associated with anticipation of immediate feeding and ingestion. Most of the insulin, however, is produced during the 'gastro-intestinal phase' when insulin is released in response to the absorption of nutrients from the digestive tract (Nelson 2000). Studies that have tracked insulin and C-peptide production in human subjects over 24 hours show that immediately following a meal, levels of these hormones increase dramatically, after which they decrease gradually over a period of up to several hours (Kruszynska et al. 1987; Simon et al. 1987; Daly et al. 1998). Because of such variation in insulin and C-peptide secretion over the course of a day, the choice of sampling protocol (i.e. when and how often to sample) is important.

In view of these data from human research it is puzzling that no significant patterns in diurnal UCP variation have so far been identified in field studies of primates. This could be for several reasons. First, primate foods may not trigger the same insulin spikes as human foods do. Human food is calorically richer, easier to digest and poorer in fiber that the average primate diet (Wrangham 2009; Wrangham and Carmody 2010; Carmody et al. 2011) and will likely lead to a more pronounced spike in insulin production than uncooked wild plant foods. Second, primate digestion may differ from human digestion in such a way as to flatten out any peaks of insulin over time. Ingesta retention time and digestive efficiency among primates vary in relation to diet quality (Clauss et al. 2008) and such variation in digestive physiology as documented for humans and chimpanzees (Milton and Demment 1988) could in theory affect the temporal pattern of insulin secretion. Third, animals in the wild forage more continuously than humans in clinical settings or captive primates, who have set feeding times (Girard-Buttoz et al. 2011). Fourth, field sampling coverage may be insufficient to detect any peaks in UCP production. Finally, single urine voids may integrate C-peptide production over a period of several hours prior to urination and thus make detection of short-term changes in CP levels difficult.

A logistical problem in primate field studies is the unpredictability of obtaining urine samples from subjects. As a result, a typical dataset used in such studies includes samples collected at different times of the day during different periods and from different individuals. Human studies show that fasting UCP levels are well correlated with 24-h UCP production and total estimated energy

intake (McDonald et al. 2009; Galgani et al. 2010) but a similar validation has not been yet performed with primate subjects. Most studies have used only first-morning voids to measure UCP and have thus avoided addressing the problem of diurnal variation in UCP levels, but there are also some that have examined it (Table 3.1).

Table 3.1. Summary of studies using urinary C-peptide (UCP) in primates and their treatment of diurnal variation in UCP production. Note that to date UCP has not been used on non-primate mammalian species in the field.

Study	Species	Subjects*	Explicitly examined diurnal variation in UCP	Sampling intensity**	Result
Wolden-Hanson et al. (1993)	Rhesus macaque	C (exp.)	No (full 12-h urine collections were pooled)	-	
Sherry & Ellison (2007)	Chimpanzee, orangutan	W, C	No (fasting samples only)	-	-
Emery Thompson & Knott (2008)	Orangutan	W	No (mostly fasting samples used)	-	-
Deschner et al. (2008)	Bonobo	C (exp.)	Yes (fasting vs. non-fasting samples)	2.0	No difference
Emery Thompson et al. (2009)	Chimpanzee	W	Yes (regression of mean UCP on time of day)	1.1[1]	No circadian pattern
Harris et al. (2010)	Guereza colobus	W	Yes (regression of mean UCP on time of day)	1.0[2]	No circadian pattern
Higham et al. (2011)	Rhesus macaque	FR	No	-	-
Girrard-Buttoz et al. (2011)	Rhesus and long-tailed macaque	C (exp.)	Yes (fasting vs. non-fasting samples)	2.0	Fasting < Non-fasting; highly correlated
Georgiev et al. (2011)	Bonobo	W	No (fasting samples only)	-	-
Present study	Chimpanzee	W	Yes (diurnal patterns and fasting vs. non-fasting samples)	2.2 - 6.5	Significant diurnal variation related to feeding behavior; fasting < non-fasting

**Subjects: W = wild, C (exp.) = captive (food-reduction experiment), FR = free-ranging*
***Sampling intensity = mean number of urine samples per individual per day*
[1] Calculated from original dataset; [2] Harris, pers. comm.
- Not applicable since these studies did not consider diurnal variation in UCP production.

In a restricted-feeding and re-feeding experiment with captive bonobos, Deschner *et al.* (2008) found that UCP levels measured in the morning before any food was eaten (8-8:30 h) did not differ significantly from UCP measured in the afternoon (at around 14:00 h, or about 4 h after the bonobos had their main meal of fruits and vegetables). The authors therefore concluded that urinary C-peptide in primates is not a good measure of short-term changes in nutrient supply. Conversely, a similar feeding experiment on captive rhesus and long-tailed macaques showed that fasting UCP levels were significantly lower than non-fasting (Girard-Buttoz et al. 2011). Girard-Buttoz *et al.* (2011) also suggested that the difference between fasting and non-fasting samples may be less substantial among wild animals since they do not have a fixed feeding schedule, they feed more continuously and their food is not as calorie-rich as that of the captive subjects they studied. Field studies have shown that UCP does not follow a distinct circadian pattern of production (Emery Thompson et al. 2009; Harris et al. 2010), unlike cortisol and testosterone, for example, which are at their highest levels early in the morning and decline linearly with time of day (Muller and Lipson 2003). How UCP production varies in relation to feeding schedules however is not known. Thus studies that use UCP to characterize energetic condition of individuals do not apply an adjustment for time of collection even if some of the samples in the dataset are not at fasting (baseline) levels (Emery Thompson et al. 2009; Harris et al. 2010; Higham et al. 2011). Such an approach would yield reliable estimates of UCP production when multiple urine samples are available and averaged for each individual on a daily scale or if not – across

a larger time frame (e.g. weeks, months or seasons). However, comparisons between individuals and periods based on daily spot-samples (i.e. single urine voids) collected at variable times may be problematic particularly if we do not understand the temporal relationship between food intake and UCP production among naturally foraging animals.

In this study I examined diurnal variation in UCP levels in wild male chimpanzees, *Pan troglodytes*, and test the hypothesis that diurnal changes in UCP production are related to feeding activity. Secondarily, I predicted that consumption of energy-rich ripe fruit explains more of the diurnal variation in UCP levels than feeding time in general. I first explored a large urine dataset collected over an 11-month period from 12 different males to assess variation in mean hourly UCP levels across the day. I then restricted the analysis to a subset of the data – days for which at least 4 urine samples were collected from the same individual – to examine how the time of sampling may affect our estimate of male energetic balance on each of the sampled days. I also evaluated the correlation of hourly feeding time and hourly ripe fruit feeding time with subsequent changes in UCP production within a subset of high-intensity urine sampling days for which behavioral activity data was also available. Establishing the time lag of UCP excretion in relation to behavioral events is crucial for appropriate interpretation of such data and the design of suitable sampling protocols (Heistermann 2010). I also illustrate the importance of understanding diurnal UCP variation by examining it during periods of different levels of ripe fruit availability. I conclude by considering the practical implications of these findings

for future field studies that rely on UCP as a measure of energetic status among primates and other mammals.

METHODS

Study site & subjects

I studied the Kanyawara chimpanzee community in Kibale National Park, Uganda between August 2009 and June 2010. Over a period of 15 years the median annual home range of the Kanyawara chimpanzees was 16.4 km^2 (Wilson et al. 2012), encompassing mixed semi-deciduous primary forest with interspersed patches of swamps and regenerating forest (Chapman and Wrangham 1993). Subjects of this study were 12 male chimpanzees: 9 of them were considered adult at the start of the study (≥ 15 years old), and three were late adolescent (one of these turned 15 during the study, and two remained 14 years old throughout the study). Ages are known to the nearest months for individuals born since 1987 or were estimated by Richard Wrangham at the start of his long-term observations. The Kibale Chimpanzee Project has studied the chimpanzee community at Kanyawara continuously since 1987 without provisioning the animals.

Behavioral data

To quantify male feeding time, I followed focal subjects from nest to nest and recorded their behavior via continuous focal sampling (Altmann 1974) to the nearest second. Focal follows were at least 8 h in duration or, if shorter, concluded with the chimpanzee making a night-nest. Mean duration of direct

observations during focal follows with at least 4 urine samples was 10.4 ± 0.3 h (range: 6.6 – 11.8, N=23 follows of 11 males). The species name and the food type (ripe fruit, unripe fruit, young leaf, pith, seeds, bark, flowers, meat, honey) were noted for all items eaten when the focal subject was in sight. Total duration of the 23 follows was 239.5 h. During 11.5 h (4.8%) of this time the focal subject was not clearly visible and was recorded as 'time out'. On average, males spent 44.8% of the time they were in sight feeding and were out of view for 4.6% of total follow time.

To calculate the monthly ripe fruit consumption scores for observation months I analyzed a total of 12,742 feeding records (15-min instantaneous scans) collected on 331 days between July 2009 and June 2010 in the home-range of the Kanyawara chimpanzees. Long-term KCP field assistants collect these data on a daily basis when they follow a party of chimpanzees (Wrangham et al. 1996; Emery Thompson and Wrangham 2008a). If at least one individual in the party under observation is feeding, the food eaten is noted. If more than one food type is eaten within the same party, the food eaten by most of the individuals in sight is scored. While this method of recording feeding behavior overestimates individual feeding time collected by continuous focal sampling, it provides a comparable index of dietary composition when used to describe the proportion of fruit in the diet of the chimpanzees over a given time period (Gilby et al. 2010).

Urine sampling & analysis

KCP field assistants and myself collected chimpanzee urine samples opportunistically during observations in the field either by placing a plastic bag under a urinating individual or collecting urine off vegetation after the event. Samples were collected only if they had not been contaminated with feces and, for vegetation samples, if it was clear that no other chimpanzee had urinated in the same spot recently. Any debris in the samples was removed with a pipette each evening in a field laboratory. All samples were frozen at -20C° the same evening in a propane freezer (i.e. within a maximum of 14 h from time of collection). They were transported on ice-packs to University of New Mexico where Melissa Emery Thompson conducted all laboratory analyses. UCP assays were performed using the Millipore™ Linco RIA kit, with all samples assayed at an initial dilution of 1:2. Sensitivity of the assay was approximately 100 pg/ml. Intra-assay coefficient of variation (CV), measured as the average CV of duplicate determinations, was 7.1%. Inter-assay CV (N=15) was 10.4% for low samples and 5.0% for high samples. C-peptide concentrations were indexed via creatinine concentration measured in triplicate with the Jaffe reaction (Taussky 1954). Eleven urine samples with low creatinine values (< 0.05 mg/ml) were discarded from analysis because of the risk of obtaining over-inflated C-peptide values. I consider all urine samples collected at before 7 am to be at fasting or baseline levels. In most cases these samples were collected at or near the night-nest upon awakening. These samples are a conservative estimate of UCP levels before any feeding has taken place on the day of observation (i.e. immediately after the overnight fast; chimpanzees do not usually feed at night).

Data analysis

Behavioral data

To calculate hourly feeding times I converted the continuous focal sampling data on chimpanzee activity to 1-min instantaneous scans. Hourly feeding time was then calculated as the percentage of scans during one hour in which a chimpanzee was seen feeding. When examining seasonal differences in diurnal variation of UCP levels, I used the median monthly ripe fruit consumption score (57.8%) during the study period to divide months into high- and low- fruit consumption months. Six months of the study period were classified as high ripe fruit consumption months and five - as low.

Urine data

The analyses in this study consider a dataset of 695 urine samples collected between 1 August 2009 and 30 June 2010. I carried out analysis at several levels (Table 3.2). First, I examined variation in hourly mean UCP production in the full dataset (Dataset A) with and without 5 extreme outliers. Second, I also examined hourly variation in UCP levels within a subset of these data comprising 46 male-days (unique male-day combinations) for which at least 4 urine samples were collected (Dataset B). Within these 46 male-days I narrowed analysis to 28 male-days (Dataset C) for which at least 4 urine samples were collected and one of them was collected very early in the morning (between 6:00 and 7:59 h depending on time of waking of the male) and was thus at fasting (baseline) levels of UCP. Finally, I examined the relationship between male feeding behavior and subsequent changes in UCP levels in a subset of 23

male-days (Dataset D), for which at least 4 urine samples collected together with matching continuous focal data. Details of urine sampling effort within each of the datasets are shown in Table 3.2. To account for inter-individual differences in UCP levels, I present the hourly variation in UCP as mean of male means for Datasets A and B. In the analyses that compare matched-samples within days, I use each day as an independent data point.

Table 3.2 Urine sampling effort by time of day in the four datasets used in analysis. Dataset A is the full dataset and subsequent sets are subsets of it. Dataset A includes all urine samples collected during the study. Dataset B includes all male-days on which at least 4 samples were collected. Dataset C includes those male-days from Dataset C that included a urine sample collected before 8:00 h. Dataset D includes male-days, on which a minimum of 4 urine samples were collected and the males were subject to focal behavioral observations.

	Dataset A (N 321 male-days)			**Dataset B (N 46 male-days)**			**Dataset C (N 28 male-days)**			**Dataset D (N 23 male-days)**		
Mean sampling intensity (samples/ male-day ± SE)	**2.15 ± 0.11**			**5.8 ± 0.38**			**5.96 ± 0.57**			**6.48 ± 0.67**		
Hour	N males	Samples/ male	N samples	N males	Samples/ male	N samples	N males	Samples/ male	N samples	N males	Samples/ male	N samples
6	12	4.42	53	8	1.75	14	8	1.75	14	8	1.38	11
7	12	5.25	63	10	2.4	24	10	2.4	24	9	1.56	14
8	11	6.18	68	10	3.2	32	9	2.33	21	9	1.78	16
9	11	4	44	8	3.25	26	6	2.83	17	7	2	14
10	12	4.08	49	9	3	27	7	2	14	9	1.89	17
11	12	4	48	7	2.86	20	6	2.67	16	6	2	12
12	10	3.9	39	6	2.33	14	5	2.2	11	4	2	8
13	11	2.64	29	8	2.13	17	7	1.86	13	7	1.86	13
14	10	3.5	35	6	3.33	20	5	1.8	9	5	2	10
15	11	8.27	91	9	2.67	24	7	1.29	9	6	1.5	9
16	12	6.42	77	8	1.88	15	4	1.25	5	3	2.33	7
17	11	5.55	61	9	2.67	24	6	2	12	7	1.86	13
18	9	3.44	31	5	2	10	2	1	2	3	1.67	5
19	2	1	2	-	-	-	-	-	-	-	-	-
Overall	**12**	**57.5**	**690**	**11**	**24.3**	**267**	**10**	**16.7**	**167**	**11**	**13.55**	**149**

In the full dataset of 695 urine samples (Table 3.2) there were 5 outliers with UCP concentration more than 5 SD of the sample mean (Table 3.3, Figure 3.1). These outliers come from urine samples collected before noon from the 3 youngest males in the community (15-16 years old) during the months of November and December 2009. I first considered the implications of these outliers for diurnal variation in UCP production. I plotted male mean UCP concentration by hour with and without these outliers to examine how they affected the data (Fig 3.2). From the graph we can see that the outliers have a strong effect on the pattern such that they contribute to a distinct peak in UCP concentrations at 10 am. Because of the large magnitude of these outliers I consider that they represent either a laboratory error or a pattern that is untypical of most of the study subjects most of the time. While the samples have not been re-analyzed to rule out laboratory error, to be conservative in the present analyses, I removed the outliers from the final dataset. Note however that retaining them would have increased the likelihood of identifying significant differences between fasting (baseline) levels of UCP and non-fasting (after feeding had taken place), even if such differences were not significant across the larger sample. Removing them was therefore a conservative approach given the predictions I was testing.

I carried out statistical analyses in SPSS 19 (© IBM Corporation) and used two-tailed tests throughout. I log-transformed data only when conducting parametric tests

Table 3.3. Outliers removed from analysis and criteria for exclusion.

Outlier ID	Hour	UCP (pg/mg Cr)	Outlier/SD
ESDec10	8	70014.08	11.93
ESDec09	10	65779.41	11.21
ESDec09	10	48266.67	8.22
PBDec20	6	44247.25	7.54
TJNov06	11	33573.91	5.72
Highest UCP value retained in analysis:			
PGDec17	8	23351.85	3.98

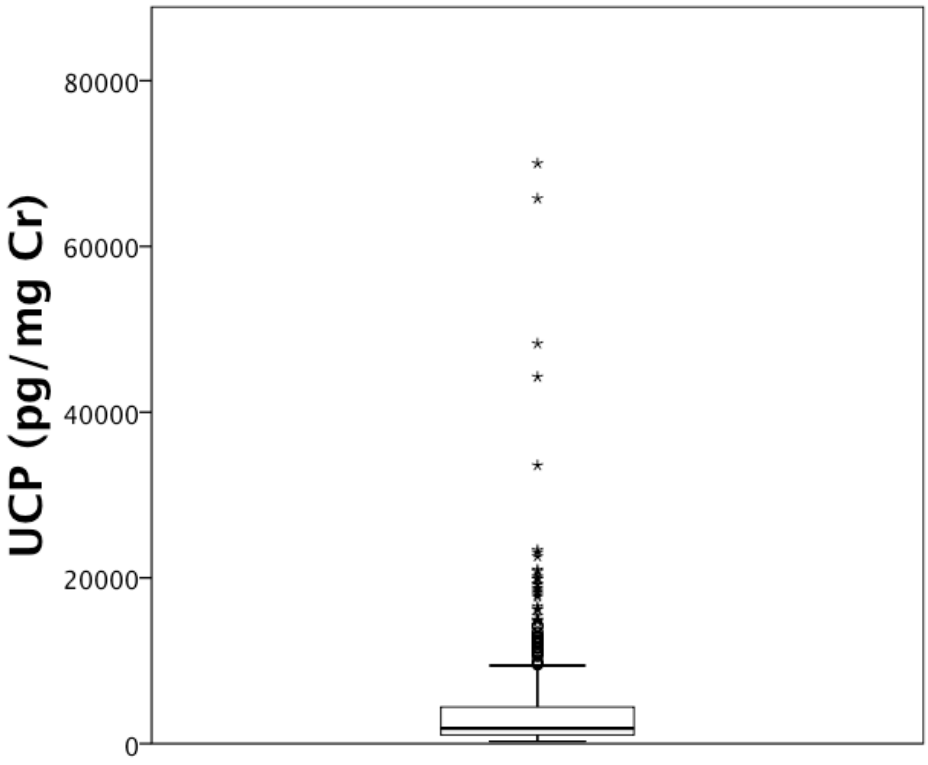


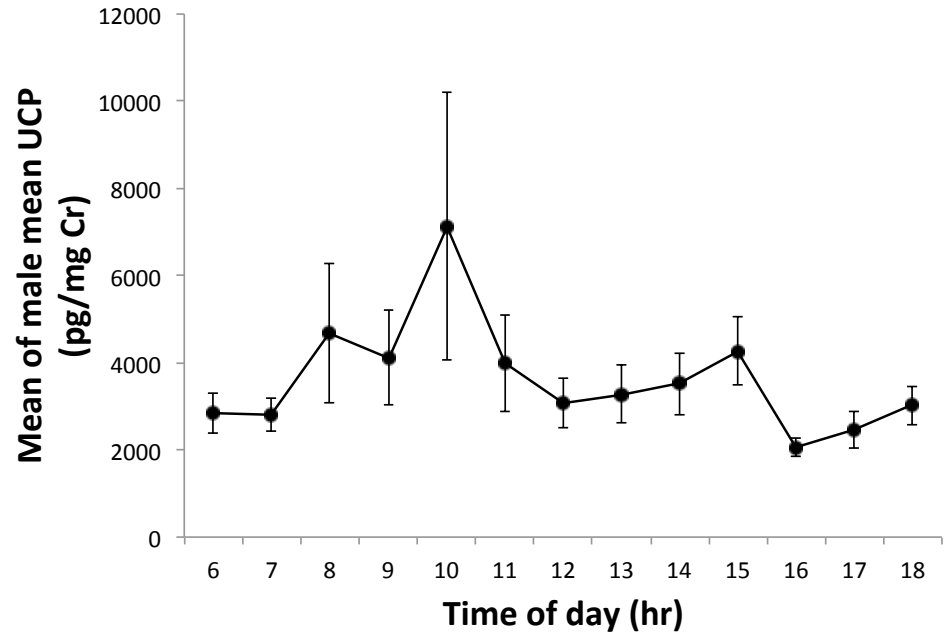
Figure 3.1 Boxplot of urinary C-peptide (UCP) concentrations in the full dataset (N=695 samples). Note the 5 extreme outliers (asterisks).

RESULTS

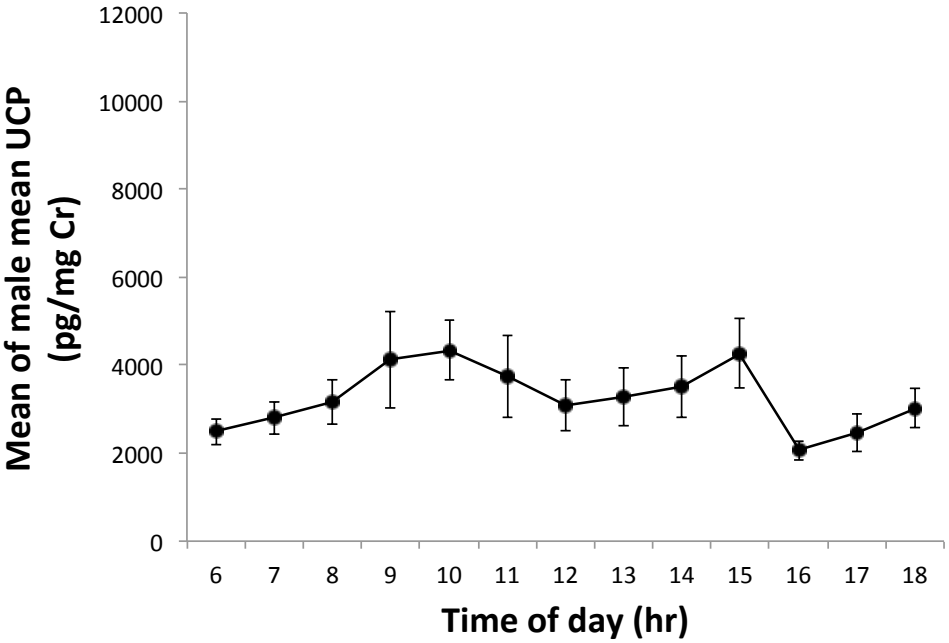
Dataset A: Diurnal UCP variation across the 11-month study period

Figure 3.2b shows the hourly mean of male mean UCP levels in the dataset containing 690 urine samples collected at mean intensity of 2.2 samples per male-day. Mean fasting (baseline) UCP levels (from urine samples collected 6:00 – 6:59 h) were significantly lower than those achieved during the peak hour of UCP production as identified from the plot (between 10:00 and 10:59 h), (related samples Wilcoxon Signed Rank Test: z=2.51; N =12 males; P = 0.012). From 11:00 to 15:59 h UCP concentrations remained relatively stable. They decreased sharply after 16:00 but then gradually began rising through 18:59 h (Figure 3.2b).

While these results suggest considerable variation in UCP levels over the course of a single day, the hourly UCP measures in this analysis are derived from a large dataset (samples collected on 188 different days over an 11-month period) and for many of these days only one urine sample per male was obtained (mean sampling intensity 2.2). Dataset A therefore does not control for within-individual diurnal variation. To address this problem I next examine diurnal patterns of UCP production within a subset of the data comprising only days of high-intensity urine sampling.



(a)

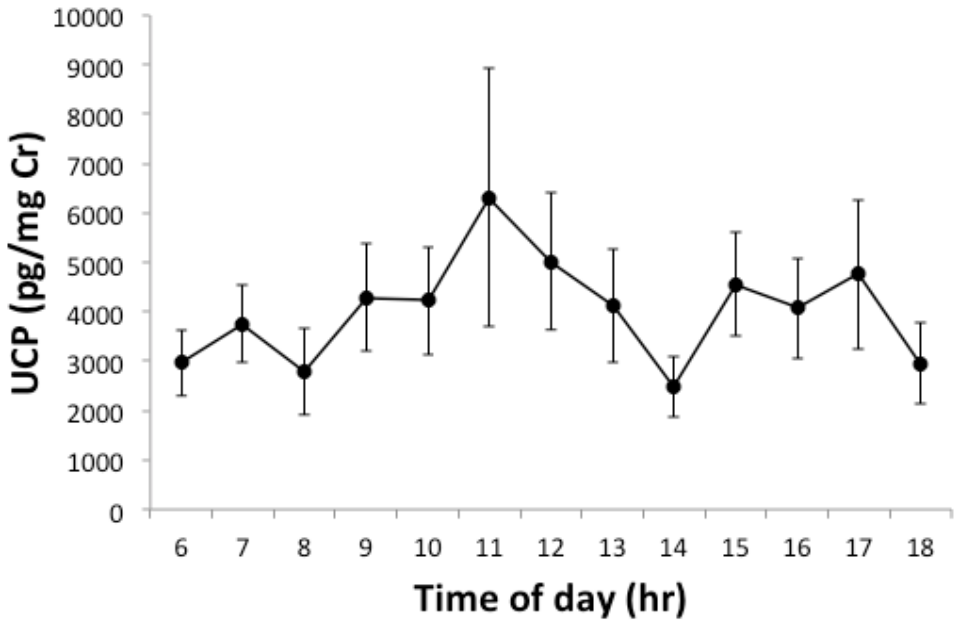


(b)

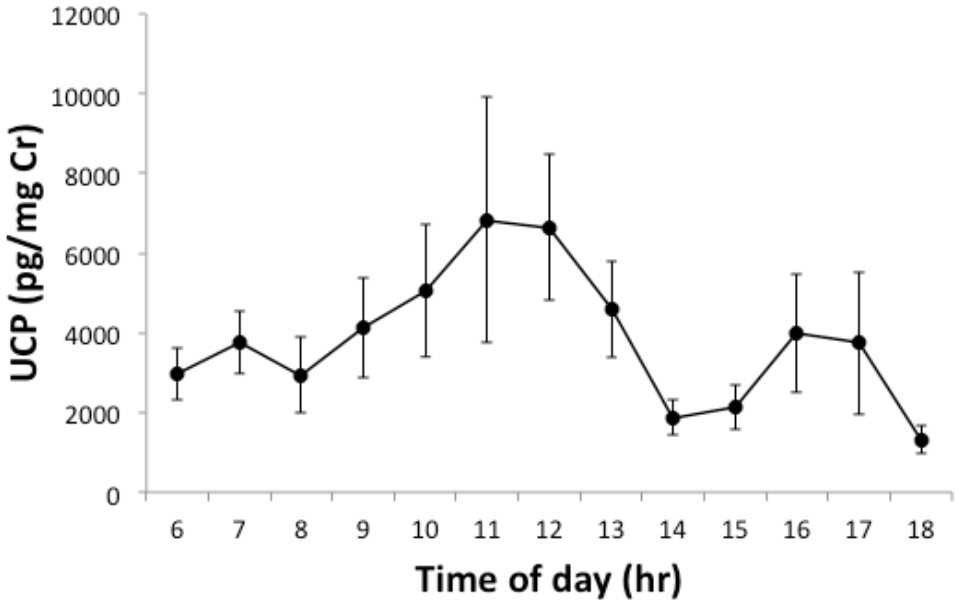
Figure 3.2 Hourly variation in urinary C-peptide (UCP) production. Values are means of male means (± SEM). (a) dataset with 5 extreme outliers (N=693 urine samples); (b) Dataset A: all urine samples except outliers (N=688). Two urine samples are not shown on the figure because they were the only ones collected after 19:00 h.

Datasets B & C: Diurnal UCP variation on intensely sampled male-days

In the full 46-male-day data set there was considerable fluctuation in UCP levels over the course of a day (Dataset B, Figure 3.3a). Within this dataset I identified 28 male-days on which there were at least 4 urine samples available, with the first sample collected very early in the morning (i.e. at approximately fasting levels between 6:00 and 7:59 h, Dataset C in Table 3.2, Figure 3.3b)



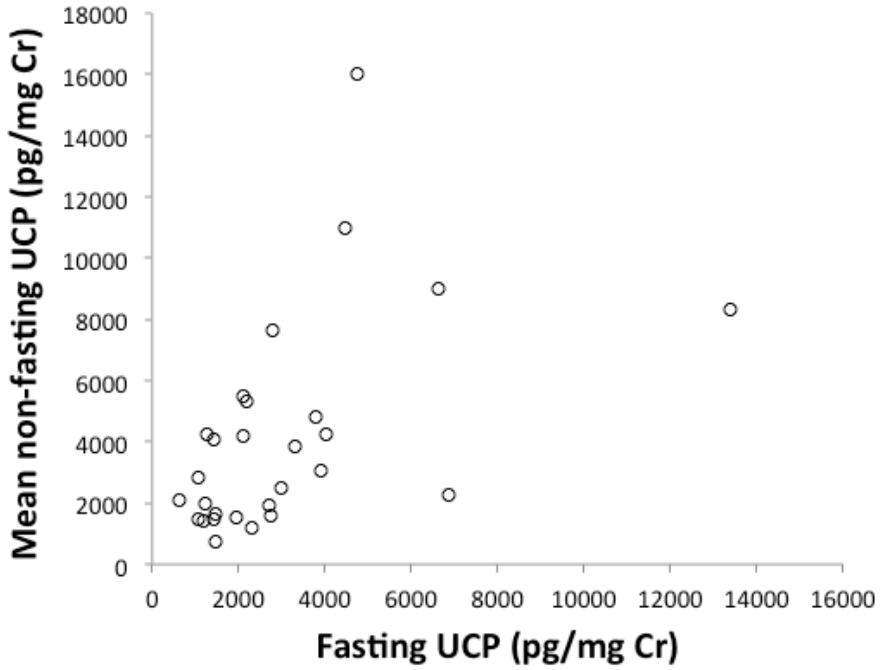
(a)



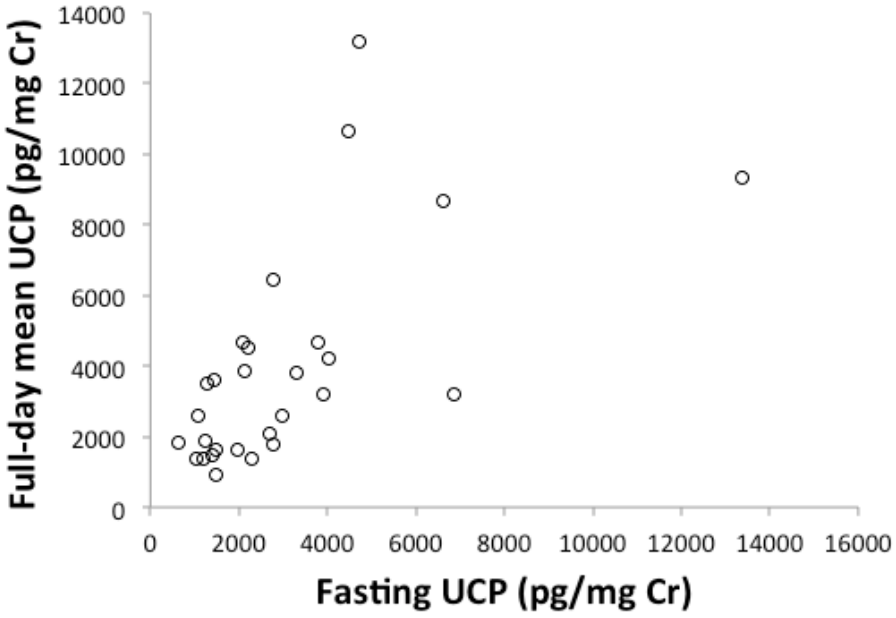
(b)

Figure 3.3 Diurnal variation in urinary C-peptide (UCP) production within (a) Dataset B, high-intensity sampled days (N=46 days; 267 urine samples) and (b) Dataset C; 28 intensely sampled days (N=167 urine samples). Values are mean of male hourly means ± SEM.

Using dataset C (28 days on which a fasting urine sample was available) I compared energetic balance between estimates that used (1) a first-morning spot-sample measurement, (2) the mean of all non-fasting samples, and (3) the mean of all urine samples collected on that day. UCP measured in fasting (first-morning) samples were correlated with both mean UCP non-fasting samples (r_s = 0.58, N = 28, P = 0.001, Figure 3.4a) and full-day mean UCP levels (r_s = 0.66, N = 28, P < 0.001, Figure 3.4b). Nevertheless, there were significant differences between these three measures of energetic balance in matched comparisons (Friedman test: χ^2=7.143, df=2, P=0.028). Post-hoc pairwise comparisons showed that UCP levels in the fasting samples were lower than those calculated from the non-fasting urine samples (z = -2.673, P_{adj} = 0.023). The differences among the other pairwise combinations were not significant (Figure 3.5). These data show that there is sufficient variance in UCP levels within a day that multiple samples are needed to obtain a reliable daily estimate. To explain the variation in hourly UCP levels I next consider their relationship to changes in individual feeding activity.



(a)



(b)

Figure 3.4 Relationship between fasting urinary C-peptide (UCP) levels (measured in a spot-sample) and (a) mean non-fasting levels calculated from all other urine samples collected on the same day; and (b) mean levels calculated from all samples on the same day (N=28 male-days).

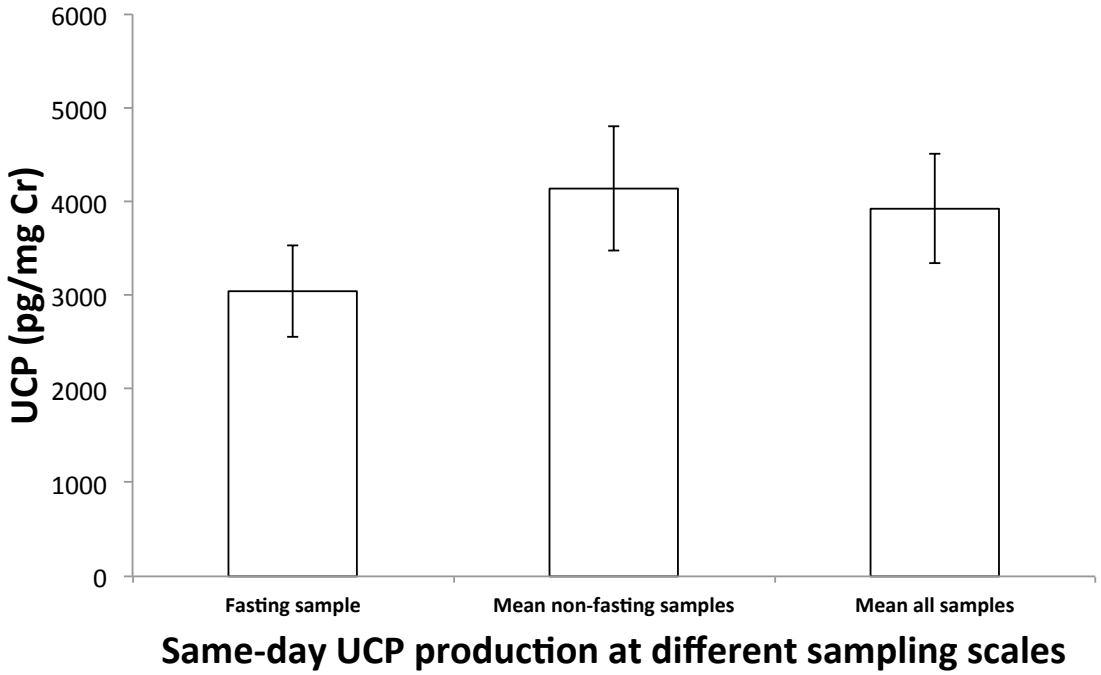


Figure 3.5 Mean urinary C-peptide (UCP) production estimated from sampling at different time-scales within a dataset of high-intensity sampled male-days (N=28 male days). UCP levels in first-morning samples were significantly lower than mean non-fasting levels. Differences in the remaining pair-wise comparisons were not significant (see text for details).

Dataset D: Hourly changes in urinary C-peptide (UCP) production in relation to feeding schedule and to ripe fruit intake

The dataset of high-frequency urine days included matching continuous focal sampling observations for 23 male-days. On average for each male-day I obtained 6.5 urine samples (SEM 0.67; range: 4 – 19; total 149 urine samples, Table 3.2). There was considerable variation in mean hourly UCP with a distinct peak between 12 and 13 h (Figure 3.6). From time of awakening, male UCP levels increased linearly between 6 and 13 h (linear regression of hourly UCP levels (log-transformed) on time of day (hour): Adj. R^2=0.87, df=6, P=0.001).

After 13 h UCP dropped precipitously but not in a linear fashion (Adj. R^2=0.19, df=5, P=0.22).

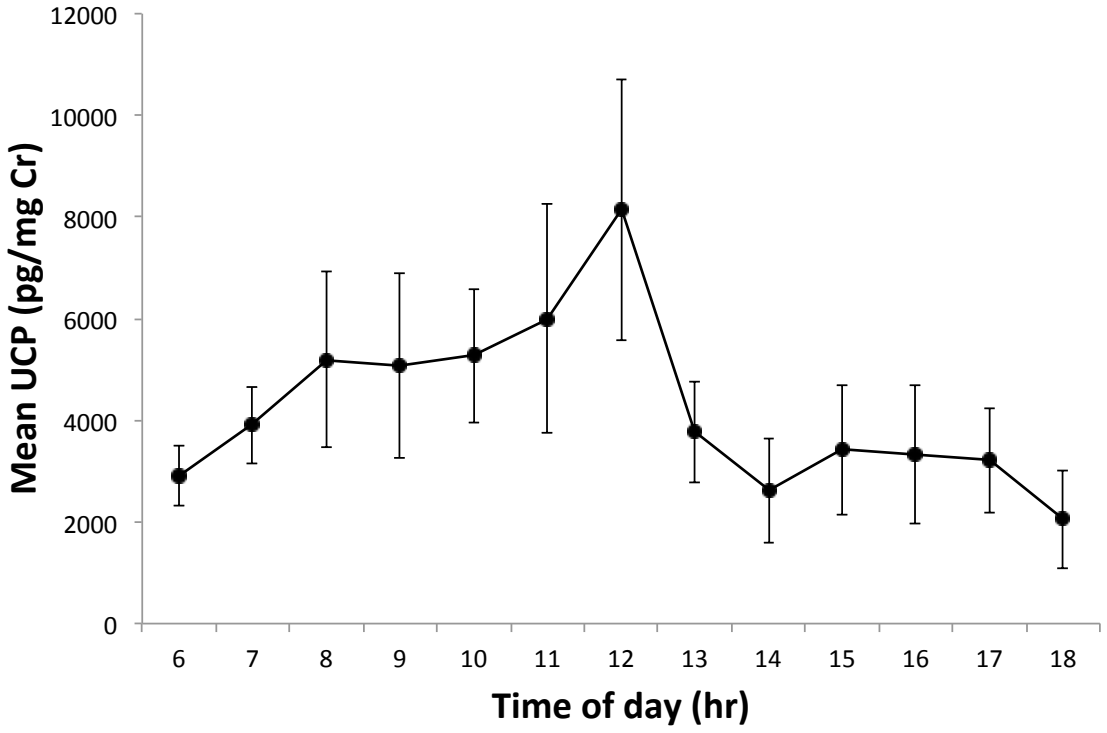
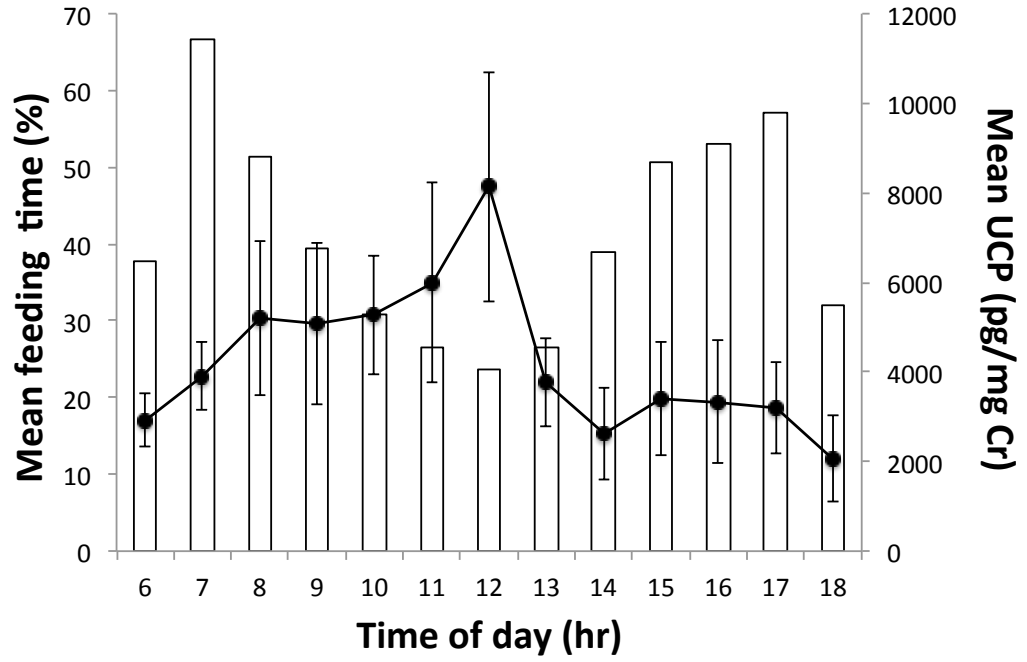


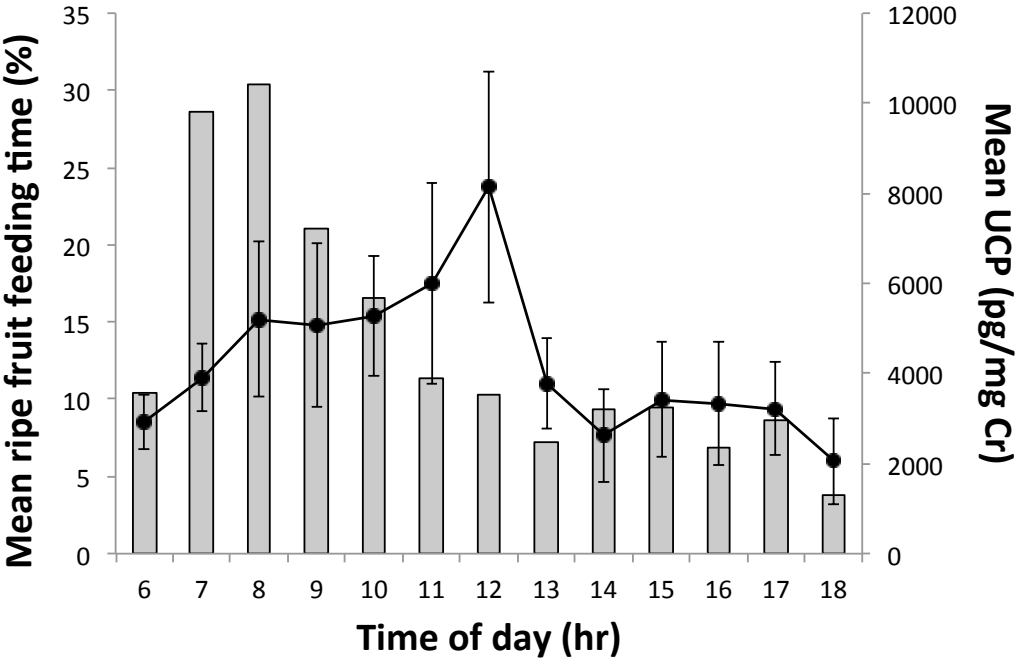
Figure 3.6 Hourly variation in urinary C-peptide (UCP) production on the 23 male-days for which both intense urine sampling and focal observations were available (Dataset D). Error bars are ±SEM.

I compared the variation in hourly UCP levels in this data set to the variation of hourly total feeding time and ripe fruit feeding time. Ripe fruit are rich in carbohydrates and their consumption should result in higher insulin and UCP production. Figure 3.7 shows the hourly percentage of time that male chimpanzees spent feeding on any food (a) and on ripe fruit in particular (b). Feeding tended to be high in the morning within the first few hours after waking up, followed by a decrease around noon. In the afternoon feeding increased in

the hours before construction of night nests (Figure 3.7a). In contrast, feeding on ripe fruit specifically was mostly restricted to the early hours of the day and decreased from around 11 am onwards (Fig 3.7b).



(a)



(b)

Figure 3.7 Hourly variation in urinary C-peptide (UCP) production (circles; error bars are ± SEM) in relation to mean hourly male (a) total feeding time (white bars) and (b) ripe fruit feeding time (filled bars). Data are from 23 male-days with intensive urine sampling and continuous focal observations.

I examined the relationship between ripe fruit consumption and UCP production on an hourly basis with different time lags.

I predicted that ripe fruit consumption would be significantly associated with UCP levels and that the association would be strongest when comparing feeding time in one hour with UCP levels in the hours following that hour. Because the exact time lag at which this relationship would emerge is not know, I examined the relationship between ripe fruit feeding time and UCP levels at different intervals (during the same hour; with a 1-, 2-, 3-, 4-, 5-, 6- and 7-h lag). Table 3.4 summarizes the results from this analysis and indicates that the association between ripe fruit consumption and UCP levels is strongest at a 2-h time lag (Table 3.4, Figure 3.8).

In contrast, total time spent feeding was a poor predictor of changes in hourly UCP levels at any of the time lags examined. There were no significant associations between hourly feeding time and hourly UCP levels (Table 3.5).

Table 3.4. Relationship between hourly average ripe fruit feeding time (%) and average urinary C-peptide (UCP) production in the dataset of intensely sampled focal follows (N=23 focal follow days). Data were log-transformed before performing the regression. Ripe fruit feeding time was compared with UCP levels at different times relative to the behavioral observation on feeding (with a 1-h step lag).

		Linear regression	
Time lag	**N (hours)**	**Adj. R^2**	**P-value**
On the hour	13	0.24	0.051
1-h time lag	12	0.33	0.03
2-h time lag	11	**0.456**	**0.013**
3-h time lag	10	0.443	0.021
4-h time lag	9	0.36	0.051
5-h time lag	8	0.072	0.261
6-h time lag	7	-0.15	0.659
7-h time lag	6	-0.228	0.803

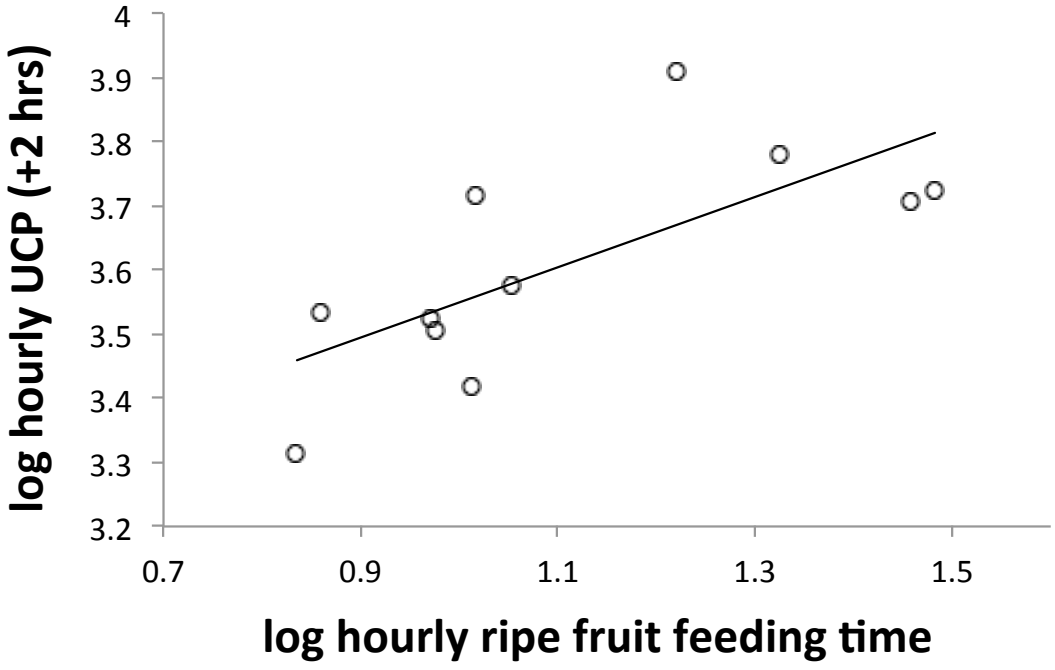


Figure 3.8 Relationship between ripe fruit feeding time (log-transformed percentage) and hourly UCP production with a 2-h time lag (log-transformed pg/mg Cr).

Table 3.5. Relationship between hourly average feeding time (%) and average urinary C-peptide (UCP) production in the dataset of intensely sampled focal follows (N=23 focal follow days). Data were log-transformed before performing the regression. Feeding time was compared with UCP levels at different times relative to the behavioral observation on feeding (with a 1-h step lag).

		Linear regression	
Time lag	**N (hours)**	**Adj. R^2**	**P-value**
On the hour	13	0.045	0.24
1-h time lag	12	-0.037	0.46
2-h time lag	11	-0.108	0.88
3-h time lag	10	-0.005	0.36
4-h time lag	9	0.295	0.075
5-h time lag	8	0.397	0.056
6-h time lag	7	-0.121	0.58
7-h time lag	6	-0.18	0.65

Seasonal differences in UCP diurnal variation

The effect of ripe fruit consumption on male UCP production was particularly clear when comparing diurnal variation in samples collected during months of high- and low ripe fruit consumption. Using the full dataset of urine samples (N=690) I plotted the hourly male means by fruit consumption season (Figure 3.9). Overall, males had higher mean daily UCP levels during months of high ripe fruit consumption (daily male means of hourly means, Wilcoxon test: z = 2.3; N = 11 males; P = 0.021). The difference between seasons was also significant when comparing UCP levels matched by hour of the day (Wilcoxon test: z = 2.34; N= 13 hours; P = 0.019). The inter-seasonal difference in UCP levels in samples collected between 6:00 and 7:59 was not statistically significant (Wilcoxon: z = 1.69; N = 11 males; P = 0.091) but comparing only samples collected from 6:00 to 6:59 showed higher fasting UCP levels during the high ripe fruit consumption months (Wilcoxon: z = 2.07; N = 9 males; P = 0.038). During

high fruit consumption UCP showed a clear peak between 9:00 and 9:59 h, while during low fruit consumption months such a morning peak was not visible (Figure 3.9). During low fruit consumption months UCP levels peaked between 14:00 and 14:59 h but the mean UCP levels achieved were lower than the morning peak of high fruit consumption months.

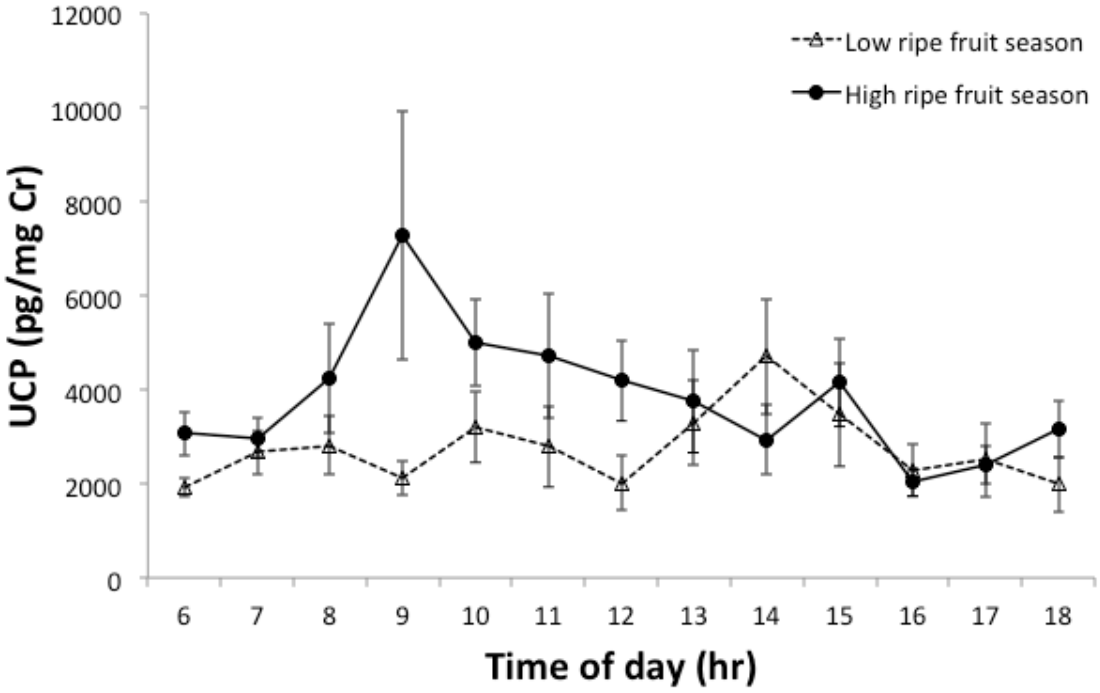


Figure 3.9 Hourly urinary C-peptide (UCP) production variation by season. Values are means of male means (± SEM).

The difference in male UCP levels was stronger when we categorized observation months according to the importance of *Ficus sur* to monthly dietary intake. In a previous analysis of male UCP production at Kanyawara, chimpanzees showed their highest UCP levels during a month in which *F. sur* was a particularly important dietary item (Emery Thompson et al. 2009). We used the median monthly *F. sur* consumption score (1.45%) to separate observation months into low (N=6) and high (N=5) levels of consumption. Males had significantly higher mean levels of UCP during periods of high *F. sur* consumption (Wilcoxon test: z = -2.9; N = 12 males; P = 0.004). Males also had significantly higher hourly UCP levels during such months (z = -3.2; N = 13 hours; P = 0.001, Figure 3.10).

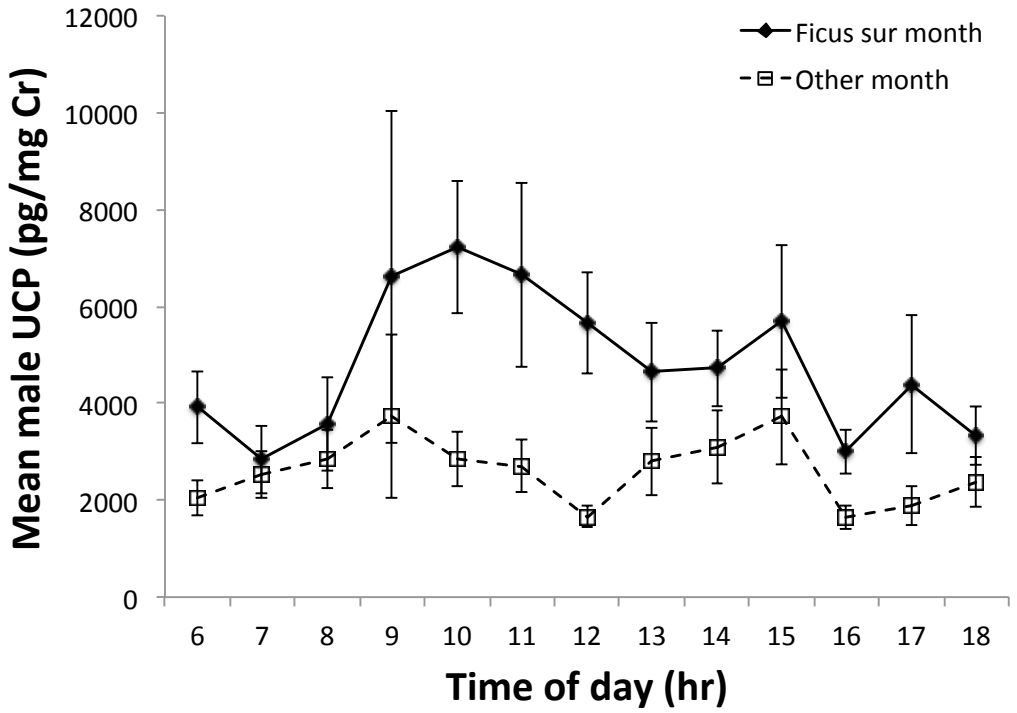
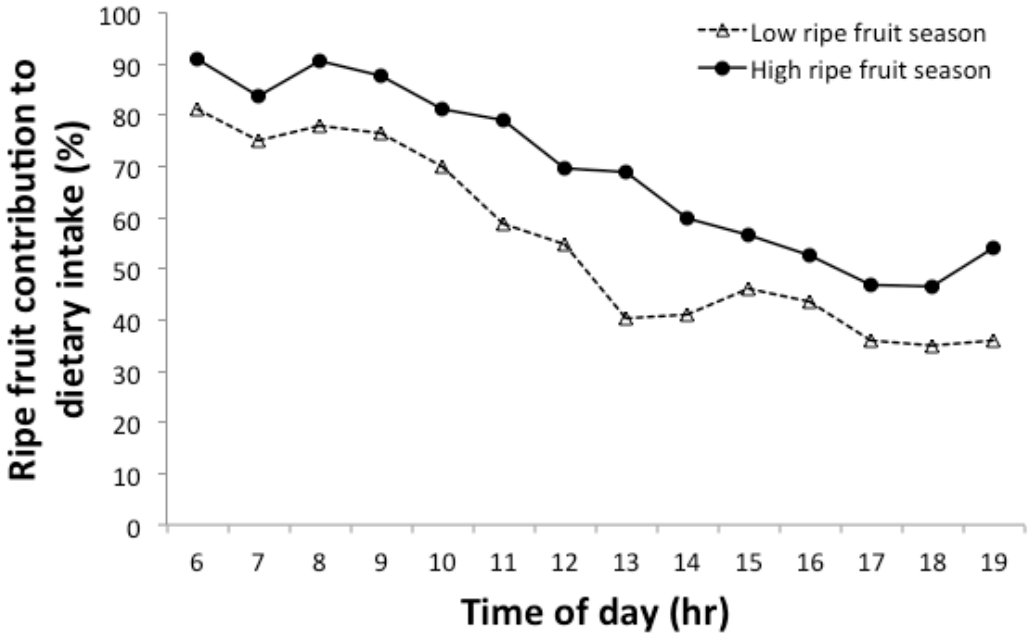
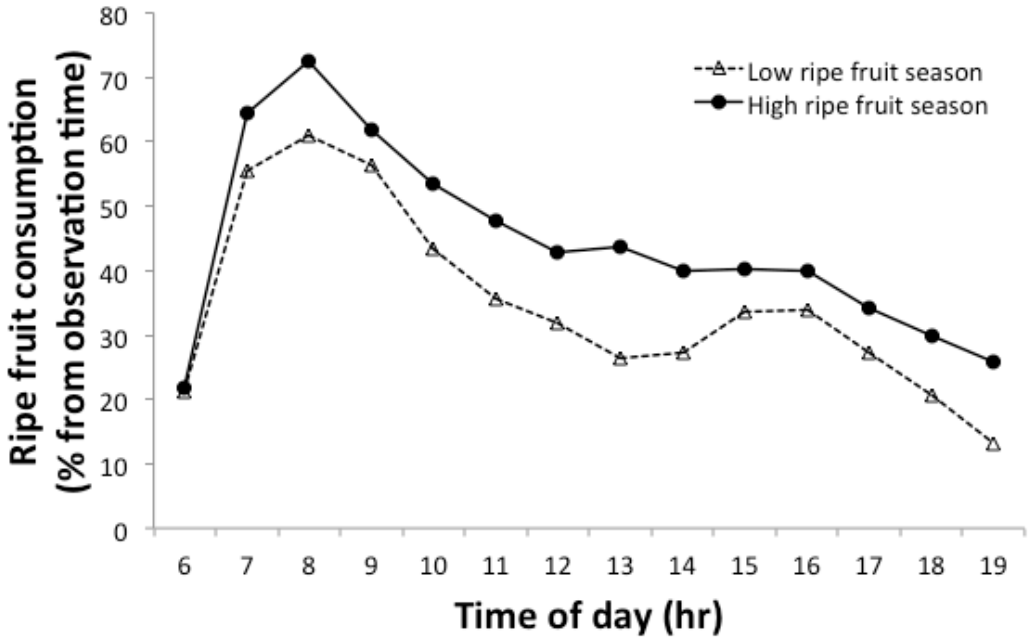


Figure 3.10 Hourly urinary C-peptide (UCP) production variation during months of high and low consumption of *Ficus sur*. Values are means of male means (± SEM).

To assess if the morning UCP peak during high ripe fruit consumption months can be attributed to chimpanzees feeding more on ripe fruit at this time of the day, relative to low ripe fruit consumption months, we examined the diurnal variation of feeding behavior in a large party-level data set of feeding records collected during the same study period. Figure 3.11 shows that despite the distinct peak in UCP production in high ripe fruit consumption months, the feeding effort allocated to ripe fruit is broadly similar in high and low ripe fruit consumption months. Mean hourly contribution of ripe fruit to dietary intake was highly correlated between high and low ripe fruit consumption months (r_s=0.94; N = 14 hours; P < 0.001) showing that ripe fruit intake rises and falls throughout the day similarly in both seasons. Mean hourly ripe fruit feeding times were also highly correlated between the two types of season (r_s=0.895; N = 14 hours; P < 0.001).



(a)



(b)

Figure 3.11 Ripe fruit consumption by time of day. Data are from 15-min scan samples of party activity (N=18,917 party scans over an 11-month period). (a) Contribution of ripe fruit to hourly feeding time; (b) Proportion of ripe fruit feeding time from total hourly observation time.

DISCUSSION

In this study I show for the first time that urinary UCP is a sensitive marker of short-term (on the scale of hours) changes in energy intake in wild non-human primates. Previous studies have revealed that average UCP levels measured over the course or weeks and months track changes in energy intake both in the wild and in captive diet restriction experiments (Deschner et al. 2008; Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Harris et al. 2010; Girard-Buttoz et al. 2011). The only two previous studies that examined UCP production in relation to the timing of meals within a single day reached conflicting conclusions. Deschner *et al.* (2008) did not find difference between fasting and non-fasting bonobo urine samples, while Girard-Buttoz *et al.* (2011) showed that fasting UCP levels were lower than non-fasting in two species of macaque. In a dataset of 23 full-day observations on male chimpanzees, for which I obtained an average of 6.5 urine samples per male per day I found significant positive relationship between hourly ripe fruit feeding time and UCP levels. The association was strongest when hourly ripe fruit feeding time was matched with UCP levels measured with a 2 hour time lag. Additionally, there was significant variation in mean hourly UCP levels across a larger dataset, spanning urine measurements made during an 11-month study albeit at a lower sampling intensity (2.2 urine samples/male-day). This study also demonstrated that fasting UCP levels in male chimpanzees were significantly lower than mean non-fasting levels.

Establishing the presence of any time-dependent patterns in the production of hormones and biological markers used in field or captive studies is a major methodological concern in behavioural endocrinology (Whitten et al. 1998; Heistermann 2010). I suggest that the reason that such a diurnal pattern has not been reported in earlier studies of UCP is that the present dataset is the first to contain days with high-intensity sampling (up to a mean of 6.5 urine samples per individual per day). Previous studies that tested for diurnal variation in UCP levels had lower sampling intensity (1-2 samples per individual per day, Table 3.1) and as a result any existing patterns might have been obscured. Additionally, the primary concern in previous analysis was detecting a circadian rhythm similar to that of urinary testosterone and cortisol, which decline linearly with time of day. In the field urine samples are collected opportunistically and obtaining multiple samples on the same day can be precluded by various factors such as the animal's behavior or its use of dense habitat as well as by precipitation. Additionally, the laboratory costs of analyzing large number of samples can be high. The difficulty in obtaining multiple samples on the same day is supported by the fact that ≥ 4 urine samples were obtained on only 14.3% of the total 321 male-days on which at least one urine sample was collected. This study therefore provides an important advance in our understanding of UCP dynamics in wild subjects subsisting on a natural diet.

A previous study that found time of sampling to be important for UCP measurement was a captive food restriction experiment on rhesus and long-tailed macaques. Urine samples collected in the afternoon, several hours after

their subjects were given their main meal of the day had higher UCP levels than fasting urine samples collected early in the morning (Girard-Buttoz et al. 2011). Because this study was conducted in captivity where animals received food at a predetermined time rather than foraging more continuously throughout the day, and the food itself was more calorie-rich than most natural primate foods, the authors suggested that UCP variation would be less pronounced across the day in wild subjects (Girard-Buttoz et al. 2011). The current study, conversely, indicates that even though chimpanzees do not forage at fixed times, there was sufficient variation in the distribution of foraging effort across the day to cause significant peaks and troughs in the daily UCP profiles of the sampled individuals.

Insulin production and thus C-peptide excretion in the urine do not reflect absolute caloric content but rather depend on the type of foods consumed. UCP is a particularly good marker of dietary carbohydrate intake (Buyken et al. 2006). Studies in human subjects have shown that, when caloric content is held constant, consuming meals high or low in carbohydrate and protein leads to, respectively, higher or lower UCP production (Hoogwerf and Goetz 1983). Given this, I predicted that hourly variation in UCP production in chimpanzees is related to time spent eating ripe fruit specifically, rather than total feeding time. Ripe fruit eaten by the study subjects at Kanyawara are rich in carbohydrates, are their preferred food (Wrangham et al. 1996; Conklin-Brittain et al. 1998; Wrangham et al. 1998; Conklin-Brittain et al. 2006) and have significant impact on female reproduction (Emery Thompson and Wrangham 2008a). An earlier long-term study has already shown that monthly measures of ripe fruit consumption predict

male UCP levels in this study community (Emery Thompson et al. 2009) but the extent to which this bio-marker could be used to monitor short-term changes in energetic status has not been appreciated until now. The fact that UCP levels reflect changes in nutrient intake on the scale of hours will allow greater time-resolution in studies of primate energetics, provided sufficient urine sampling intensity can be achieved.

An alternative explanation for the observed pattern in UCP production in relation to ripe fruit feeding is however also possible. In humans given identical meals three times a day, despite plasma glucose levels experiencing similar increases after each meal, the level of plasma insulin rose much higher after the morning meals but not after subsequent meals (Malherbe et al. 1969). Other studies, including one of captive vervet monkeys, *Cercopithecus aethiops*, (Meis et al. 1983), have also documented that insulin response varies with time of day even when identical meals are ingested (Jarrett 1972; Ahmed et al. 1976; Mejean et al. 1988; Kalsbeek and Strubbe 1998; Peter et al. 2010). Thus, the peak of UCP production in our data observed in the later morning and around noon may not be just a result of differing fruit consumption across the day but may also incorporate lower insulin responsiveness to caloric consumption later in the day.

These findings have several important methodological implications. First, studies that want to use UCP to measure short-term changes in behavior (on the scale of days, rather than months) should aim for high-intensity sampling coverage. Characterizing the ‘average daily energetic status’ of an individual cannot be made reliably from a single urine sample. In the absence of behavioral

data on the foraging activity of the focal animals on the day in question, the only urine sample that can reliably be compared to a sample collected from another individual or from the same individual at a different time periopds is the first morning urine sample (at fasting levels). Thus whenever practical, the collection of such samples should be a priority.

All this is not to say that studies that do not control tightly for time of sample collection cannot be useful for understanding the energetics of primate behavior and ecology. Large datasets, with randomly sampled UCP levels from many individuals on multiple days at different times of the day can reveal meaningful biological patterns in the data, given a low 'signal-to-noise ratio' (Goyman and Trappschuh 2011). It is, however, worth emphasizing the importance of thorough exploratory analysis to assess the 'randomness' of sampling in regards to individual representation across time-blocks and across conditions of interest (e.g. age-sex class, reproductive state, social rank, food availability periods, mating vs. non-mating periods, dry vs. wet seasons). Establishing the range of variance within- and between individuals and conditions will help not just establishing the adequacy of sampling effort in specific datasets but can also be essential for a sound interpretation of results. It is difficult to give any specific guidelines, given that the diurnal patterns identified in the datasets presented here differ from one another depending on which dataset is used (i.e. peaks of UCP are at 9, 11 or 12 hours depending on the subset of samples plotted: Figs. 3.2, 3.3, 3.6). Patterns in feeding behavior and UCP response may also vary between populations or between dietary regimes. Fasting UCP levels

seem the least ambiguous measure because we can be sure that during the night the animals have not been feeding (in most cases) and thus such urine samples can be compared to each other both at the inter- and intra-individual level. Because feeding patterns differ from one day to another, it would be also difficult to rely on average species or population-level activity data to generate time-windows, which will be comparable across individuals in all circumstances. The best recommendation that emerges from this study is that the more urine samples obtained from each individual study subject per day, the more accurate the assessment of its energetic condition via UCP would be.

CHAPTER 4

Estrous females and male feeding behavior in chimpanzees: foraging constraints of mating effort in a promiscuous non-seasonally breeding primate

ABSTRACT

In many vertebrates male-male competition for mating opportunities leads to energetic costs, through extra energy expenditure, reduced energy intake or both. Polygynous and promiscuous mammals with high breeding seasonality can be so strongly affected that males cannot persist in maximal reproductive efforts for long durations and suffer high mortality. In contrast, our understanding of such costs is limited and contradictory in species without breeding seasonality. Since males in such species face a trade-off between temporary loss of condition and future ability to compete I investigated how mating effort influences feeding behavior in chimpanzees – a promiscuous non-seasonal breeder. On days when parous females were in estrus all males fed less. Feeding time was negatively correlated with the frequency of aggressive behavior and copulations, and the total reduction in feeding time observed in the presence of parous females in estrus was not compensated by increased reliance on foods of higher quality. Increased mating effort therefore appeared to be responsible for reduced food intake. The magnitude of these feeding costs was not related to male dominance rank even though high-ranking males obtained more copulations. I discuss several possible explanations for a lack of a rank-related scaling of foraging

constraints as they apply to the specific circumstances of estrous female availability and fecundity during this particular study period, and in the broader context of the mating system of chimpanzees. I suggest that chimpanzees illustrate how lack of breeding seasonality, the existence of a stable male dominance hierarchy maintained at all times, male-male bonding and indirect long-term sexual coercion of females by males may contribute to a relatively low-cost mating effort that allows year-round reproductive effort over many years of a male's reproductive career.

INTRODUCTION

The conflicting energy allocation demands of reproduction and self-maintenance in many male animals are most obvious during periods of mating competition (Stearns 1992; Andersson 1994). At such times males often sacrifice feeding time in order to engage in behaviors more directly related to increasing their paternity success, notably aggression, courtship, mate-guarding and copulation (Winter 1976; Clutton-Brock et al. 1982; Mace 1989; Poole 1989; Westneat 1994; Alberts et al. 1996; Chuang-Dobbs et al. 2001; Komdeur 2001; Matsubara 2003; Mysterud et al. 2004; Willisch and Ingold 2007; Pelletier et al. 2009; Ancona et al. 2010; Higham et al. 2011). Reduced feeding time is known to lead to a lower intake of energy and to contribute to loss of body mass during breeding periods, particularly among those males that compete most intensively (Bercovitch and Nurnberg 1996). The case is particularly well illustrated among extremely polygynous southern elephant seals, *Mirounga leonina*, who cease

feeding altogether during their breeding season: alpha males expend more energy than subordinates and their body mass predicts their tenure on the beach, as well as their mating success (Galimberti et al. 2007; Crocker et al. 2012). The ability of males to meet such energetic costs of reproduction may thus underlie the particular mating strategies they adopt and in turn their lifetime fitness. The magnitude and intensity of male-male competition and the associated costs of such competition are also known to affect male life expectancy. In species with more intense male-male mating competition males die younger than females, while in species with less severe competition males and females have similar mortality schedules (Clutton-Brock and Isvaran 2007; Bronikowski et al. 2011). Studying the constraints that mating effort places on male feeding behavior is therefore useful for assessing the strength of sexual selection, particularly in species whose low or moderate sexual dimorphism would suggest that intra-sexual competition for reproduction is relatively relaxed (Plavcan and van Schaik 1992). Quantifying the inter-individual variation in short-term costs of male mating effort also helps to interpret differences in male life-history traits across taxa.

In this study I test the hypothesis that mating effort constrains male feeding time in a promiscuous primate, the chimpanzee. Chimpanzees are an interesting case for several reasons. On the one hand, behavioral observations reveal intense intra-sexual competition for reproduction. Males are more aggressive when highly attractive (parous) females are in estrus (Tutin 1979; Emery Thompson and Wrangham 2008b), they engage in mate-guarding to

prevent other males from copulating (Watts 1998) and higher-ranking males, the alpha male in particular, are responsible for most copulations and fertilizations in all studied populations (Goodall 1986; Boesch et al. 2006; Inoue et al. 2008; Wroblewski et al. 2009; Newton-Fisher et al. 2010). Males also behave aggressively towards estrous females and they are successful in constraining their choice of mates via sexual coercion, at least in some populations (Muller et al. 2011).

On the other hand, moderate reproductive skew among males (Kutsukake and Nunn 2009) and the moderate levels of sexual body dimorphism in chimpanzees (Plavcan 2004), relative to other primates and mammals in general, indicate that levels of competition are not as extreme as those seen in many species with seasonal polygynous mating. Furthermore, chimpanzee male life history patterns differ importantly from those in species that engage in energetically costly mating competition during distinct breeding periods. Male rhesus macaques, *Macaca mulatta,* for example have a highly seasonal period of reproduction during which males feed less, engage in energetically exertive multiple-mount ejaculatory copulations and lose condition in proportion to the effort expended (Bercovitch and Nurnberg 1996; Bercovitch 1997; Higham et al. 2011). Even though male rhesus are able to prepare for the mating season by storing fat during the birthing period when no females are in estrus, the intensity of their reproductive effort has been suggested as an explanation for the higher levels of male mortality during the breeding, relative to the birthing season (Hoffman et al. 2008). Male chimpanzees have a shorter life span than females

(Hill et al. 2001) but otherwise do not suffer increased mortality during a specific period of the year, perhaps because females do not display estrus synchrony. Females may come into estrus at any time of the year (Goodall 1986; Emery Thompson and Wrangham 2008a; Matsumoto-Oda and Ihara 2011) and periods of intense mating competition are usually brief, focused on the female's peri-ovulatory period lasting ca. 5 days (Deschner et al. 2004; Emery Thompson 2005; Emery Thompson and Wrangham 2008b). Alpha male chimpanzees are able to maintain their high rank and high levels of reproductive effort over many years despite the apparent physiological costs associated with their status such as increased testosterone and cortisol levels (Muehlenbein et al. 2004; Muller and Wrangham 2004a; Muller and Wrangham 2004b). A recent study has in fact concluded that alpha chimpanzee males live longer than non-alpha males (McCarthy et al. 2011) thus questioning the notion that maintaining high dominance rank and high reproductive effort and success in this species is traded off at high energetic and physiological costs. This apparent inconsistency leads to the prediction that mating effort among male chimpanzees is not as costly as among other primates and mammals. One reason that this might be so could be their lack of breeding seasonality.

Data from other non-seasonally breeding primate species seem to support such an interpretation. Mating effort does not consistently constrain foraging in chacma baboons, *Papio ursinus*, (Weingrill et al. 2003; Henzi et al. 2010) and has only a slight effect on male feeding behavior among yellow baboons, *Papio cynocephalus*: their feeding bouts were shorter when consorting with estrous

females but total feeding time was not affected (Alberts et al. 1996). The evidence of foraging constraints among high-ranking males is more consistent in seasonally breeding primates (Matsubara 2003; Higham et al. 2011). Yet high reproductive success may also come at little energetic cost even among extremely seasonal breeders. Sifaka males, *Propithecus verreauxi*, guard estrous females and manage to sire almost all infants in their groups despite the presence of other males but no costs of doing so have been detected: during periods of mate-guarding their feeding was not constrained and there was only a trend for increased rates of aggression (Mass et al. 2009). At the other extreme are African elephants, which are non-seasonal breeders in the sense that there are some females coming into estrus and conceiving during every month of the year (Poole et al. 2011). Despite the potential year-round availability of mating opportunities, elephant bulls do not maintain reproductive effort year-round. Bulls come into breeding condition during distinct periods of musth lasting from days to several weeks at sporadic intervals among young males and 2-5 months on an annual basis for old males (Poole 1987). During musth they feed less, travel more and lose weight. Weight loss increases with musth duration and bulls that were in poorer condition terminated musth quicker (Poole 1989). Maintaining reproductive effort year-round is evidently too costly for male elephants, unlike for male chimpanzees.

In summary, male chimpanzees offer an interesting opportunity for examining costs of mating effort in a non-seasonally breeding mammal that is able to maintain reproductive effort year-round continuously for multiple years (as

shown by the long duration of alpha tenures). Accordingly, in this study I test four predictions related to the consequences of mating effort for energy intake in male chimpanzees. My predictions are: first, that male mating effort constrains feeding behavior; second, that male aggressive and mating behavior are the proximate causes of reduced feeding time in mating contexts; third, that males are not able to compensate the reduction in overall feeding time on mating days by focusing their foraging effort on high-quality food (ripe fruit); and fourth, that high-ranking males experience greater reduction in feeding time due to the greater proportion of copulations they manage to perform.

METHODS

Study site & subjects

The Kanyawara chimpanzee community inhabits the north-western edge of Kibale National Park, Uganda (Isabirye-Basuta 1988). During a period of fifteen years, they ranged over a total of 41 km^2 with an annual median home range of 16.4 km^2 (Wilson et al. 2012). The territory of the Kanyawara chimpanzees is a mix of mostly primary unlogged forest, with areas of partly logged forest, papyrus swamps and former pine plantations (Chapman and Wrangham 1993; Struhsaker 1997). Since 1987 Wrangham and colleagues of the Kibale Chimpanzee Project (KCP: http://kibalechimpanzees.wordpress.com/) have been conducting a long-term continuous study of the Kanyawara chimpanzees (Wrangham et al. 1991). During this study (August 2009 – June 2010) the community numbered 49 - 50 individuals, all fully habituated to human

observers. My subjects were 9 adult and 3 late-adolescent males (aged between 14 and an estimated 55 years at the start of observations; median and mean age: 31.5 ± SE 3.9 years). One of the adolescents turned 15 (the criteria for being considered adult) during the study. Ages were determined either from actual birth records or based on estimates at the beginning of long-term monitoring by KCP in 1987 made by Richard Wrangham and colleagues.

Behavioral observations

I quantified male foraging behavior from continuous focal observations (Altmann 1974). I recorded chimpanzee activity with the CyberChimps behavioral sampling software developed and customized by Paco Bertolani. This program ran on a Samsung i200 smart phone and allowed changes in focal activity to be noted to the nearest second. Feeding behavior consisted of handling, ingesting and chewing food items while stationary. If a focal subject continued chewing a wadge while walking, this was noted as travel. During feeding sessions, the onset and termination of each feeding bout was recorded to the nearest second, whenever possible. Using these data I calculated daily feeding time (proportion from observation time), ripe fruit contribution to diet (proportion from feeding time), mean daily feeding bout duration (min) and the total number of bouts per day.

Ideally, each focal follow started when the target left his night-nest, but if a desired focal target was not seen at the sleeping site, a session was initiated at a later time upon first encounter. I observed focal subjects until they made a nest in the evening, were lost during the day, or were lost shortly before nesting in the

dark. I restricted analyses to follows that were either complete nest-to-nest observations or at least 8 h long. The median duration of focal follows in the data-set was 11.57 h (mean 11.03 ± SD 1.42 h; range: 6.7–12.9 h, N=85 days). The choice of a focal individual was semi-random because a pre-determined target was not always located on the day of the follow, and sampling was flexible to allow observations of males that were seen less regularly.

Party composition & female reproductive state

Previous research has shown that chimpanzees feeding in larger foraging groups (parties) tend to spend less time feeding (Bygott 1974; Wrangham and Smuts 1980; Pandolfi 2004). To control for this possible confounder on male feeding time I recorded the number of adult and adolescent individuals within 50 continuous meters of each other at 15-min intervals. Mean daily party size is the average number of individuals recorded during all 15-min scans on that day. I noted the reproductive state of all cycling females by assigning a score to the ano-genital swelling (1 = deflated; 2 = partially swollen; 3 = fully swollen, swelling tense and shiny). Only fully tumescent females are considered as 'estrous' because it is during this stage of their cycle that they are most attractive to males and the likelihood of mating is highest (Emery and Whitten 2003; Deschner et al. 2004; Emery Thompson 2005; Emery Thompson and Wrangham 2008b). Females differ in their attractively as mating patterns depending on their parity. Young, nulliparous females are at the onset of their reproductive careers and have not produced any offspring yet. Because their cycles tend to be less fertile, they do not excite much male mating interest. In contrast, older, parous females

(those that have produced at least one infant) are highly attractive and incite intense mating competition (Muller et al. 2006). I classify days on which at least one parous female was in estrus as 'parous mating days', days on which at least one nulliparous female (but not a parous one) was in estrus as 'nulliparous mating days' and days on which neither type of estrous female was seen as 'non-mating days'. In practice whenever a parous female was in estrus males remained with her for a significant part of observations and often for the entire day. Classifying days into mating and non-mating days provides a reliable estimate of current opportunities for mating.

Aggression & sex

During focal follows I recorded all agonistic and sexual interactions involving the target male (Martin and Bateson 2007). Aggressive behaviors noted were: vocal and non-vocal displays, charges, chases and physical attacks (slaps, hits, kicks and bites). Only male-female copulations were considered sexual interactions. Rates for both types of behavior were calculated per hour of observation time for each focal follow. Supplementary *ad libitum* and all-occurrences (Martin and Bateson 2007) data on aggression and copulations were collected by KCP field assistants during the entire study period. These data were used to calculate the dominance hierarchy and the overall mating success of the 12 focal males because they presented a larger, more comprehensive record of the interactions of my study subjects.

Food availability & dietary quality

At Kanyawara consumption of ripe fruit, and drupes in particular, is a robust index of dietary quality and positive energy balance (Emery Thompson et al. 2007b; Gilby and Wrangham 2007; Emery Thompson and Wrangham 2008a; Emery Thompson et al. 2009). I used a monthly score for non-fig fruit (NFF) consumption to describe dietary conditions and preferred food abundance. This NFF-score was calculated as the mean monthly percentage of feeding records (15-min party scans) during which drupes were consumed in all chimpanzee parties observed during that month by KCP field staff and myself (N = 12,742 feeding scans observed on 331 days during this study). This dietary score at Kanyawara correlates significantly with fruit availability measured twice a month on phenology transects (Wrangham et al. 1996). It also correlates with focal estimates of dietary composition (Emery Thompson 2005; Gilby et al. 2010) and is thus a useful measure of both habitat-wide fruit availability and individual fruit intake. Controlling for dietary quality is necessary in all analyses because chimpanzees tend to spend more time feeding during periods of ripe fruit scarcity (Knott 2005) and this may mask the effect of mating effort on male feeding time if females are more likely to come into estrus during periods of high dietary quality as has been shown for this study population (Emery Thompson and Wrangham 2008a).

Dominance hierarchy

I used pant-grunt exchanges and decided dyadic agonistic interactions to assign male dominance ranks. I analyzed all interaction data with MatMan 1.1 (Noldus Information Technology, Wageningen, The Netherlands) to arrange the

males in a 12x12 matrix according to their rank (de Vries et al. 1993). This method for constructing dominance hierarchies, which is known as the I&SI method (de Vries 1998), minimizes the number of inconsistencies in the pattern of dyadic wins and losses. The use of this method is only possible for linear hierarchies. I therefore first conducted a test of linearity in MatMan 1.1. Based on 535 decided dominance interactions among the Kanyawara males observed from August 2009 through June 2010, the corrected Landau's index of linearity was h'= 0.83, indicating that the male dominance hierarchy was significantly linear ($P < 0.0001$). After reordering the matrix, there was only one inconsistent relationship involving the former alpha male (MS). Even though the I&SI method only provides a rank order, rather than a rank score as calculated by the Batchelder, Bershad & Simpson (BBS) method (Jameson et al. 1999), it makes fewer assumptions and produces more robust results (de Vries and Appleby 2000). The 12 males observed during this study were assigned ranks 1 through 12, with 1 being the highest (alpha) position and 12 – the lowest. This means that in analyses 'a negative effect of dominance rank' should be read as 'a positive effect of high dominance rank' (i.e. that high-ranking males score higher than lower-ranking males on the variable in question).

Data analysis

To examine the effect of presence of estrous females on male feeding behavior I used linear mixed effects models (LMM) in R version 2.13 (R Development Core Team 2011) with the 'lmer' function of the lme4 package version 0.999375-39 (Bates et al. 2011). LMM allow to control for pseudo-

replication resulting from the repeated observations on the same subjects and also permit unequal sampling of subjects across experimental or observational conditions (Zuur et al. 2009). Time spent feeding (proportion from daily observation time), ripe fruit feeding time (proportion from daily observation time) and ripe fruit contribution to daily dietary composition (proportion of ripe fruit feeding time from total daily feeding time) were logit-transformed prior to analysis to address the fact that values were bounded by 0 and 1 (Warton and Hui 2011). Feeding bout durations (min) and number of daily feeding bouts were log-transformed to achieve normal distribution. I controlled for repeated observation of the same 12 males by specifying the identity of focal subjects as a random effect in the model. To account for the possible temporal auto-correlation of consecutive observations on the same individuals I included an auto-correlation term as a fixed effect in the models (R-code for the derivation of this term written by Roger Mundry). This auto-correlation term was derived following Barelli et al. (2011). First, I ran the full models with maximum likelihood estimation and obtained residuals from it. Second, for each data-point, I calculated the weighted average of the residuals of all other data points, with the weight being equal to time lag to the other data points^{-1}. Time lag between observations was measured in days.

I used maximum likelihood estimation (ML) for evaluating full model fits against the null model (consisting only of the random effect and the auto-correlation term) with the likelihood ratio test (function 'anova' in R). The following fixed effects were considered: male dominance rank (z-transformed ordinal rank),

monthly dietary quality (% non-fig fruits in the diet), mean daily party size, presence of estrous females (and whether they were parous or nulliparous) and the interaction between rank and estrous female presence. Additionally, one model included male rates of aggression and mating behavior (act/hr) as predictor variables. I report fixed effect estimates from the full models using restricted maximum likelihood estimation (REML). Following Barelli *et al.* (2011) we used a Markov chain Monte Carlo (MCMC) simulation (Baayen 2008) with the function 'pvals.fnc' from the R package languageR (Baayen 2011) to obtain reliable P-values for all fixed effects in the models. Likelihood test for fixed effects in mixed models are potentially unreliable especially when the number of cases per level of the random effect (e.g. male identity) is low (Bolker et al. 2008) and the MCMC simulation offers an improved confidence in interpreting the results. Non-parametric analyses were performed in SPSS 19 (© IBM Corporation). All tests were two-tailed. Figures show raw data values.

RESULTS

Mating patterns

Estrous females were available on 33 of 85 full-day focals. On 12 of those days only nulliparous females were available and on 21 days, one of the estrous females was parous. The average daily number of females observed on estrous days was 1.15 (std 0.69) and the average daily number of parous females was 0.53 (SD 0.45). All mating observations were confined to multi-male parties (i.e.

no consortship data were collected although two consortships were inferred to have taken place during the study period).

KCP observers noted a total of 696 copulations during the entire study period between fully swollen (estrous) females and the 12 focal males (129 with nulliparous and 567 with parous females). When all females were considered, the lowest ranking male (12^{th} rank) copulated most (106 copulations; 15.3% of all copulations), out-performing even the alpha male who copulated 105 times and obtained 15.1% of all copulations. When we consider only parous females however, the alpha male was responsible for most matings (103 copulations; 18.2%) and there was a significant negative correlation between rank and the percentage of matings with parous females obtained (r_s = -0.67, N = 12, P = 0.027, Figure 4.1). Despite the fact that only one parous female was in estrus at a time the alpha did not monopolize mating with her. Subsequent observations by KCP field staff showed that only one of the parous females conceived during this study period. However, this conception occurred during a time when she was continuously absent from the main group of male chimpanzees that we followed. Because one of the adult males was absent during the same time and they returned together we consider that the conception occurred during a consortship. None of the behavioral observations reported on here cover her likely time of conception. All days on which I observed male mating effort therefore are considered non-conceptive mating periods.

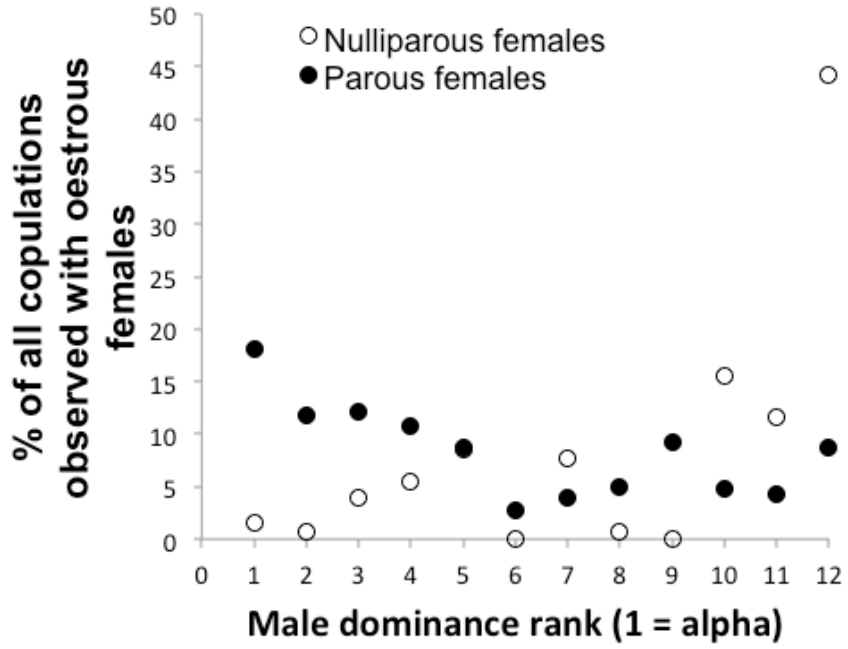


Figure 4.1 Relationship between dominance rank and the relative mating success (% copulations obtained by each male from the total number of copulations with estrous females observed; 696 copulations) of males at Kanyawara (Aug 2009 – June 2010).

Male feeding behavior: general description

During the 85 focal follows in this data-set, males spent a mean of 39.9% (± SE 1.3) of daily observation time feeding (range: 13.4 – 78.0%). They fed on ripe fruit for a mean of 24.9% (± 1.4) of daily observation time (range: 2.1 – 70.5%) and ripe fruits accounted for a mean of 62.9% (± 2.4) of daily feeding time (range: 3.7 – 100%).

The mean daily party size of focal males was 7.2 independent individuals (± 0.5; range: 1 – 15.4). As expected, mean party size was larger on estrous days (estrous days: 10.6 ± 0.6; non-estrous days: 5.1 ± 0.4; Mann-Whitney U

test: z = 5.8, N = 85, P < 0.001). This difference was due to the increased mean number of males in the party, which nearly doubled on estrous days (estrous days: 6.4 ± 0.3; non-estrous days: 3.3 ± 0.5; MWU test: z = 5.8, N = 85, P < 0.001).

I expected that several factors, in addition to the presence of estrous females, would affect male feeding time. Males spent less time feeding in larger parties (r_s = -0.3, N = 85, P = 0.005). Feeding time was also negatively associated with monthly dietary quality (r_s= -0.282; N = 85; P = 0.009). Additionally, mean daily party size was positively associated with monthly dietary quality (r_s = 0.38, N = 85, P < 0.001). Thus in order to distinguish which of these variables affect male feeding time most, in all further multivariate analyses I controlled for gregariousness and availability of preferred fruit species.

Foraging constraints

From the explanatory variables considered in the LMM (log-likelihood ratio test, LRT: χ^2 = 24.63, df = 7, P = 0.0008) the one that contributed most to explaining variation in male feeding time was the presence of parous estrous females. On days when at least one such female was present, males spent less time feeding (Figure 4.2). None of the other variables considered had a significant effect on male feeding time. The interaction between male rank and presence of estrous females was also not significant (Table 4.1) suggesting that the effect of parous estrous female presence did not differ among males at different position in the dominance hierarchy (Fig 4.3).

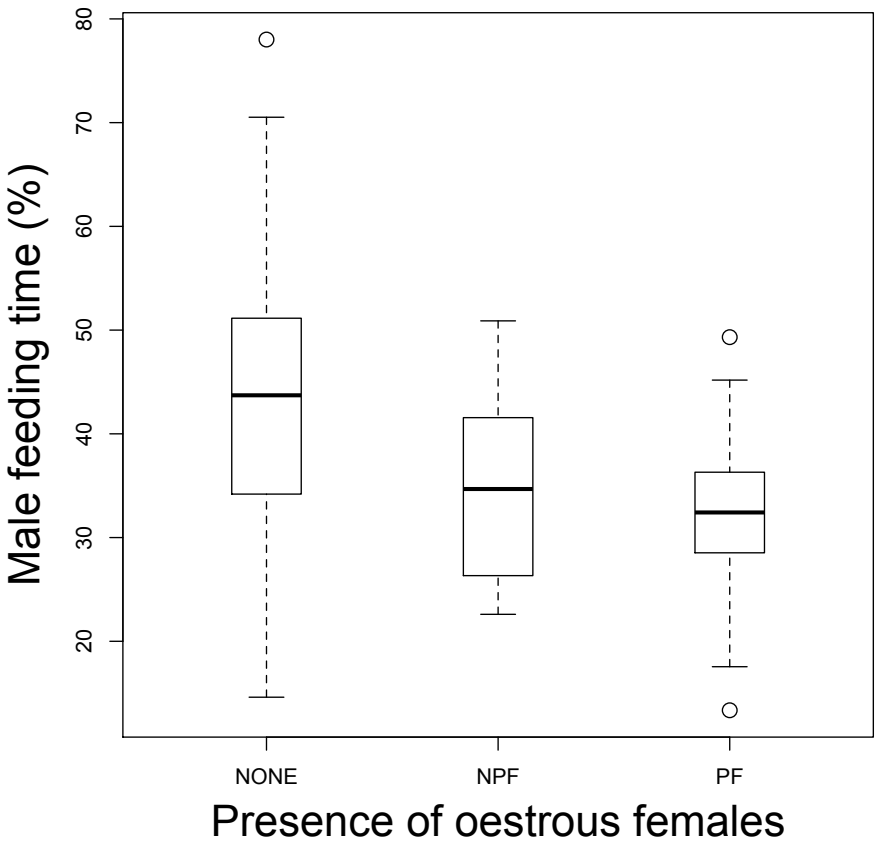


Figure 4.2 Male feeding time on days with and without estrous females. Raw data are shown. In comparison to days on which no females were in estrous (NONE), males fed for significantly less time in the presence of parous estrous females (PF) but not when only nulliparous females were present (NPF). Boxplots show the median (thick line), the interquartile range of the data (the box), 1.5 times the interquartile range of the data (whiskers) and any outliers (circles).

Table 4.1 LMM analysis of factors affecting male daily feeding time (logit-transformed % of daily observation time).

	Estimate	**SE**	**P_{MCMC}**
Intercept	-0.088	0.057	0.120
Parous females	**-0.200**	**0.075**	**0.009**
Male dominance rank	0.063	0.033	0.064
Nulliparous females	-0.123	0.072	0.112
Monthly dietary quality (%NFF)	-0.001	0.001	0.195
Nulliparous females * Rank	-0.069	0.063	0.276
Mean daily party size	0.004	0.008	0.615
Parous females * Rank	-0.015	0.061	0.850
Auto-correlation term	**0.062**	**0.023**	**0.007**

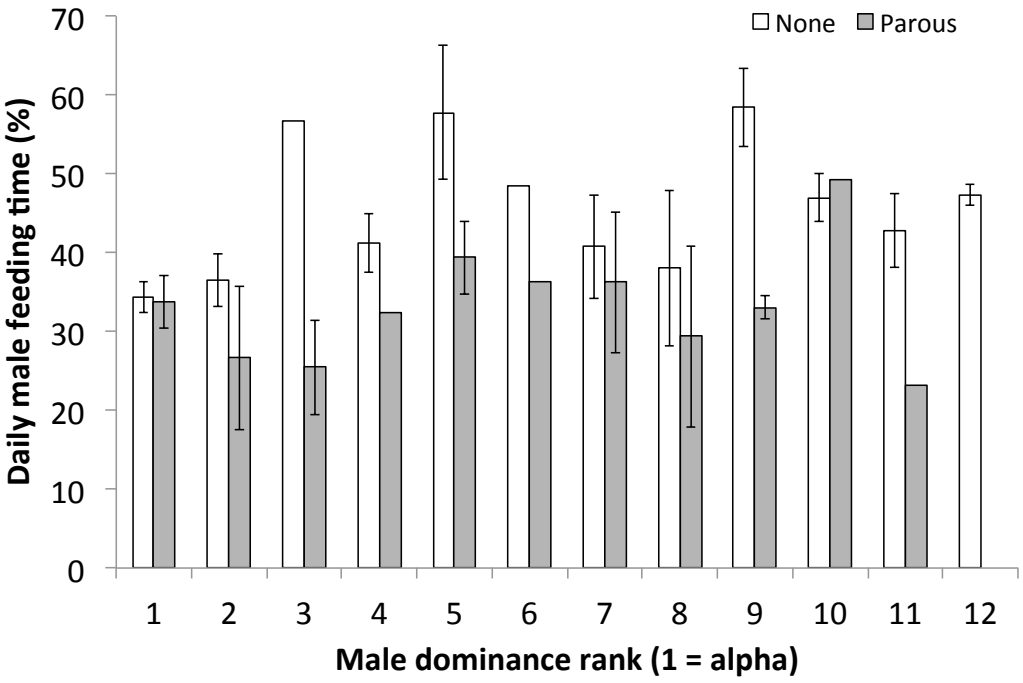


Figure 4.3 The reduction of feeding time on days when parous females were in estrus (gray bars) applied similarly to all males regardless of their dominance rank (interaction rank*parous females: N.S.; see Table 4.1).

The reduction of male feeding time on days when parous females were in estrus was explained by the males feeding in shorter bouts rather than having fewer bouts over the course of the day. The full model for mean daily bout duration was significant when tested against the null (LRT: $\chi^2 = 15.07$, df = 7, P = 0.04). Two factors affected male feeding bout duration. Males in larger parties had longer bouts. When parous estrous females were present however, males fed in shorter bouts (Figure 4.4). None of the other terms considered in the model contributed to explaining the variation in the data (Table 4.2). In contrast, the model for daily number of feeding bouts was not significant when tested against

the null (LRT: χ^2 = 2.94, df = 7, P = 0.9). Thus, none of the factors considered affected the number of daily feeding bouts.

Table 4.2 LMM analysis of factors affecting mean daily duration of male feeding bouts (log-transformed minutes).

	Estimate	**SE**	**P_{MCMC}**
Intercept	0.822	0.052	0.0001
Parous females	**-0.153**	**0.059**	**0.011**
Mean daily party size	**0.015**	**0.006**	**0.028**
Male dominance rank	0.055	0.038	0.091
Nulliparous females * Rank	-0.086	0.049	0.101
Nulliparous females	-0.068	0.055	0.258
Parous females * Rank	0.030	0.049	0.638
Monthly dietary quality (%NFF)	0.000	0.001	0.928
Auto-correlation term	-0.034	0.018	0.128

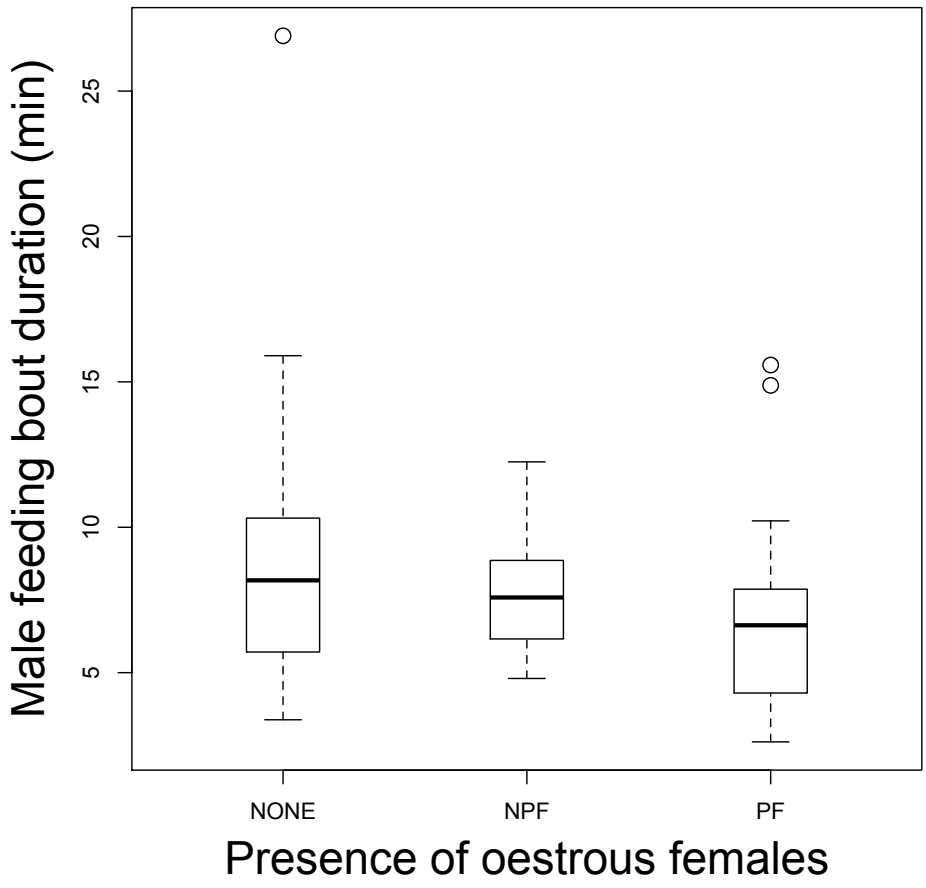
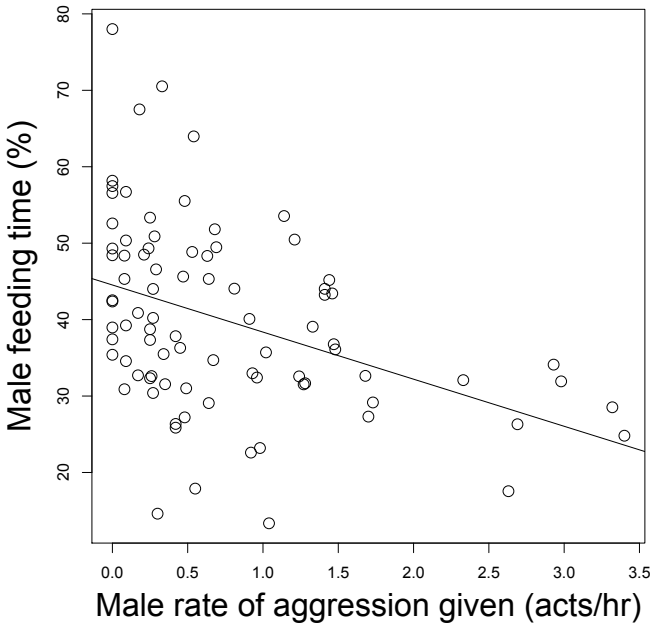


Figure 4.4 Mean daily duration of male feeding bouts in the presence and absence of parous females. Raw data are shown. In comparison to days on which no females were in estrous (NONE), male feeding bouts were significantly shorter in the presence of parous estrous females (PF) but not when only nulliparous females were present (NPF).

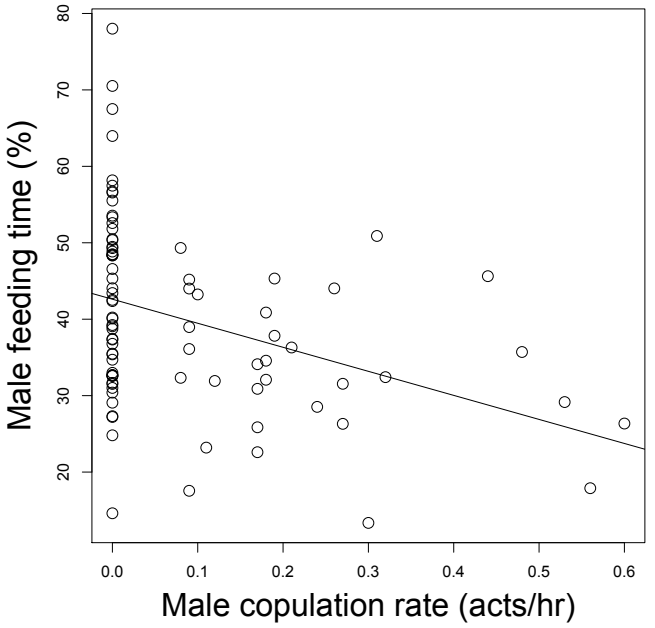
Proximate causes

To determine the proximate causes of the observed reduction of male feeding time in the presence of estrous parous females, I examined the effects of aggression and mating behavior in a separate model. I considered these to be the most likely proximate factors affecting feeding time, since the rates of these behaviors tend to be higher on days when estrous females are available and they

are an important component of male mating effort. I constructed a LMM, in which instead of using the presence of estrous females to quantify male mating effort, I examined the effect of male rates of aggression given and received, and of copulation rates with any females (whether fully swollen or not). I also included male rank in the model, since high-ranking males tend to be more aggressive, which could confound the effect of aggression on feeding time. This full model had a significantly better fit to the data when tested against the null (LRT: χ^2 = 30.92, df = 6, $P < 0.0001$). After accounting for mean daily party size, monthly dietary quality and male rank (all non-significant effects, Table 4.3), male rates of aggression given and copulation rates had a negative effect on male daily feeding time (Figure 4.5). Rates of aggression received did not influence male feeding time (Table 4.3).



(a)



(b)

Figure 4.5 The effect of two measures of mating effort on male feeding time. Rates of aggression given (a) and copulation rates with any females (b) were associated with a significant decrease in male feeding time. Regression line is shown for illustrative purposes only. See Table 4.3 for results of LMM analysis.

Table 4.3 LMM analysis of proximate effects on male feeding time. No differentiation made between mating with parous and nulliparous females.

	Estimate	SE	P_{MCMC}
Intercept	-0.062	0.054	0.246
Copulation rate (with all females)	**-0.446**	**0.174**	**0.011**
Aggression given	**-0.079**	**0.032**	**0.017**
Male dominance rank	0.042	0.031	0.148
Aggression received	-0.180	0.152	0.216
Monthly dietary quality (%NFF)	-0.001	0.001	0.302
Mean daily party size	0.005	0.007	0.458
Auto-correlation term	**0.061**	**0.021**	**0.004**

To further elucidate the relationship between mating behavior and male feeding time, I examined the effect of copulation rates with parous and nulliparous females in a separate model (LRT: $\chi^2 = 20.06$, df = 7, P = 0.0001). Rates of aggression given were still associated with a significant decrease in male feeding time, but the effects of copulation rates with females were no longer significant. Between the two measures of mating behavior, however, rates of copulation with parous females approached statistical significance in their negative impact on male feeding (Table 4.4)

Table 4.4 LMM analysis of proximate effects on male feeding time. The effect of rates of copulation with parous and nulliparous females are considered separately.

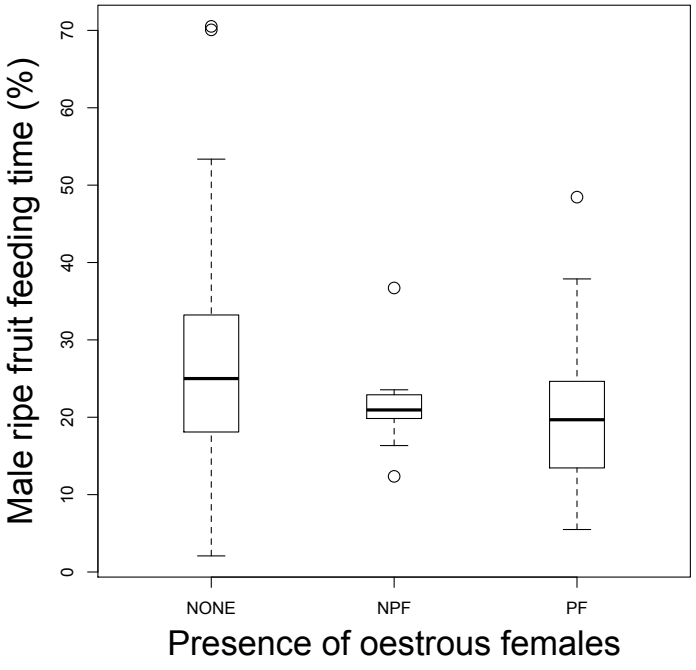
	Estimate	SE	P_{MCMC}
Intercept	-0.053	0.055	0.321
Aggression given	**-0.083**	**0.032**	**0.016**
Copulation rate (parous females)	-0.396	0.211	0.051
Copulation rate (nulliparous females)	-0.221	0.140	0.133
Male dominance rank	0.042	0.031	0.158
Aggression received	-0.197	0.155	0.191
Monthly dietary quality (%NFF)	-0.001	0.001	0.320
Mean daily party size	0.004	0.007	0.569
Auto-correlation term	**0.061**	**0.022**	**0.007**

Dietary compensation

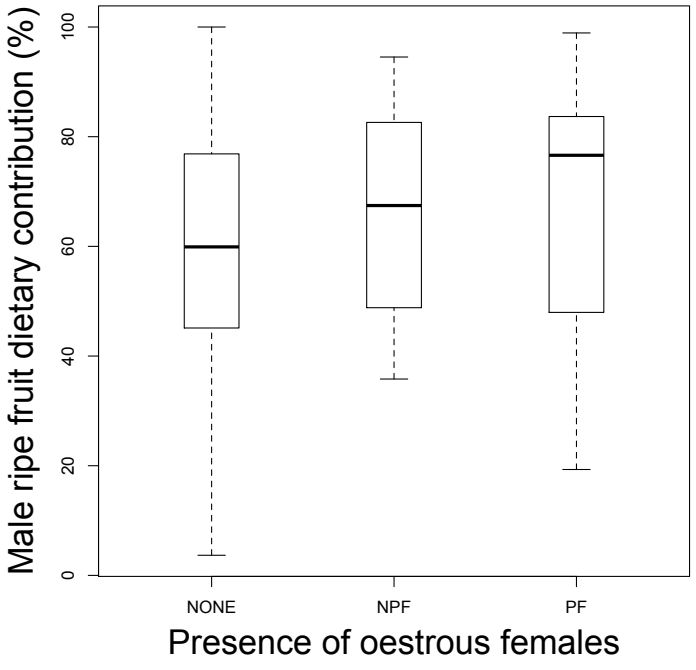
Since chimpanzees utilize a variety of foods, a reduction in total feeding time might represent maintenance of the same caloric intake if males increase feeding time on ripe fruit, their preferred and highly nutritious food. To test this possibility I examined the effect on ripe fruit feeding time of the same factors described earlier. I constructed two models to assess the prediction that males compensate for their reduced feeding time when parous females are in estrous by focusing their foraging on ripe fruit. The first model used the proportion of time spent feeding on ripe fruit (from daily observation time) as a response variable,

while the second used the contribution of ripe fruit to daily dietary intake (measured in time spent feeding). Neither of these was significantly better than the null model in explaining variation in the response variables (time spent feeding on ripe fruit, LRT: $\chi^2 = 6.96$, df = 7, P = 0.43; ripe fruit dietary contribution, LRT: $\chi^2 = 9.1$, df = 7, P = 0.25).

In sum, these results show that male chimpanzees do not engage in behavioral compensation on mating days: males did not increase the quality of their food intake by concentrating their foraging effort on ripe fruit when faced with the time-constraints of mating effort (Figure 4.6).



(a)



(b)

Figure 4.6 Ripe fruit consumption was not affected by the presence of estrous females: (a) time spent feeding on ripe fruit (% from daily observation time) and (b) ripe fruit daily dietary contribution (% from daily feeding time). Raw data are shown.

DISCUSSION

This study provides the first evidence that mating effort constrains male foraging in chimpanzees. In the presence of a parous estrous female males reduced their feeding time by an average of ca. 26% relative to days on which no females were in estrus. This result held after controlling for the effects of scramble feeding competition (as assessed by mean daily party size), availability of preferred fruit (mean monthly non-fig consumption score) and male social status (ordinal dominance rank). Increased rates of aggressive and sexual behavior were significantly associated with reduced feeding time among males, providing a proximate explanation for the reduction of male feeding time on days on which parous females were in estrus. There was no evidence that changes in dietary composition helped to offset the reduction in energy intake inferred from activity budget data: ripe fruit feeding time did not increase when mating effort constrained total time available for feeding. Taken together these results suggest that mating effort involved substantial energetic shortfalls in a promiscuous non-seasonally breeding primate, even without accounting for a concomitant increase in energy expenditure due to the higher rates of male aggression during periods when highly attractive females were in estrus.

The scale of these short-term costs of mating effort is particularly striking since none of the parous females towards which males directed mating effort conceived during the periods behavioral data were collected. Because male chimpanzees show sensitivity to a female's current fecundity (Emery Thompson and Wrangham 2008b) we can expect that reproductive competition for parous

females during conceptive cycles would have even greater impact on male feeding time than this study showed. The effect of the availability of multiple highly fecund females on male activity budgets also remains an important question for future studies. During this study only one parous female was in estrus at any one time and she was thus the focus of attention of all the males in the mating party. If multiple females are in estrus simultaneously, a situation similar to what is seen among seasonal breeders, male feeding time can either be constrained further (because their effort is multiplied by the number of females available for mating) or the feeding constraints of mating effort might be reversed (because male attention is split among different females and there is less opposition from other males). The chimpanzee mating system therefore may yet offer further insight into how breeding seasonality mediates levels of male-male competition and into the proximate causes of the costs of male mating effort in different reproductive contexts.

Documenting the costs or benefits of chimpanzee consortships also remains an important challenge for further study. While males may not have to incur feeding constrains when alone with an estrous female as much as they do in multi-male mating parties, the fact that they are trying to avoid being seen by other members of their community means they have to spend time in areas of the territory which are peripheral and likely poor in fruiting trees. Forming a consortship with an estrous female, a strategy that provides a male with the highest level of paternity certainty, may also prove to be the most metabolically costly form of mating effort.

While the effect of estrous parous female presence on male feeding time was negative and significant across all males, there was no evidence that high-ranking males were more constrained in their foraging than lower-ranking individuals during mating periods. The alpha male in particular did not decrease his feeding time at all (Figure 4.3), even though he did feed less than lower-ranking individuals overall (as suggested by the near significant effect of male rank in LMM analysis, Table 4.1). This is puzzling given that high-ranking males are generally more aggressive than lower-ranking males, particularly so during periods when mating opportunities are available (Chapter 5) and this study showed that rates of aggression are negatively associated with feeding time. Furthermore, during this study, and as found in general, high-ranking males obtained more copulations with parous females than lower-ranked individuals, the alpha male particularly so (Figure 4.1). This suggests that the tactics for securing copulations employed by high-ranking males were more cost-effective in securing copulations or that these data are atypical of chimpanzee mating patterns in general. I will thus discuss five possible explanations for the similar magnitude of foraging constraints across the dominance hierarchy shown in this study.

First, the lack of the predicted relationship between male rank and feeding time during mating periods may be explained by the quality of the estrus cycles I observed. It is possible that competition for matings did not reach its maximal possible intensity during this study period since none of the data on mating effort come from periods that led to a conception. If males were competing over a

fecund female, which were to conceive, high-ranking males might increase their effort more and it is only at such times that they would incur greater reduction in their feeding time, similar to what has been reported in seasonal breeders (Higham et al. 2011). The scenario in which the males were aware of the current fecundity of females and did not compete very intensely during the period of this study however fails to explain the observation that feeding costs were significant only when parous females were in estrus, and not nulliparous. The increased rates of aggression on parous days among high-ranking males (Chapter 5) also suggest that males did not disregard the potential conceptive value of the matings they obtained, even though no infants were eventually produced. A longer study, which would quantify the foraging costs of male mating effort during conceptive cycles, will help resolve this question.

Second, high social status may allow males to secure matings at a lower opportunity cost, while lower-ranking individuals have to incur proportionately higher costs (reduced feeding time) for obtaining a smaller mating share with highly attractive parous females. The existence of long-term dominance relations among males, which are maintained even outside periods of direct mating competition might account for this observation. At the same time the fact that high-ranking males tended to feed less overall, regardless of the presence of mating opportunities suggests that high-ranking males continuously invest in status competition and maintenance at the cost of feeding effort. This may be a strategy to dissipate costly reproductive effort over a longer time-frame so as to reduce the negative impact of extreme food limitation and increased energy

expenditure, which can have a dramatic effect on both body condition and patterns of mortality among seasonally breeding species such as the rhesus macaque (Bercovitch and Nurnberg 1996; Hoffman et al. 2008; Higham et al. 2011). Maintaining relatively low-cost but continuous reproductive effort may be one way for high-ranking male chimpanzees to maintain their social status for multiple years and sometimes for over a decade. This will be particularly beneficial from the perspective of an alpha male in a primate with slowly reproducing females such as the chimpanzee (Emery Thompson et al. 2007a).

Third, the relative benefits to the high-ranking males of incurring extremely high costs may not be as large in chimpanzees, as in other species. A life-history perspective can explain inter-specific differences in reproductive strategies among non-seasonal breeders. Even though estrous females are available year-round, the allocation reproductive effort differs drastically between male chimpanzees and elephants, for example. The energetic costs of musth (the breeding condition) in elephants are so high that bulls are not able to maintain it for long. Old males manage several months at a time and as a result they enjoy considerable reproductive benefits. However, from the elephant males' point of view the annual musth period can only work (in terms of fitness outcomes) if there are enough females in estrus at the time when they happen to come into musth. Since musth periods are not synchronized among males, and some estrus females are available in most weeks of the year (Poole et al. 2011) the timing of this male reproductively active period is important. During any given week of the year there are more estrus females available for fertilization to a

male elephant, in comparison to a male chimpanzees (e.g. 0-10 confirmed elephant conceptions per week, median = 1, at Amboseli, Kenya: Poole et al. 2011 vs. 0 - 1 confirmed conceptions per week, mean = 0. 02; median = 0, at Kanyawara, Uganda: this study). Hence a high-cost strategy of male effort can be beneficial for elephants when sufficient number of females is available in the population but chimpanzees need to 'spread out' their mating effort to adjust over the long-term to the much lower number of females available for fertilizing at any given time in their communities. The fact that two so different male strategies (elephant musth vs chimpanzee mating year-round) have evolved in mammals that are both very long-lived and have females who do not show distinct breeding seasonality suggests that multiple factors can shape the optimal strategy a male can adopt for maximizing his reproductive success.

Fourth, male-female relations can also affect the relative costs that high-ranking males incur during mating competition. A factor that may help explain why the male chimpanzees who obtained most copulations during this study did not feed less than the other males when competing is that male chimpanzees systematically dominate and direct aggression towards females even when they are not in estrus (Muller et al. 2007). Such male-female aggression acts as indirect and long-term sexual coercion and effectively limits female mate choice during their peri-ovulatory periods (Muller et al. 2009; Muller et al. 2011). This aggressive conditioning of females may allow high-ranking males to be more efficient in their mate-guarding behavior and obtain high mating rates without suffering extreme reductions in their feeding time. To test this hypothesis, it may

be useful to consider male costs of reproduction in species in which males do not practice sexual coercion. Unlike chimpanzees, rhesus macaques are a female-philopatric species and females can exert significant impact on male reproductive success via their mating preferences (Manson 1992). When females show preference towards lower-ranking males, higher-ranking males direct aggression towards them but that does not alter the females' choice (Manson 1994). If this pattern of interactions during macaque breeding seasons is common enough it may help explain why male feeding time is negatively associated with male rank (i.e. high-ranking males feed less) in rhesus macaques during their breeding season (Higham et al. 2011) but not among male chimpanzees (this study). Differences in reproductive strategies between and within species, in addition to breeding seasonality, may thus also affect how the variation in costs of mating effort within and between individual males.

Finally, male-male relations can further contribute to explaining cross-species variation in costs of male mating effort. In species where dominant males incur higher costs of mating effort than subordinates such as rhesus and Japanese macaques (Matsubara 2003; Higham et al. 2011) there is a distinct lack of male-male bonding and cooperation. Conversely, male chimpanzees are highly social and cooperate extensively (Muller and Mitani 2005) even when competing for mates (Watts 1998; Duffy et al. 2007). To some extent the existence of such affiliative relationships may allow the intensity of competition to be toned down among chimpanzees, relative to species without male-male bonding.

Further studies of the costs of male mating effort in this species in a variety of contexts as outlined above will help resolve these remaining questions. Comparative analyses of the relationship between costs of mating effort on the one hand, and sea sonality of reproduction, male and female mating tactics as well as the prevalence of male-male bonding will allow testing some of the ideas raised here in a broader mammalian perspective.

CHAPTER 5

The energetic costs of sex and violence: urinary C-peptide analyses of mating effort and dominance competition in male chimpanzees

ABSTRACT

Male-male mating competition in many polygynous and highly sexually dimorphic species that do not mate promiscuously incurs significant energetic costs. As a result male reproductive success is strongly dependent on energy status. The degree to which energy may constrain reproductive effort in promiscuously mating species is less appreciated, partly because detecting energetic costs of aggression and mating effort may be difficult if such costs do not produce noticeable changes in body mass. In this study I test the hypothesis that male competitive dominance is energetically constrained in chimpanzees, a species in which aggression plays an important role in obtaining status and access to females, and yet the energetic impact of agonism is unknown. Using behavioral observations and non-invasive measurements of urinary C-peptide levels (UCP) I show that male energy balance was negatively affected by aggressive behavior and time spent traveling. Males in large parties have lower UCP levels. Because parous estrous females attracted large parties and caused an increase in male aggression rates, males had low UCP levels during periods of mating competition. Abundance of high-quality food in the habitat allowed males to increase energetic allocation to aggressive behavior, independently of the presence of estrous females. In sum, I provide several lines of evidence in

favor of the argument that energy balance is important for male intrasexual competition in a promiscuous primate.

INTRODUCTION

Male reproductive effort, even in the absence of paternal care, can be costly (Post and Greenlaw 1982; Key and Ross 1999; Ellison 2003). A significant proportion of such costs result from mating effort, in the form of increased metabolic demands of male-male aggression (Clutton-Brock et al. 1979; Marler and Moore 1991) or display effort (Vehrencamp et al. 1989), as well as the constraints imposed by different mating tactics on male feeding behavior (Alberts et al. 1996; Pelletier et al. 2009; Brivio et al. 2010; Chapter 4). These reproductive costs are expected to mediate the relationship between male condition (individual quality) and reproductive success (Wilson and Nussey 2010). The strength of that relationship will depend on the severity of such costs. In this study we measure the energetic costs of male-male competition in chimpanzees to test the hypothesis that male competition in this species has a substantial effect on individual energy balance and thus that male competitive ability is constrained by access to energetic resources and by physical condition.

Male condition is particularly important in highly polygynous species, in which contest is the main mode of mating competition. In pinnipeds and ungulates with such mating systems male competitive ability is tested in numerous challenges and fights. Males who are in the best condition (defined by their size and/or energy reserves) tend to win most of these contests and

maintain their mating effort the longest (Clutton-Brock et al. 1982; Galimberti et al. 2007). Thus, such males succeed in siring the greatest number of offspring (Fabiani et al. 2004). High degree of polygyny is however not the only type of mating system in which male condition has a major impact on reproductive success. Condition is also a key determinant of male fitness in black bears, *Ursus americanus*, which are solitary but nevertheless compete over females when they locate them (Kovach and Powell 2003). Likewise males in species living in multi-male, multi-female groups engage in 'endurance rivalry' for copulations over extended mating seasons (e.g. rhesus macaque, *Macaca mulatta*: Bercovitch 1997; Higham et al. 2011). Male condition also matters in species with a scramble competition mating system: among male North American red squirrels, *Tamiasciurus hudsonicus*, energy expenditure increases during mating seasons and is at its highest when such periods coincide with high food abundance (Lane et al. 2010).

There are two main reasons why male condition declines during periods of male-male competition: male feeding is reduced, and rates of aggression escalate. Feeding is compromised because finding and monitoring fertile females places a time-constraint on the males' ability to feed uninterrupted. Aggression escalates because aggressive interactions are the main way in which male conflicts are resolved, dominance hierarchies are established or maintained and ultimately, access to fertile females is negotiated. Aggressive behavior is physically exerting. It carries significant metabolic costs in a variety of species as shown by increased oxygen consumption (Briffa and Sneddon 2007). Together, a

reduction in energy intake because of constrained foraging and/or an increase in energy expenditure on aggression explain the loss of male body mass in many different species during their respective breeding seasons, regardless of the type of mating system involved (Clutton-Brock et al. 1979; Komdeur 2001; Matsubara 2003; Isaac 2005; Low 2006; Pelletier et al. 2006; Galimberti et al. 2007; Pelletier et al. 2009; Sailer and Fietz 2009; Schubert et al. 2009; Ancona et al. 2010; Huchard et al. 2012). In sum, superior body condition often allows males to invest more energy in male-male contest competition for both status and receptive females.

Ecological conditions may therefore play an important role in determining the outcome of male-male competition. When direct competition over feeding resources can result in improved nutritional state, animals tend to become more aggressive if hungry and during periods of food shortage (Ducey and Heuer 1991; Janson and Vogel 2006). Conversely, animals tend to reduce their aggressive interactions when energy resources are limited and direct competition cannot improve access to food. This applies both to intra-group and inter-group aggression in primates, as shown in experimental studies of food-deprived captive and free-ranging rhesus macaques (Southwick 1967; Loy 1970; Marsden 1972). Experimentally-restricted access to carbohydrates led to a significant decrease in aggressive behavior and overall activity among Argentine ant, *Linepithema humile*, colonies (Grover et al. 2007) and in territorial defence in blue-throated humming-birds, *Lampornis clemenciae* (Powers and McKee 1994). Conversely, territorial defence in cooperatively breeding pied babblers, *Turdoides*

bicolor, increased after food-supplementation even outside their breeding season (Golabek et al. 2012). These experimental studies demonstrate an important link between energy status and competitive behavior.

Additional support for the hypothesis that males have limited resources to invest towards mating effort comes from studies that demonstrate that males selectively compete for females who are of higher reproductive value. Male mating preference for females with greater reproductive potential has been demonstrated among primates (Alberts et al. 2006; Muller et al. 2006; Parga 2006; Emery Thompson and Wrangham 2008b; Gomez et al. 2012) and ungulates (Preston et al. 2005; Mainguy et al. 2008). High-ranking males in many species tend to focus their mating effort on more fertile females and this allows lower-ranking males to sire offspring with less desired females (Newton-Fisher et al. 2010). While such patterns in mating and reproductive skew are open to different interpretations and are likely mediated by a number of factors (Clutton-Brock 1998; Henzi et al. 2010), they also invariably involve prioritising energy expenditure towards those mating opportunities most likely to produce maximum fitness returns.

Against to the hypothesis that male mating effort is energetically constrained, there are some studies that have failed to identify an increase in male costs during periods of mating competition. Dominant male sifakas, *Propithecus verreauxi*, for example, do not incur foraging costs when mate-guarding and show only a slight increase in their agonistic behavior (Mass et al. 2009). Although these males have higher levels of glucocorticoids during the

mating season (Fichtel et al. 2007), whether that results from increased energy expenditure is not clear. Similarly, no evidence was found that male mating effort carries energetic costs among moustached tamarins, *Saguinus mystax* (Huck et al. 2004). During consortships males did not become more aggressive, nor did they reduce their feeding time. They were however more conspicuous while following receptive females, potentially increasing their vulnerability to predators (Huck et al. 2004). Male Alpine ibex, *Capra ibex*, improve their survivorship during the breeding season by significantly reducing aggressiveness (Willisch and Neuhaus 2010). Time spent in agonistic interactions and the number of fights were lower during the rutting period and subordinate males were reluctant to challenge dominants. A pre-established and stable dominance hierarchy thus allowed a reduction of the energetic costs of male mating effort (Willisch and Neuhaus 2010).

Finally, there are also studies that argue against an ecologically-mediated model of male competitive ability. Variation in ripe fruit availability did not affect intragroup agonism among spider monkeys (Asensio et al. 2008) or inter-group aggression in blue monkeys (Lawes and Henzi 1995). Similarly, although boundary patrols in chimpanzees are energetically costly (Amsler 2010) there is no evidence that they are limited by ripe fruit shortages at Ngogo, Kibale National Park (Mitani and Watts 2005). Although these observations suggest that food scarcity does not necessarily restrain male agonism, it is worth noting that they do not rule out a link between food abundance and competitive dominance. The chimpanzees at Ngogo, an unusually large community numbering more than 150

individuals, enjoy stable year-round production of high-quality foods (Watts et al. 2012), particularly in comparison to Kanyawara, some 10 km to the north (Potts et al. 2011). With such continuously high levels of food availability, chimpanzees at Ngogo may never have to restrain their competitive drive in order to save energy. Indeed, the Ngogo chimpanzees have been exceptionally successful in their inter-group interactions, killing many of their neighbors and annexing parts of their territory (Mitani et al. 2010).

The experimental studies reporting a decrease in aggressive behavior during food limitation were carried out under extreme conditions that are uncommon in wild populations. For example, in one of these experiments a reduction in aggression among rhesus monkeys was noted only after food supply was cut from 75% of baseline levels to 50% (Southwick 1967). Under these conditions high-ranking males were always the first to feed and the lowest ranking individual obtained little to no food. One of these subordinate individuals lost 35% of its body mass and was removed from the experiment to be hand-fed back to health (Southwick 1967). In view of the data from Ngogo and other primate field studies (Lawes and Henzi 1995; Mitani and Watts 2005; Asensio et al. 2008), it is uncertain whether subtle variation in food abundance, as seen under natural foraging conditions, would have the same impact on male competitiveness.

In summary, despite strong evidence from a variety of study systems supporting the importance of male condition for reproductive success and the pervasiveness of energetic costs of mating effort, there are also a number of

examples that suggest alternative low-cost male reproductive strategies. Significant inter- and intra-specific variation in mating costs, and thus in the importance of male condition for male reproductive success, probably exists and therefore needs an explanation.

In this study we provide a test of the energy-constraint hypothesis in chimpanzees, a species, the social and mating systems of which allow the formulation of contradictory predictions about the importance of male condition for reproductive success. In favor of the energy-constraint hypothesis, male mating and reproductive success in chimpanzees is predicted relatively well by dominance rank (Boesch et al. 2006; Wroblewski et al. 2009; Newton-Fisher et al. 2010). Aggression plays a key role in achieving and maintaining high social status (Nishida 1983; de Waal 1986; Goodall 1986). Periods of mating competition are characterized by increased male-male and male-female agonism and aggressive sexual coercion of females is prevalent (Muller et al. 2011). High-ranking males often engage in mate-guarding and such activities are suggested to carry important trade-offs in terms of reduced feeding time (Tutin 1979; Watts 1998). Indeed, the presence of highly attractive (parous) females was associated with significant decrease in male feeding time and rates of aggression given were negatively associated with feeding time (Chapter 4). While the data on feeding suggests that costs apply equally across the dominance hierarchy, without taking energy expenditure into account it is not possible to rule out the alternative that significant differences in costs of mating effort exist between high and low

ranking males. Mating competition therefore is expected to incur significant energetic costs in this species and these costs could be rank-related.

Preliminary support for the hypothesis that mating effort is costly to male chimpanzees also comes from a study of male cortisol production in relation to dominance status. The highest-ranking males who are most aggressive and most reproductively successful have significantly higher cortisol levels than their subordinates (Muller and Wrangham 2004b). However, although cortisol levels are associated with glucose mobilization, they can also be elevated by psycho-social stress alone (Sapolsky 1994; Abbott et al. 2003; Creel and Sands 2003).

Preliminary data from Kanyawara indicate that dominant males also have the lowest levels of urinary C-peptide (Emery Thompson et al. 2009). Urinary C-peptide (UCP) is a measure of insulin production that provides a complementary measure of metabolic stress. Taken together, the cortisol and the UCP data suggest that high levels of aggression, typical of dominant males, carry significant energetic costs and that these costs may be particularly high during periods of mating competition. The exact relationship between male mating effort and UCP has not yet been established in chimpanzees, however.

Finally, male chimpanzee hunting of colobus monkeys increases during periods of high dietary quality, after controlling for the confounding effects of male party size and presence of estrous females (Gilby and Wrangham 2007). This suggests that in periods of nutritional surplus, males would be more likely to expend energy on physically exerting and risky behaviours. A similar mechanism may thus also modulate male intra-specific aggression.

Against the energy-constraint hypothesis, chimpanzees do not breed during an extended mating season since estrus synchrony among females is low (Matsumoto-Oda et al. 2007). Compared to males of seasonally breeding species that engage in endurance rivalries for several months at a time and suffer high energetic costs (Higham et al. 2011), chimpanzee males may be better able to pace themselves over the shorter periods when sexual opportunities are available. This would dissipate the costs of mating effort and thus limit their impact on condition. Additionally, although females do not come into estrus at the same time, they are more likely to do so during periods of high dietary quality (Emery Thompson and Wrangham 2008a). During such periods males may be able to sustain high mating effort despite any time constraints imposed on feeding activity by ingesting more calories per unit time than during periods when high-quality fruit are not available. So even though we found that males fed less when parous females were in estrus (Chapter 4), it is also plausible that their energy balance remained unaffected.

Although aggression is key in obtaining and maintaining dominance rank in male chimpanzees, body size is not. There are no consistent differences in body mass among males of low and high rank (Pusey et al. 2005). Relatively small males may sometimes achieve alpha status by strategically building alliances rather than by the use of physical force alone (Riss and Goodall 1977; Goodall 1986; Foster et al. 2009). Maintaining alliances is important not just in status competition but also in securing access to estrous females (Watts 1998; Duffy et al. 2007). The high costs of mating competition observed in species with

weak to non-existent male-male bonding such as rhesus macaques (Higham et al. 2011) and southern elephant seals (Galimberti et al. 2007) may thus be reduced in species, in which male-male bonding, cooperation and even some reproductive tolerance are as important as they are in chimpanzees (Muller and Mitani 2005; Gilby and Wrangham 2008).

In this study I examine the energy-constraint hypothesis by comparing male UCP production during periods of high and low male-male competition, with the prediction that UCP levels are lower on days with high agonistic engagement and specifically on days when parous estrous females are available. I further predict that high-ranking males will show greater energetic costs on such days due to their increased investment in agonistic competition. Alternatively, lower-ranking males will suffer greater energetic costs on such days, if the social status of high-ranking males allows them to secure copulations at lower energetic cost.

I also probe the relationship between UCP levels and male behavioral activity in order to establish the cause of differences in UCP levels on days with low and high male-male competition. I predict that (1) behaviors related to energy acquisition (i.e. feeding and, specifically, ripe fruit consumption) are positively associated with UCP, and that (2) behaviors leading to energy expenditure (i.e. travel and aggression) are negatively associated with UCP. Finally, I also examine the relationship between habitat-wide abundance of drupe (non-fig) fruit (rich in carbohydrates, high-quality food) and male rates of aggression. I predict that if male competitive ability is energetically constrained, males will increase their aggressive behavior during periods of high drupe fruit abundance – whether

that competition occurs for mates or for status. Conversely, if male-male aggression is important during periods of food scarcity, I expect that aggression would be negatively associated with drupe fig abundance.

By analyzing the behavioral and physiological data at several levels (see Data analysis) I further evaluate the effect that different intensities of urine sampling have on the results and conclusions in studies that employ urinary C-peptide (UCP) to assess the energetic balance of wild primates.

METHODS

Study site and subjects

I studied male chimpanzees between Aug 2009 and June 2010 at the Kanyawara long-term site (Wrangham et al. 1996), Kibale National Park, Uganda. Nine adult (≥15yo) and 3 late-adolescent (14 yo) males were subject to behavioral observations and non-invasive urine sampling. The Kanyawara community is fully habituated to human observers and has been studied since 1987, when the Kibale Chimpanzee Project was established (Wrangham et al. 1991; Chapman and Wrangham 1993; Wrangham et al. 1996). The history of research at this site and the ecology of Kibale National Park are summarized by Watts (2012).

Behavioral observations

I recorded chimpanzee activity with the CyberChimps behavioral sampling software developed and customized by Paco Bertolani. This program ran on a Samsung i200 smart phone and allowed noting changes in focal activity to the

nearest second. I used continuous focal animal sampling (Altmann 1974) to record whether males were resting, feeding, traveling, or engaging in other behavioral activities. I also noted all aggressive interactions (vocal and non-vocal displays, charges, chases and physical attacks) and copulations, in which focal subjects were involved. Rates for both types of behavior were calculated per hour of observation time for each focal follow.

I restricted analyses to focal follows that were either complete nest-to-nest observations or were at least 8 hrs long. The median duration of focal follows in the dataset was 11.57 hrs (mean 11.03 ± SD 1.42 hrs; range: 6.7–12.9 hrs, N=85 days). The choice of a focal individual was semi-random because a pre-determined target was not always located on the day of the follow and sampling was flexible to allow observations on chimpanzees that were rarely seen.

Party size and female reproductive state

I scored party size of the focal male throughout observations at 15-min intervals by recording the number of adult and adolescent individuals within 50 continuous meters of each other. I noted the reproductive state of all cycling females by assigning a score to the tumescence of ano-genital swellings (1 = deflated; 2 = partially swollen; 3 = fully swollen, swelling tense and shiny). Only fully tumescent females are considered as ‘estrous’ because it is during this stage of their cycle that they are most attractive to males and the likelihood of mating is highest (Emery and Whitten 2003; Deschner et al. 2004; Emery Thompson 2005; Emery Thompson and Wrangham 2008b). All analyses involving focal behavior used party size scores recorded concurrently during the

focal follow by the same observer. Separately, in some additional analyses I also use mean monthly party size values derived from KCP records that were recorded following the same protocol but by multiple observers.

Dominance hierarchy

I calculated male dominance ranks in MatMan 1.1 (Noldus Information Technology, Wageningen, The Netherlands) (de Vries et al. 1993; de Vries 1998) based on 535 decided dominance interactions (pant-grunt exchanges, displacements, dyadic aggression) among the Kanyawara males recorded from August 2009 through June 2010 (see Chapter 4). The corrected Landau's index of linearity was h'= 0.83, indicating significant linearity of the hierarchy ($P < 0.0001$). In linear-mixed model analyses I entered male dominance rank as a z-transformed score.

Food availability and diet quality

For the model examining effects on daily energetic balance I used focal data on ripe fruit intake to measure diet quality on the day of observation (% ripe fruit feeding time from total feeding time). The model for monthly C-peptide levels included the monthly proportion of ripe fruit in the diet observed throughout the home-range of the Kanyawara chimpanzees since this dietary metric has previously been show to have the greatest effect on male C-peptide variation at this study site (Emery Thompson et al. 2009). To examine how diet quality affected male rates of aggression, I calculated the percentage of non-fig fruit (NFF) in the diet on 13 days preceding focal observation and the day of focal observation (a 14-day index of NFF consumption). This behavioral measure of

preferred fruit consumption correlates strongly with data on food availability in the forest (Wrangham et al. 1996) and has strong effects on chimpanzee physiology and behavior at Kanyawara (Gilby and Wrangham 2007; Emery Thompson and Wrangham 2008a). To calculate the monthly-ripe-fruit and the 14-day-NFF consumption score I analyzed a total of 12,742 feeding records (15-min instantaneous scans) collected on 331 days between July 2009 and June 2010 in the home-range of the Kanyawara chimpanzees (Gilby et al. 2010).

Urine sampling & analysis

Urine samples were collected opportunistically by myself and Kibale Chimpanzee Project field assistants by either placing a plastic bag under a urinating chimpanzee or collecting urine off vegetation after the event. Samples were collected only if they have not been contaminated with feces. Samples were pipetted from vegetation only if it was clear that no other chimpanzee had urinated in the same spot recently. Any debris in the samples was removed with a pipette each evening in a field laboratory. All samples were frozen at -20C° the same evening in a propane freezer (i.e. within a maximum of 14 hrs from time of collection). They were transported on ice-packs to University of New Mexico where Melissa Emery Thompson conducted all laboratory analyses. C-peptide was assayed with commercially available kits. Briefly, assays were performed using the Millipore™ Linco RIA kit, with all samples assayed at an initial dilution of 1:2. Sensitivity of the assay was approximately 100 pg/ml. Intra-assay average coefficient of variation (CV), calculated as the average CV of duplicate determinations was 7.1%. Inter-assay CV (N=15) was 10.4% for low samples

and 5.0% for high samples. C-peptide concentrations were indexed via creatinine concentration measured in triplicate with the Jaffe reaction (Taussky 1954). Eleven urine samples with low creatinine values (< 0.05 mg/ml) were discarded from analysis because of the risk of obtaining over-inflated C-peptide values.

In the full data-set of 695 urine samples (Table 3.3) there were 5 extreme outliers (with UCP concentration more than 5 times the standard deviation of the mean (see Chapter 3). These outliers come from urine samples collected before noon from the 3 youngest males in the community (15-16 years old) during the months of November and December 2009. After removing the outliers (Chapter 3), the resulting data-set contained 690 urine samples. UCP values were log-transformed for parametric analyses but not for non-parametric tests.

Statistical analyses

I used several approaches to analyze data. Because of the significant diurnal variation in UCP levels (Chapter 3), I first conducted a set of conservative tests, for which I averaged the greatest number of urine samples available to derive single means for each male. In analyses based on monthly mean UCP values I obtained those by averaging the individual male means for each month. In tests comparing male means I used matched-samples non-parametric tests (Wilcoxon signed rank test or Friedman test) and compared male mean UCP values under one of the conditions to the mean values of the same male under the other condition (e.g. days with or without parous estrous females). The sample size for such tests differs depending on how many males were observed in each of the observational conditions of interest. Spearman rank correlations

and simple linear or multiple regressions were used to analyze relationships between individual- or month-based variables. In cases of parametric testing, I log-transformed UCP values, when it was the response variable. Non-parametric and linear regression analyses were performed in SPSS 19 (© IBM Corporation). All tests were two-tailed.

Although such a statistical approach is robust in the sense that it relied on a large set of urine samples to obtain mean male or mean monthly UCP values, it also had two major drawbacks: (1) it did not allow to control for confounding variables; and (2) averaging UCP values across males and months obscured much of the intra-individual variation in relation to changing socio-ecological and behavioral factors that I wanted to capture. Therefore, in the second stage of analyses, I conducted linear-mixed model analyses of UCP levels at the level of single-day focal observations and mean male-month values.

I applied linear mixed effects models (LMM) in R version 2.13 (R Development Core Team 2011) using the 'lmer' function of the lme4 package version 0.999375-39 (Bates et al. 2011). Linear mixed effects models allow controlling for pseudoreplication resulting from the repeated observations on the same subjects and also permit unequal sampling of subjects across experimental or observational conditions (Zuur et al. 2009). I controlled for repeated observation of the same 12 males by specifying the identity of focal subjects as a random effect in the model. Because of possible autocorrelation in models, which used daily values of UCP as the response variable, I included an auto-correlation term as a fixed effect in such models (R code for the derivation of this term

written by Roger Mundry). This auto-correlation term was derived following Barelli et al. (2011). First, I ran the full models with maximum likelihood estimation and obtained residuals from it. Second, for each datum, I calculated the weighted average of the residuals of all other data points, with the weight being equal to time lag to the other data points^{-1}. Time lag between observations was measured in days.

I used maximum likelihood estimation (ML) for evaluating full model fits against the null model (consisting only of the random effect and the auto-correlation term, where applicable) with the likelihood ratio test (function 'anova' in R). I report fixed effect estimates from models using restricted maximum likelihood estimation (REML). To obtain reliable P-values for the fixed terms within models I applied Markov chain Monte Carlo (MCMC) sampling (Baayen 2008) with the function 'pvals.fnc' from the R package languageR (Baayen 2011). I simplified full models by dropping non-significant terms until only terms, which contributed to explaining significant variation, remained. After removing each non-significant term I evaluated the fit of the simplified model vs. the null and compared its AIC to the more complicated model. I continued model simplification until the value of the AIC of the simplified model was lower, relative to that of the previous, more complicated model. I report the non-significance of terms by adding them one a time to the final minimal model. Because model simplification procedures can lead to an inflated rate of type I errors (Mundry and Nunn 2009; Forstmeier and Schielzeth 2011) I also report the total number of

terms included in the initial model before simplification was applied as well as indicate if any of the terms in this full model were significant.

I carried out separate LMM analysis on three data-sets. In the first model I used monthly male average UCP values as the response term (N = 92 male-months; all 690 urine samples). In the second and third model I restricted the analysis to days, for which focal behavioral data were also available. The second model contained all available male-days (unique male-day combination; N = 81). The third model was restricted to male-days, on which at least 3 urine samples were collected (N = 40 days). I did this because of the uncertainty in estimating mean daily UCP values based on single urine samples (Chapter 3). Therefore, in addition to testing the hypotheses of interest, in this study I also evaluate how different urine sampling regimes affect the outcome of analyses.

RESULTS

MALE-MALE COMPETITION AND UCP LEVELS

Are periods with high levels of competition associated with reduced male energy balance?

To assess the effects of male competitive investment on energetic balance I compared male UCP levels on days that differed in their relative engagement in aggressive and competitive interactions. I examined the effects of presence of estrous females and the effects of an overall increase in aggression rates, regardless of availability of mating opportunities. Because male dominance

rank is a measure of individual competitive ability, I also evaluated the relationship between male dominance rank and UCP levels.

Males had lower UCP levels on days with high levels of aggressive competition

I used the median rate of aggression given in this data-set (0.47 acts/hr; range 0 – 3.32 acts/hr) to divide the 81 male-urine days into two categories – days of high and low rate of aggression. Days with rates below the median were classified as low aggression days (40 days) while those with rates of aggression of 0.47 or higher, were considered high aggression days (41 days). I calculated mean male UCP levels across all days of high and low rate of aggression and used non-parametric statistics to test the difference. Males had lower UCP levels on high aggression days than on low aggression days (Wilcoxon test: $z=2.31$, $N = 9$ males, $P = 0.021$, Figure 5.1).

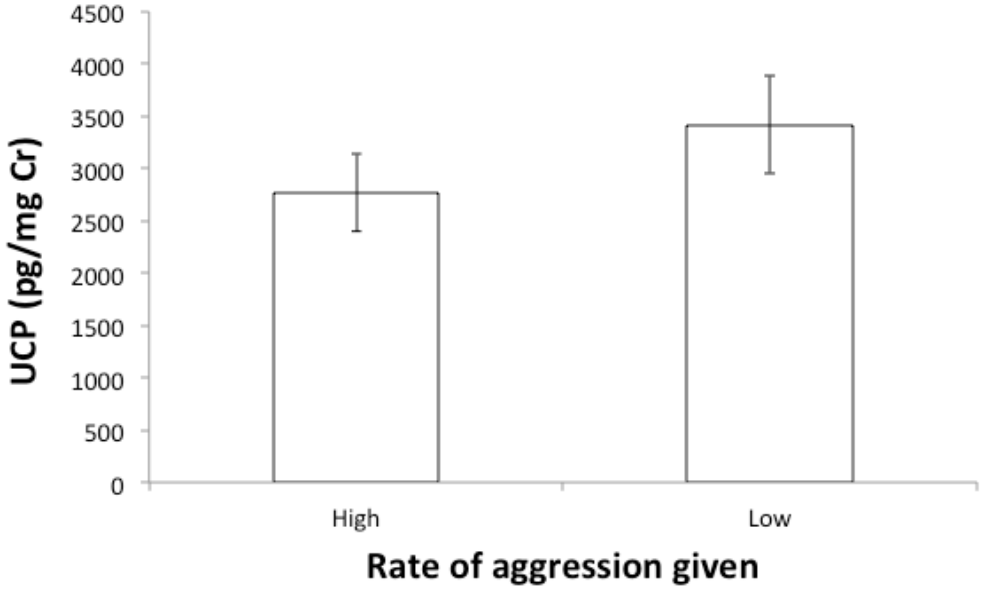


Figure 5.1 Urinary C-peptide (UCP) levels on days with high (≥0.47 acts/hr) and low aggression rates (<0.47 acts/hr). Statistical analysis was performed at the level of individual males (Wilcoxon test). Males had lower UCP levels on high aggression days.

To examine the energetic consequences of being a target of aggression, I also compared mean male UCP levels on days with no, medium or high levels of aggression directed towards them. Splitting the dataset in three groups was needed because the number of days on which the focal males did not receive any aggression was high (44.4% days, N =81). Thus I compared male energetic status on days on which they were not targets of any agonistic acts (36 days), with days on which they were subjects of aggression at low levels (22 days: rate of aggression received 0.08 – 0.13 acts/hr) and high levels (23 days: 0.16 – 0.91

acts/hr). Differences in mean male UCP levels across the three conditions were not significant (Friedman test: z = 4.22, N = 9 males, P = 0.12, Figure 5.2). This suggests that agonistic interactions are more energetically costly for the aggressors rather than the victims.

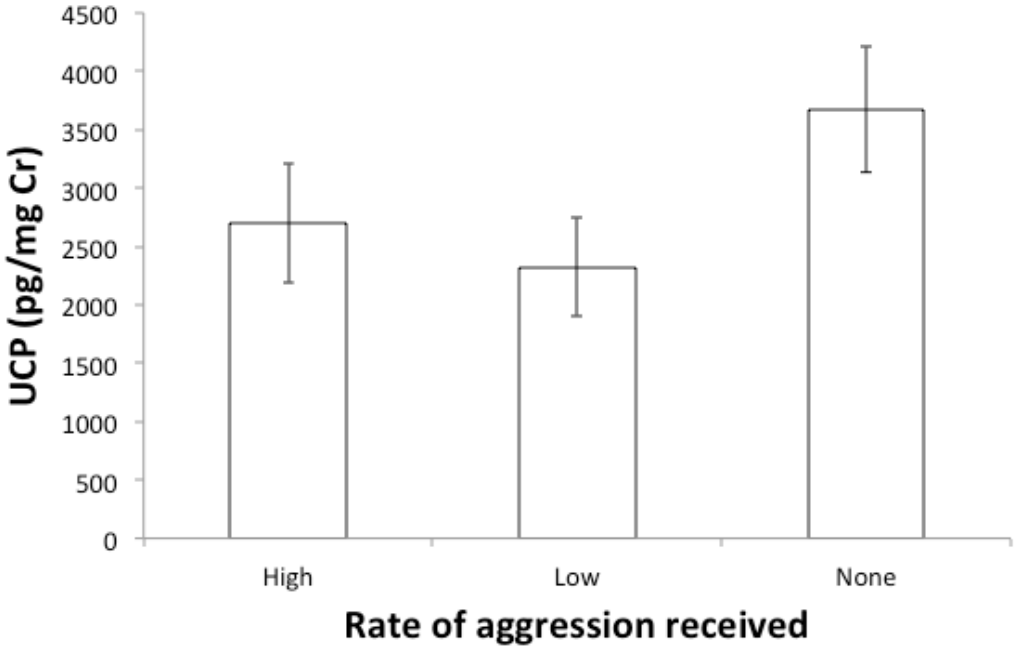


Figure 5.2 Mean urinary C-peptide (UCP) levels on days, on which focal males received aggression at high rates, low rates and none (Friedman test).

Males had lower UCP levels on days when parous females were in estrus

Using all urine samples collected during this study (N= 690 urine samples collected on 321 male-days) I calculated the mean male UCP levels on days without any estrous females (132 male-days), days when only nulliparous females were in estrus (71 male-days) and days when at least one parous female was in estrus (118 male-days). There were significant differences in mean male UCP levels across the three conditions (Friedman test: $z = 10.4$; $N = 10$ males; $P = 0.006$). Post-hoc pairwise comparisons showed that male UCP levels were similar on days without any estrous females and days with nulliparous females ($P_{adj} = 1.0$) and also on days with nulliparous and days with parous females in estrus ($P_{adj} = 0.076$). However, UCP levels were significantly lower on days when parous females were in estrus, relative to days when no estrous females were available ($P_{adj} = 0.005$, Figure 5.3).

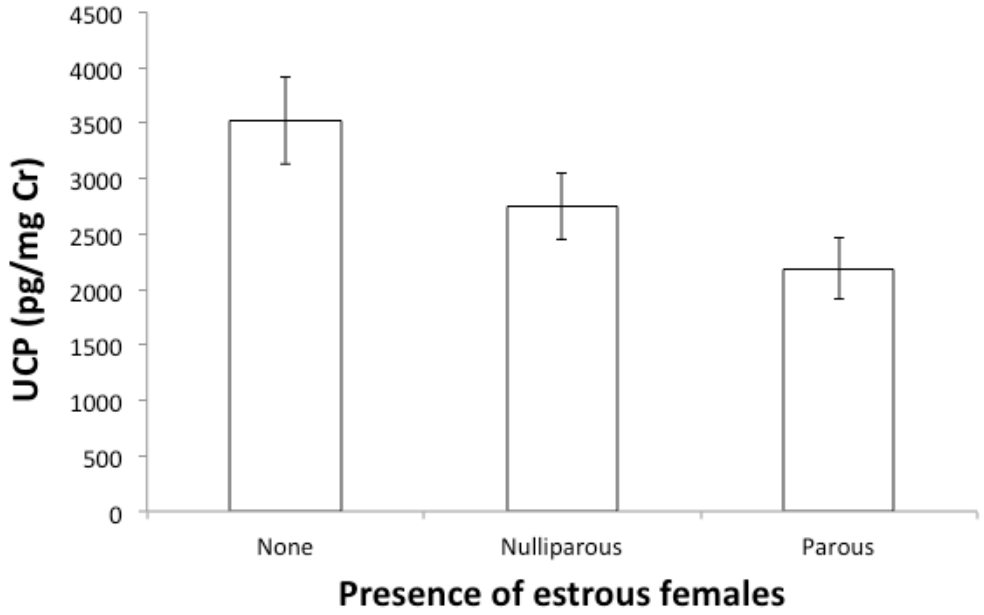


Figure 5.3 Male urinary C-peptide (UCP) in relation to presence of estrous females (Friedman test; 690 urine samples collected on 321 male-days).

High-ranking males had lower UCP levels but only in the absence of estrous parous females

Within the same data-set of 321 male-days, rank also had a significant effect on log-transformed mean UCP levels (Adj. R^2 = 0.42; df = 11; P = 0.014) with the highest-ranking males having the lowest UCP levels (Figure 5.4). The relationship between rank and UCP levels was no longer significant when I examined UCP in three separate analyses: for days when no female was in estrus (Adj. R^2 = 0.24, df = 11, P = 0.061); for days when only parous females were in estrus (Adj. R^2 = 0.061, df = 11, P = 0.22); and for nulliparous days (Adj. R^2 = 0.29, df = 9, P = 0.063). Note that the variance explained by male

dominance rank in the data was lowest for the days when parous females were in estrous (0.061). Given the nearly significant P-values for the other two categories of days, this result suggests that when parous estrous females are available all males have a similar energy balance and that any differences in their UCP levels are only apparent on days with no or very little mating effort and competition (i.e. when no or only nulliparous females are in estrous). Indeed, when I re-analyzed the data by grouping days without any estrous females and days when only nulliparous females were present into one category, the relationship between rank and UCP was significant (Adj. R^2 = 0.37, df = 11, P = 0.021, Figure 5.5). No such relationship was found on days when parous females were in estrous, as shown before.

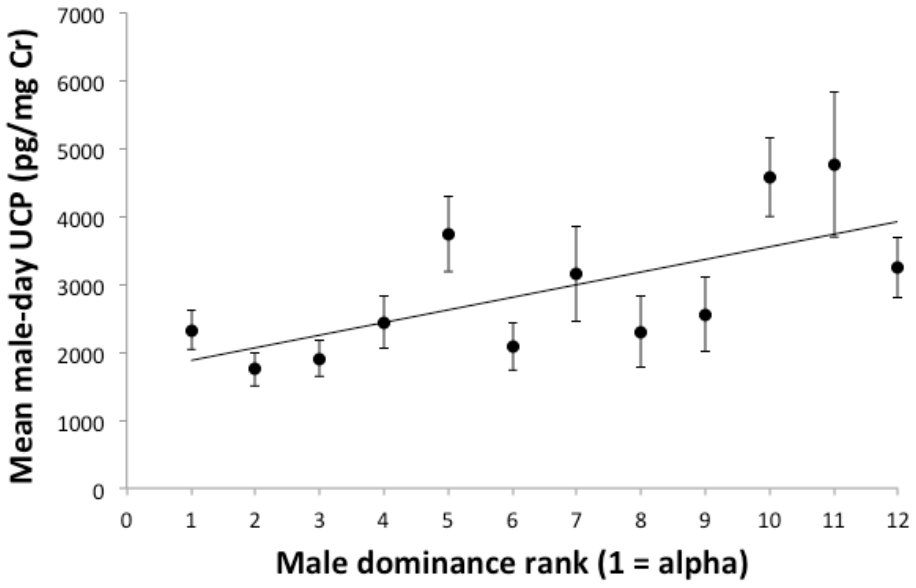


Figure 5.4 Relationship between mean urinary C-peptide levels (UCP) and male dominance rank. Values are means of male daily means (N = 321 male-days; error bars = ±1 SEM). See text for statistics.

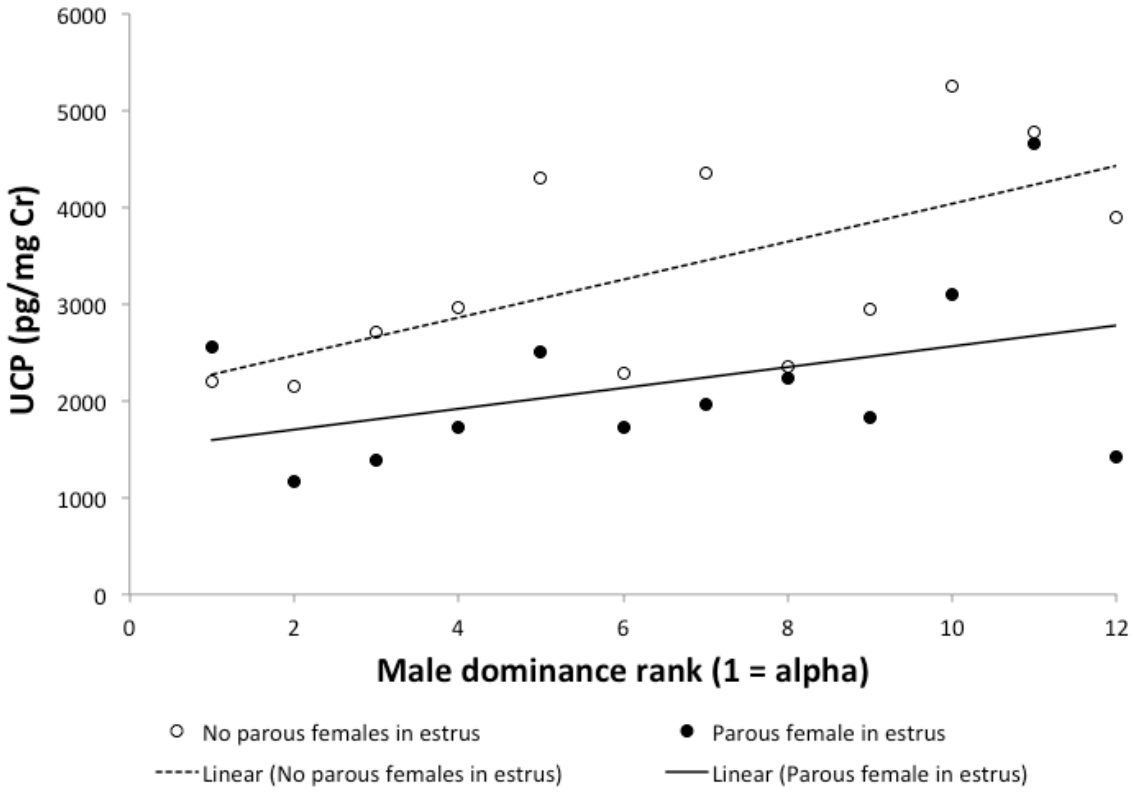


Figure 5.5 Relationship between male dominance rank and urinary C-peptide (UCP) levels on days with and without parous estrous females. Values are means of male daily means (N = 321 male-days; error bars = ±1 SEM). See text for statistics.

EXPLAINING VARIATION:

What accounts low male energetic status during periods of intense mating competition?

I expected that two distinct groups of factors might be responsible for the reduced male UCP levels during periods of mating competition: 1. Socio-ecological factors (party size, presence of estrous females, individual dominance rank and food availability/diet); and 2. Behavioral activity factors (time spent

traveling, feeding, rates of aggression and copulation rates). I first examine these factors in relation to average male monthly UCP levels and then apply linear mixed modeling techniques to daily values of UCP to examine if different levels of analysis yield consistent results.

SOCIO-ECOLOGICAL FACTORS AND MALE UCP LEVELS

In bivariate linear regressions, male monthly log-transformed UCP levels were negatively associated with mean monthly party size (Adj. $R^2 = 0.323$; $F_{1,9} = 5.77$; beta = -0.625; P = 0.04) but not with the monthly number of days on which parous females were in estrus (Adj. $R^2 = 0.071$; $F_{1,9} = 1.76$; beta = -0.404; P = 0.217). There was a nearly significant trend, however, for a negative association between mean monthly UCP and the total number of days on which any females (either parous or nulliparous) were in estrus (Adj. $R^2 = 0.278$; $F_{1,9} = 4.86$; beta = -0.592; P = 0.055). UCP was not affected by monthly diet quality, measured as the contribution of non-fig fruit to the monthly feeding observations in the community (Adj. $R^2 = 0.219$; $F_{1,9} = 3.8$; beta = -0.545; P = 0.083) or the total amount of ripe fruit in the monthly diet (Adj. $R^2 = -0.048$; $F_{1,9} = 0.54$; beta = -0.238; P = 0.480). The monthly contribution of fig fruit to the diet, however, had a significant positive effect on male UCP levels (Adj. $R^2 = 0.337$; $F_{1,9} = 6.07$; beta = 0.635; P = 0.036, Figure 5.6).

To evaluate the effect of all these factors in concert, I carried out a multiple linear regression analysis including 6 predictor variables: mean monthly party size, ripe fruit score, non-fig fruit score, fig score, parous estrous females

score, and nulliparous estrous females score. These data were calculated from all KCP behavioral observations at Kanyawara for each month during which male urine samples were collected. Estrous female scores are the total number of days on which estrous females were observed in parties containing at least one male in a given month.

The full model was not significant (Adj. R^2=0.382; $F_{5,5}$ = 2.24; P = 0.199). This may be due to the high covariance between some of the factors in the model (e.g. ripe fruit score = non-fig score + fig score). I carried out model simplification until the model fit became significant (Adj. R^2=0.517; $F_{3,7}$ = 4.56; P = 0.045). Mean monthly party size was the only term that significantly affected male monthly UCP levels (Table 5.1).

Table 5.1 Factors affecting monthly variation in male UCP levels.

	beta	**P-value**
Constant		<0.001
Party size	-1.84	0.015
Ripe fruit score	0.72	0.078
Parous female days	0.78	0.126

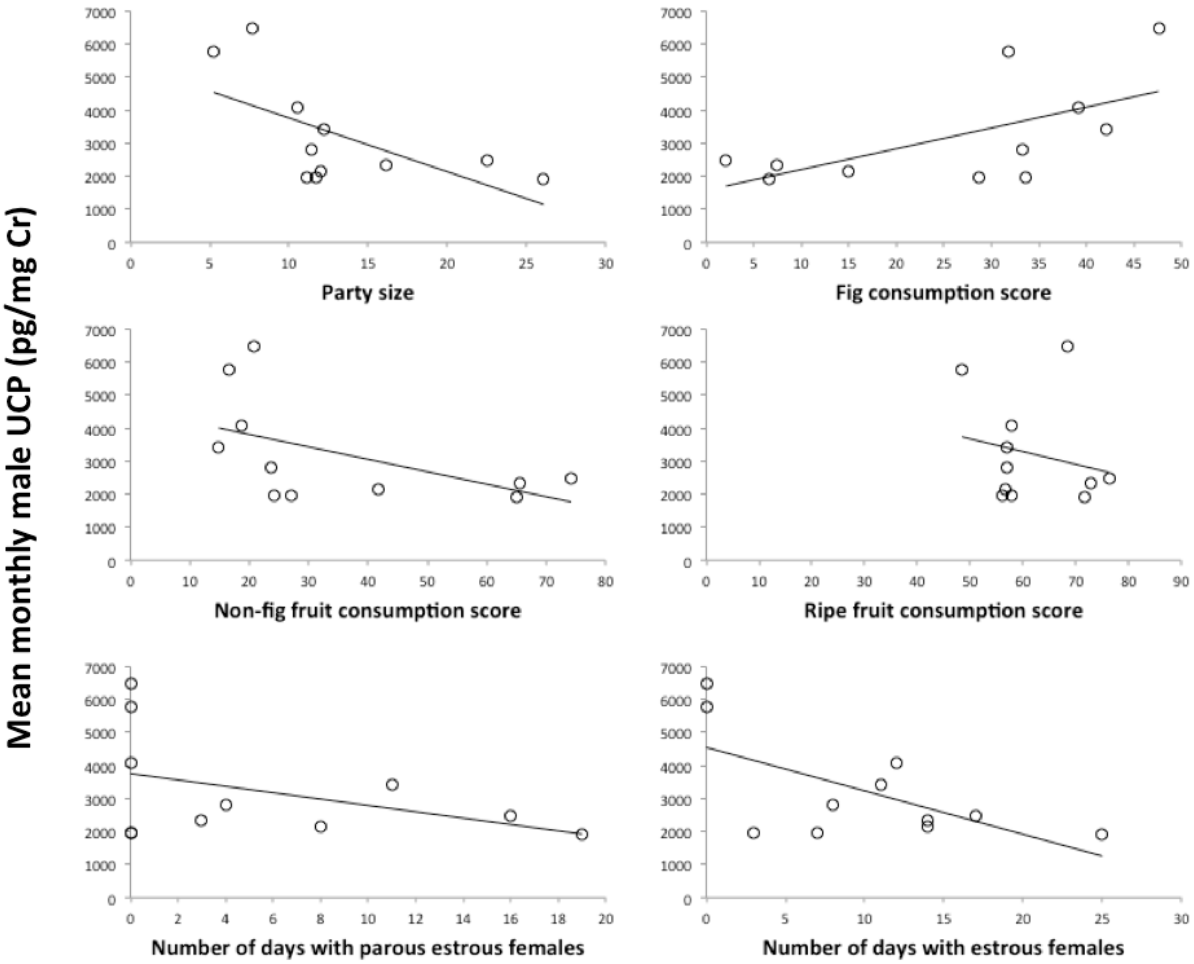


Figure 5.6 Relationships between monthly male UCP levels (means of male means) and a set of socio-ecological factors. Plots show bivariate correlations. Regression lines are for illustrative purposes only. See text for details.

Male behavior and energetics in large parties

To evaluate the cause of the negative relationship between mean party size and male UCP levels, I next considered how male activity patterns differed between days that they spent in relatively large parties, compared to days they spent in smaller ones. I split the focal data-set (N=85 follows) into two by the

median party size observed (6.66): days with small parties (<6.66; 42 days) and days with large parties (≥ 6.66; 43 days).

Males in larger parties spent significantly less time feeding (Wilcoxon, z = 2.4; N = 11 males; P = 0.016) but did not differ in the amount of time allocated to feeding on ripe fruit specifically (z = 0.178; P = 0.859). Males in larger parties were more aggressive (z = -2.312; P = 0.021); received aggression at higher rates (z = -2.943; P = 0.003); and spent significantly more time traveling (z = -2.045; P = 0.041). Finally, males in larger parties had lower UCP levels (z = 2.49; P = 0.013). This finding parallels the observation that males had lower UCP on days when parous females were in estrus so it is important to establish if these two variables (presence of estrous females and party size) are confounded.

The distribution of large vs. small parties across days without any estrous females, days with only nulliparous females and days with parous females was significantly different from random (χ^2= 30.17; df = 2; P < 0.001). Larger parties were observed more often than smaller parties whenever a parous female was present (Figure 5.7).

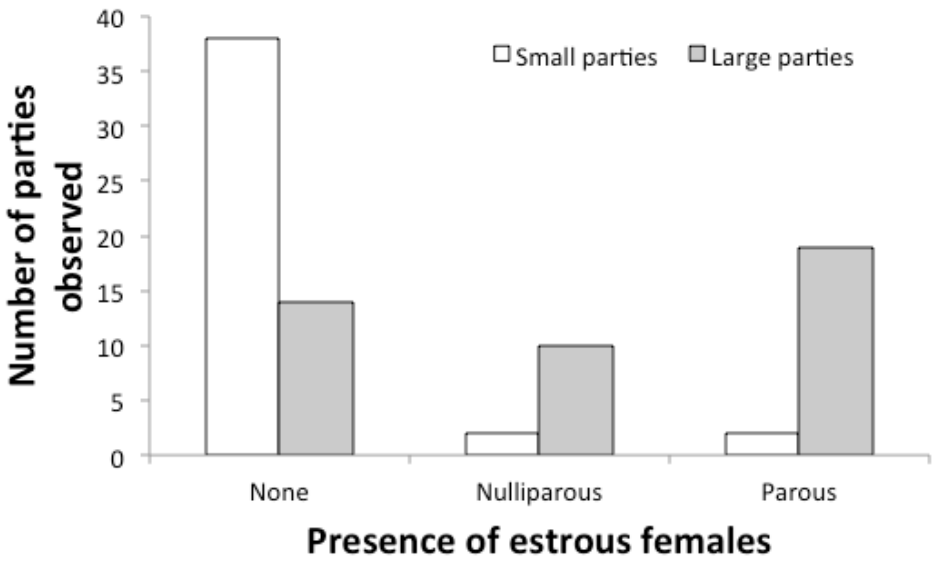


Figure 5.7 Distribution of observations by party size and presence of estrous females in the focal dataset (N = 85 focal follows).

I next examined the effect of party size on male UCP levels, independently of the presence of estrous females (i.e. considering only days without parous females). On days when males were in larger parties, their UCP level tended to be lower, almost significantly so (Wilcoxon test: z = 1.96; N = 8 males; P = 0.05). This suggests that beyond the effect of mating competition, being in a large party on itself can have a negative effect on male energy balance. Since almost all parties observed in the presence of estrous females were large, I could not test if greater party size has the same effect on male energy balance on days with mating competition.

To establish the effect of mating competition on male energetic status I examined mean male UCP levels in large parties only. I compared large parties, in which there were no parous estrous females with large parties, in which at

least one parous estrous female was present. Mean male UCP levels did not differ among the two types of party ($z = -0.561$; N = 10 males; $P = 0.575$). I also compared mean UCP levels between parties without any estrous females (either parous or nulliparous) with parties with parous estrous females but did not find a difference in male UCP levels, either ($z = 0$; N = 8 males; $P = 1.0$). While this non-parametric analysis cannot control for other factors that may affect UCP levels (e.g. ripe fruit availability), it does suggest that the effects of parous estrous female presence were secondary to the costs of simply being part of a larger grouping of chimpanzees.

Is the effect of party size on UCP masking the relationship between presence of estrous females and UCP levels?

The strong effect of party size on monthly male UCP levels raises the question of what factors are responsible for the formation of large parties. In a multiple regression model (Adj. $R^2 = 0.884$; $F_{4,6} = 19.97$; $P = 0.001$, Figure 5.8), the single factor that had a significant positive effect on mean monthly party size was the number of days on which parous females were in estrus during that month (Table 5.2).

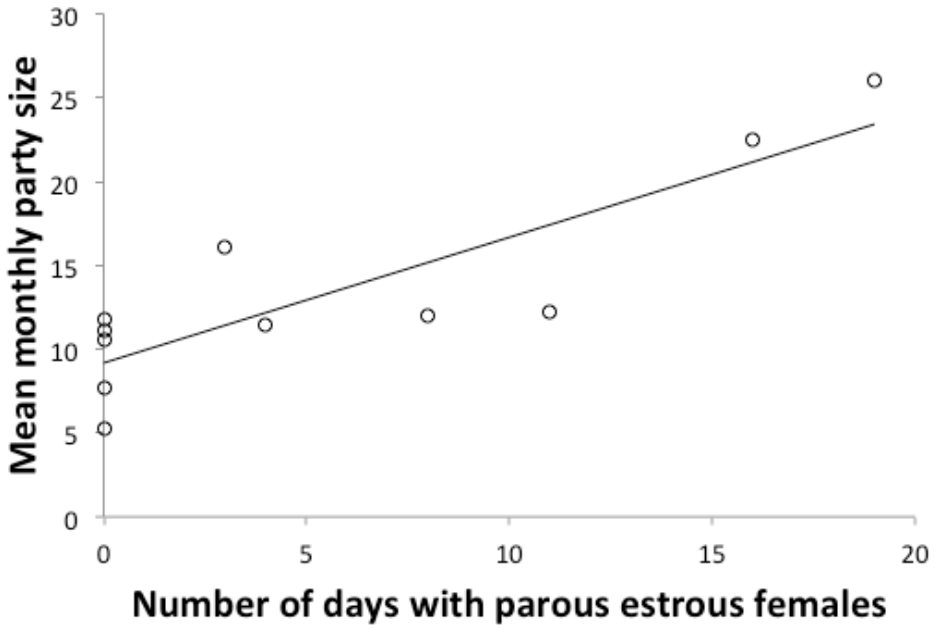


Figure 5.8 Relationship between monthly number of days with parous estrous females and mean monthly party size at Kanyawara. See text for details.

Table 5.2 Results from multiple linear regression examining the effects of socio-ecological variables on chimpanzee party size at Kanyawara.

	beta	P-value
Constant		0.808
Parous female days	0.639	0.008
Nulliparous female days	0.219	0.136
Ripe fruit score	0.286	0.086
Fig score	-0.157	0.391

NFF score was excluded from the regression model

Factors affecting male daily party size: linear-mixed model analysis

To confirm the results based on the analysis of mean monthly party size, I also carried out a linear mixed model analysis using the focal data set of 85 focal follows. I examined the effects of male dominance rank, dietary quality and the

presence of estrous females (none, nulliparous, or parous) on the party size of males at the daily level. The full model was significant when tested against the null (χ^2 = 65.19; df = 4; P < 0.0001). Two factors affected party size of focal males: the presence of estrous females (Figure 5.9) and dietary quality (Figure 5.10). The effect of parous female presence was particularly strong: mean daily party size increased by 5.6 individuals on average relative to days when no females were in estrous. The dominance rank of the focal male did not affect the mean daily number of individuals with whom he traveled (Table 5.3).

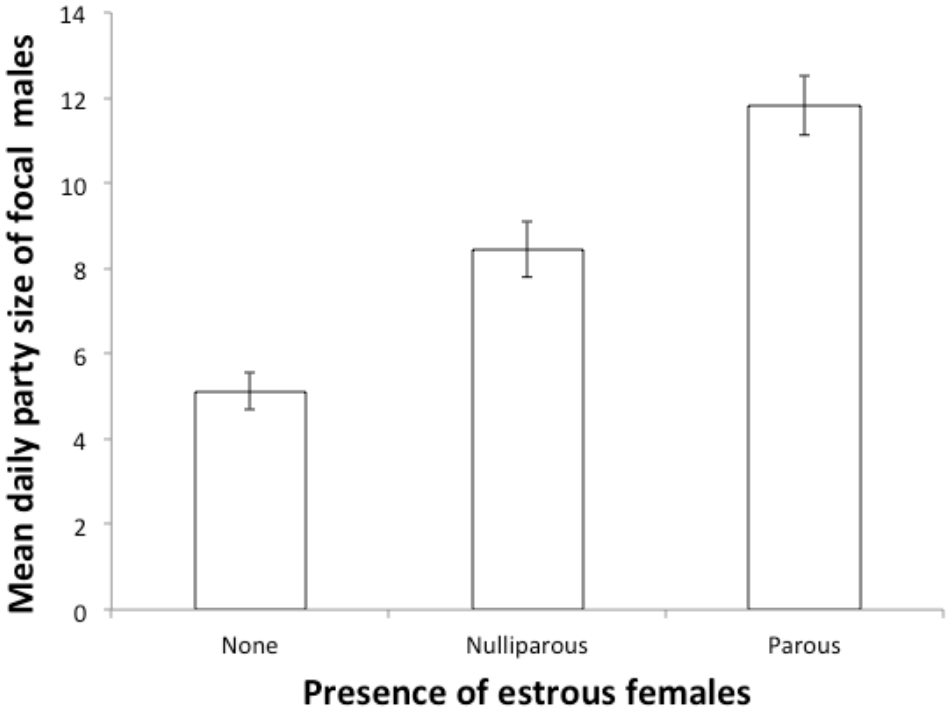


Figure 5.9 Male party size on days with and without estrous females. N = 85 focal follows.

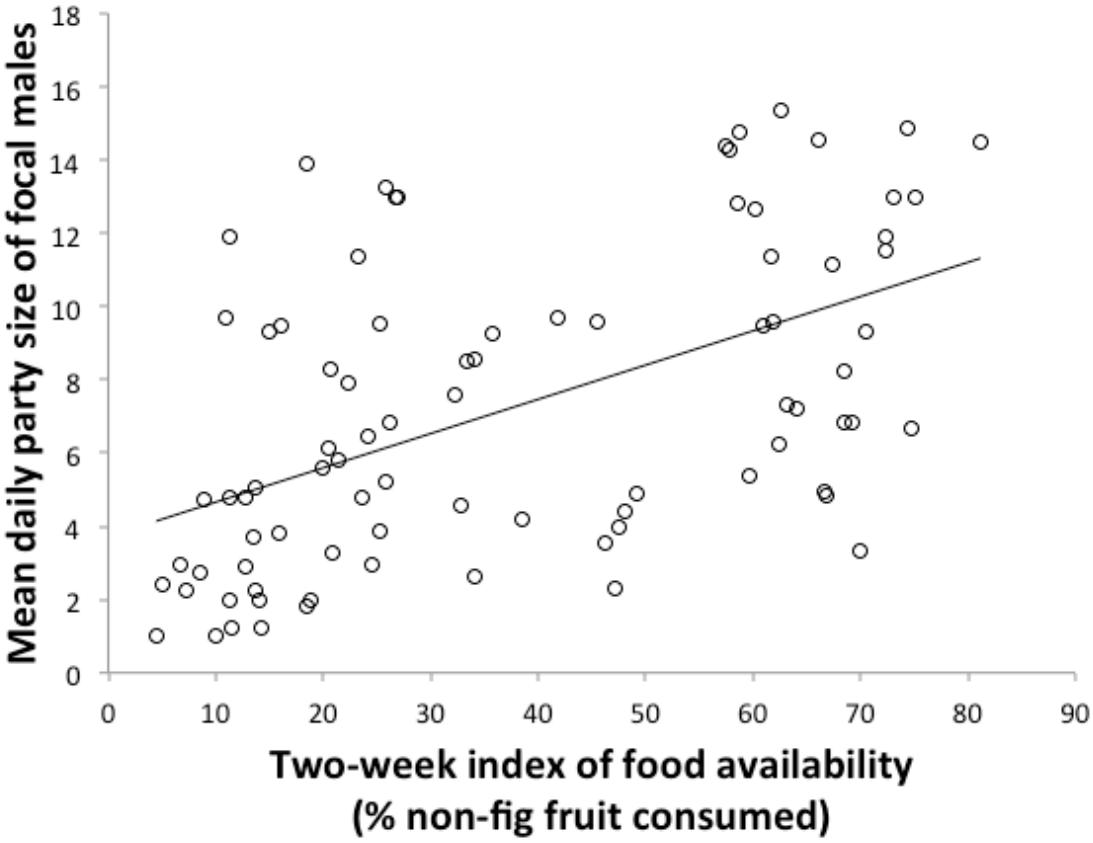


Figure 5.10 Relationship between dietary quality and party size of focal males. Regression line shows the bivariate relationship. See text for details on LMM analysis.

Table 5.3 Results from linear mixed model analysis of socio-ecological factors affecting the number of independent individuals focal males asssociated with on daily basis (their mean daily party size). N = 85 focal follows.

	Estimate	**SE**	**P_{MCMC}**
Intercept	3.532	0.66	0.0001
Parous females	5.633	0.82	**0.0001**
Dietary quality (14-day NFF score)	0.054	0.01	**0.0002**
Nulliparous females	2.429	0.93	**0.016**
Male rank	-0.028	0.46	0.919
Autocorrelation term	-0.196	0.33	0.893

On the basis of these two sets of analyses, I conclude that mating opportunities via increasing party size result in significant energetic costs for male chimpanzees. The strong correlation between monthly number of days, on which parous females were in estrus and mean monthly party size (r_s = 0.86; N = 11 months; P = 0.001), as well as the fact that the parous estrous female presence was the best predictor of mean monthly party size (see above) suggest that the regression model examining both factors may be affected by colinearity. To overcome this issue, I constructed an additional model of mean monthly UCP level. Instead of using mean monthly party size as one of the explanatory variables, I entered the standardized residuals of mean monthly party size from a regression of party size on parous females (i.e. the variance in mean monthly party size that was not explained by presence of estrous females). This model explained a significant amount of the variation in monthly UCP levels (Adj. R^2 = 0.52; $F_{3,7}$ = 4.56; P = 0.045) and indicated that both the number of parous

estrous females and variation in party size affected male energetic condition (Table 5.4).

Table 5.4 Factors affecting male monthly UCP levels

	beta	P-value
Constant		0.001
Residuals of party size	-0.945	0.015
Parous female days	-0.797	0.029
Ripe fruit score	.722	0.078

Linear-mixed model analysis of socio-ecological factors affecting male UCP levels

To further evaluate the relationship between different socio-ecological factors and male UCP levels I also carried out linear-mixed model analysis. In the first set of analyses I used 92 mean monthly male UCP values as a response variable; in the second – I used 81 male-days on which I obtained at least 1 urine samples; and in the third – I used 40 male-days with a minimum of 3 urine samples from each of the males under observation.

Model 1: 92 male-monthly means of UCP levels

The 690 urine samples analyzed for this study were obtained on 321 different male-days. For days on which multiple samples were obtained, I averaged their UCP values. These mean daily averages were then averaged for each male over the course of each month (mean number of male-days per male-month = 3.47 +- SE 0.25; range: 1-13). In each month I sampled an average of 8.36 males (SE +/- 0.66; range: 5-12). The number of male-months used in this

analysis is thus 92 (mean number of months per male = 7.67 ± 0.62; range = 3-10).

To assess the effect of mating effort on mean monthly UCP levels, I calculated the number of days in each month on which at least one parous female was in estrus. I included this as a predictor variable in a model, which also accounted for mean monthly party size, male dominance rank, and food availability, and also included the interaction between rank and presence of parous estrous females. The full model was significant when tested against the null (χ^2 = 21.89; df = 5; P = 0.0005). Two factors from this model affected male UCP levels significantly: party size (estimate = -0.47; P = 0.001) and ripe fruit consumption score (estimate = 0.015; P = 0.01). Following model simplification, the optimal model (with the lowest AIC score) was also significant when tested agaisnt the null (χ^2 = 21.81; df = 4; P = 0.0002). Apart from the signficant effects of the factors identified from the full model, after model simplification, the effects of male dominance rank also became significant. The number of days on which parous females were in estrus, however did not reach statistical significance in its effect monthly male UCP levels (Table 5.5).

Table 5.5 Summary of Model 1

Final model:	**Estimate**	**SE**	**P_{MCMC}**
Intercept	2.9764	0.269538	0.0001
Mean monthly party size	-0.0473	0.013467	0.0008
Monthly ripe fruit consumption score	0.0155	0.00581	0.0118
Male rank	0.0747	0.036203	0.0394
Parous estrous days	0.0147	0.008823	0.1034
Terms removed from the full model:			
Interaction Rank * Parous estrous days	-0.0013	0.004205	0.7486

One potential issue with this model, however, is the high correlation between mean monthly party size and number of days with parous estrous females described earlier. I thus constructed two separate models to evaluate the influence of party size and mating effort in more detail.

The full model (Model 1a), which included male rank, parous estrous days and monthly ripe fruit score as fixed effects was significant when tested against the null (χ^2 = 10.1; df = 3; P = 0.02). Male rank had a significant positive effect (high-ranking males having lower UCP leves) on UCP levels, while the number of parous females had a significant negative effect (Table 5.6).

Table 5.6 Summary of Model 1a.

Full model:	**Estimate**	**SE**	**P_{MCMC}**
Intercept	3.3803	0.259833	0.0001
Male rank	0.0819	0.036557	0.0334
Parous estrous days	-0.0108	0.005327	0.0496
Monthly ripe fruit consumption score	0.0011	0.004396	0.8214

The model which included rank, party size and ripe fruit score, to the exclusion of parous female days (Model 1b), was also significant when tested against the null (χ^2 = 18.95; df = 3; P = 0.0003). Male rank and ripe fruit consumption score had significant positive effects on male UCP levels, and mean monthly party size a significant negative effect (Table 5.7) as shown in the previous model (Model 1, Table 5.5).

Table 5.7 Summary of Model 1b.

Full model:	Estimate	SE	P_{MCMC}
Intercept	3.0329	0.270057	0.0001
Male rank	0.0768	0.036543	0.0376
Mean monthly party size	-0.0288	0.007695	0.0004
Monthly ripe fruit consumption score	0.012	0.005469	0.0286

Both of the models above are significant when tested against the null model for this data-set (i.e. they provide a good explanatory fit to the data). However, the model featuring mean monthly party size has a lower AIC score than the model with parous estrous days (37.8 vs. 46.7) indicating a better fit to the data.

Model 2: 81 focal follow UCP means

For this analysis I calculated mean daily UCP levels for focal males observed on 81 days. Mean sampling intensity was 3.21 ± 0.31 samples per follows (rage: 1 – 19) but on 27.2% of days only 1 urine sample was available per follow.

I considered the effect of male daily party size, the presence of estrous females (nulliparous and parous), male dominance rank, monthly ripe fruit score (an index of fruit availability and dietary quality) and the interaction between male rank and presence of estrous females. The full model, containing all terms was significant, when compared against the null (χ^2 = 18.33; df = 7; P = 0.01) but none of the factors that it contained reached statistical significance (all fixed effects $P_{MCMC} > 0.05$). To evaluate which of the terms in this model contributed most to variation in daily male UCP concentrations I simplified the model. The

minimal model was significantly better than the null (χ^2 = 15.28; df = 2; P = 0.0005). Only daily party size had a significant negative effect on male UCP levels (Table 5.8). Dominance rank approached significance with a trend for higher-ranking males to have lower UCP levels (Table 5.8). One issue with this analysis, as before, is that mean daily party size of the focal males was much higher on days when estrous parous females were present in comparison to days when no females were in estrous (Wilcoxon test: z = 2.93; N = 11 males; P = 0.003, Figure 5.11) and thus the strong negative association that appears to exist between male party size and daily UCP levels may in fact be masking the similarly strong negative impact of mating competion on days when parous females were in estrous.

Table 5.8 Summary of Model 2. N = 81 focal follows.

Final model	**Estimate**	**SE**	**P_{MCMC}**
Intercept	3.561	0.070	0.0001
Party size	-0.028	0.008	0.0006
Rank	0.077	0.035	0.067
Autocorrelation term	0.045	0.035	0.345
Terms removed from the full model:			
Nulliparous estrous females	-0.154	0.109	0.15
Parous estrous females	-0.080	0.112	0.473
Monthly ripe fruit score	0.002	0.005	0.659
Rank*Nulliparous females	-0.066	0.100	0.511
Rank*Parous females	0.015	0.094	0.895

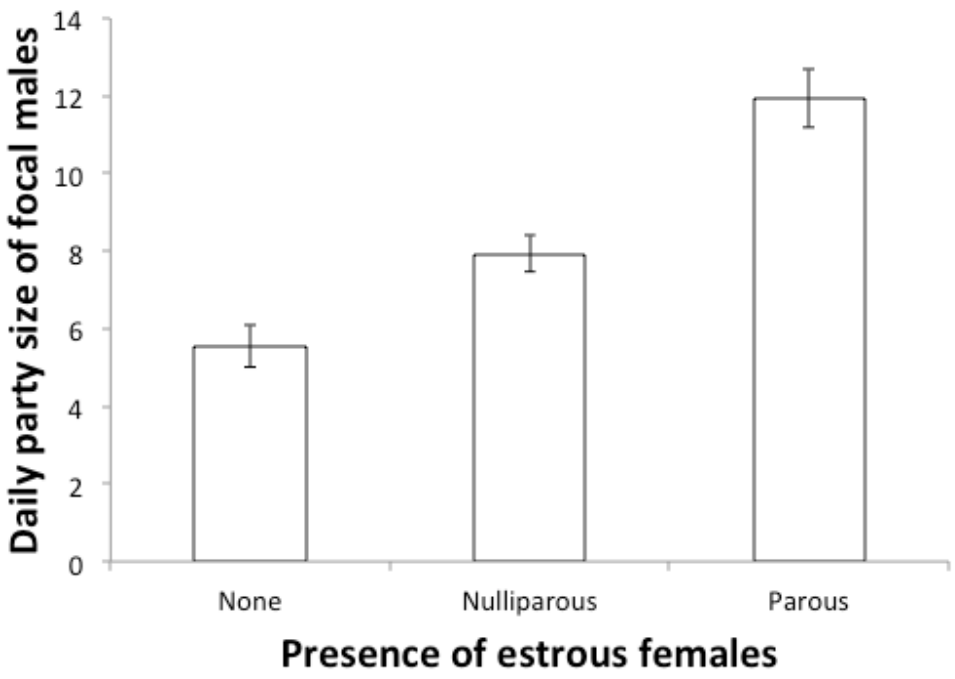


Figure 5.11 Mean daily party size of focal males (±SEM) on days with and without parous females in the data-set used for urinary analysis (81 focal follows). Party size is defined as all independently traveling individuals (i.e. infants and juveniles excluded).

I examined the effect of presence of estrous females on male UCP levels in a model that did not contain party size as a fixed effect to avoid problems of collinearity (Model 2a). The model included the following fixed effects: male dominance rank, presence of estrous females (none, nulliparous or parous), monthly ripe fruit score, and the interaction between male rank and presence of estrous females. This full model (Model 2a) was significant when tested against the null (χ^2 = 14.2; df = 6; P = 0.027). The only significant fixed effect in this full model was that of presence of parous estrous females (estimate: -0.21± 0.09; P

= 0.04). To obtain a better fit of this model and to establish if any other factors have effect on daily male UCP levels, I applied model simplification. The final model was significantly better than the null (χ^2 = 13.43; df = 3; P = 0.004). Two of the remaining factors had significant effect on male UCP levels: presence of parous and of nulliparous estrous females (Table 5.9; Figure 5.12).

Table 5.9 Summary of Model 2a. N = 81 focal follows.

Final model:	**Estimate**	**SEM**	**P_{MCMC}**
Intercept	3.44	0.05	0.0001
Parous females	-0.22	0.08	0.0126
Nulliparous females	-0.21	0.11	0.0354
Male dominance rank	0.08	0.04	0.0784
Autocorrelation term	0.04	0.04	0.4832
Terms removed from the full model:			
Monthly ripe fruit score	-0.0005	0.005	0.8838
Interaction Rank * Nulliparous females	-0.08	0.1	0.4598
Interaction Rank * Parous females	0.01	0.1	0.8782

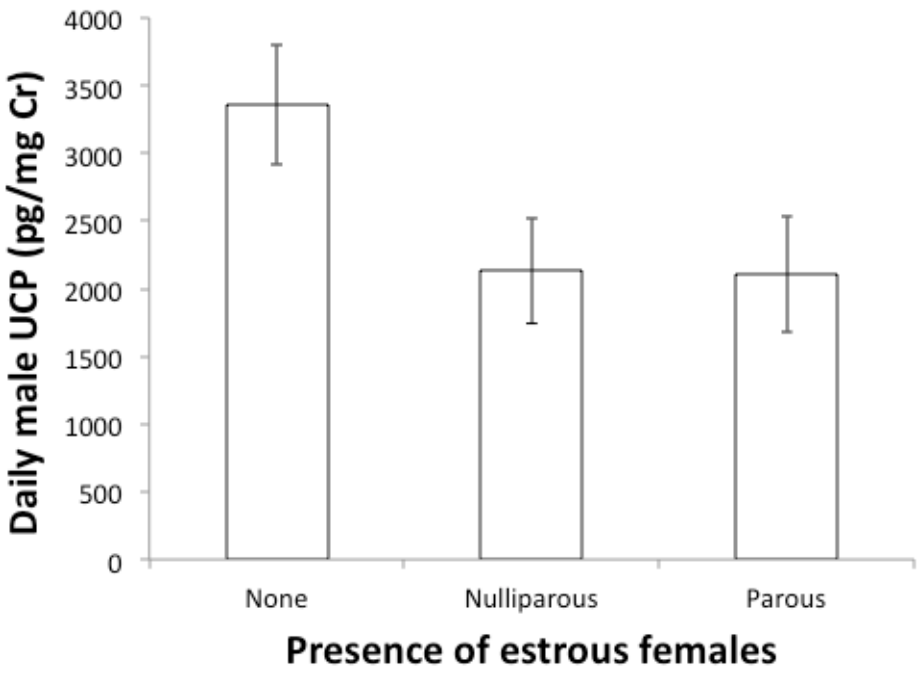


Figure 5.12 Mean daily UCP levels on days with and without sexual opportunities. N = 81 focal follows. Means show raw data; see text for statistical analysis.

I evaluated how these two 'alternative' models (Models 2 and 2a) compared in their explanatory power in regards to mean male daily UCP levels by examining their AIC scores. The model containing party size as a fixed effect had a lower AIC score than the model containing presence of estrous females as a fixed effect (48.04 vs. 51.89) and thus was a better fit to the data. The difference in AIC values was relatively small. This set of analyses suggest that in the present data-set both party size and presence of estrous females have a negative effect on male UCP levels but the effect of party size is easier to detect. The close association between these two factors (high party size in the presence

of parous estrous females) makes it difficult to distinguish which of these social effects on male energetic condition is more important.

Model 3: 40 focal follow UCP means

Because of the high proportion of single-sample days in the previous data-set (27.2% of the total of 81 focal follows), I restricted analysis to focal follows, on which at least 3 urine samples per male were obtained. The average sampling intensity within this reduced dataset was 5 samples per day ± 0.5 (range 3 – 19, N = 40).

I performed the same analysis as with the previous dataset. The full model was significant when tested against the null (χ^2 = 17.24; df = 5; P = 0.03). The only factor with a significant negative effect on male UCP was party size (P = 0.01, Figure 5.13). After model simplification (χ^2 = 10.15; df = 2; P = 0.006) the only factor with a significant negative effect on male UCP was still mean daily party size (Table 5.10).

Table 5.10 Results from LMM analysis of factors affecting mean daily urinary C-peptide (UCP; log-transformed pg/mg Cr) levels in male chimpanzees. Data set: 40 male-days with at least 3 urine samples per day.

Final model:	**Estimate**	**SE**	**P_{MCMC}**
Intercept	3.1985	0.360281	0.0001
Party size	-0.0406	0.012596	0.0024
Monthly ripe fruit score	0.0085	0.006185	0.1726
Autocorrelation term	0.0467	0.046523	0.385
Terms removed from the full model:			
Male rank	0.03	0.048509	0.5736
Parous estrous females	0.032223	0.130746	0.8226
Rank * parous females	-0.1444	0.123515	0.2336

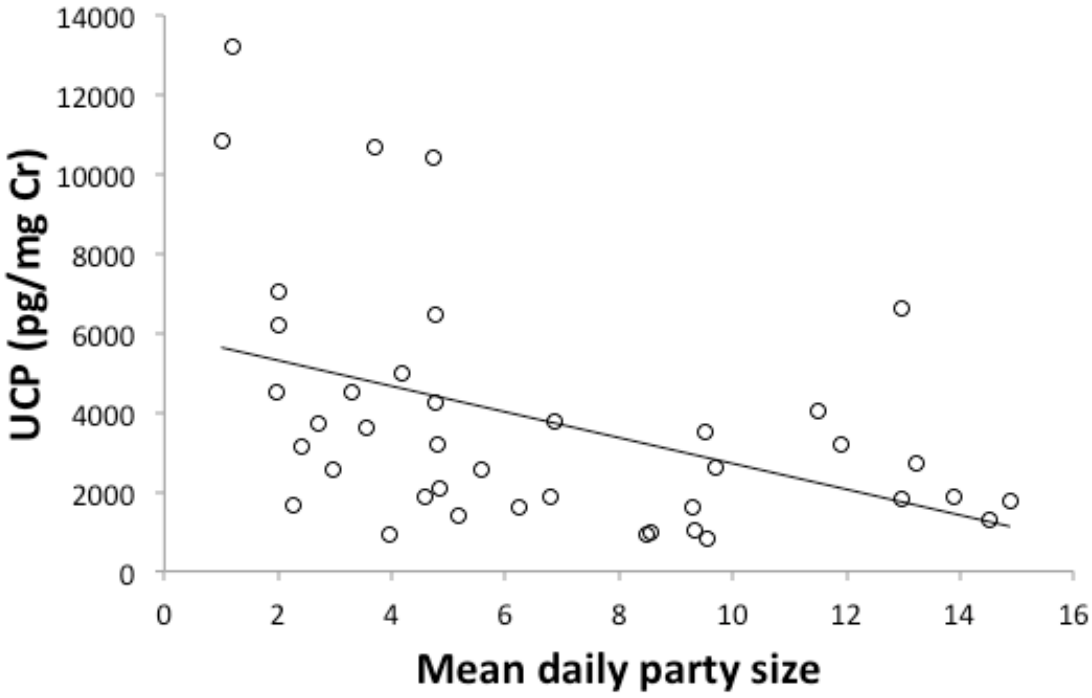


Figure 5.13 Relationship between mean daily party size of focal males and daily UCP levels. N = 40 days on which at least 3 urine samples were obtained. Regression line is for illustrative purposes.

BEHAVIORAL ACTIVITY FACTORS AND MALE UCP LEVELS

In this section, I examine the relationship between male activity and UCP levels to understand the proximate factors responsible for the negative association between male party size and the presence of parous estrous female on the one hand, and mean UCP levels on the other.

Correlational analysis of the influence of behavioral activity factors on male UCP levels: N=12 males

Within the focal dataset for which urine samples were collected (N=81 focal follows), mean male UCP levels were not correlated with any of the

behavioral variables examined: daily travel time (r_s = -0.39, N = 12 males; P = 0.208); daily feeding time (r_s = 0.329, P = 0.297); daily ripe fruit feeding time (r_s = 0.196, P = 0.542); rate of aggression given (r_s = 0.042, P = 0.897); rate of aggression received (r_s = -0.238, P = 0.457); and copulation rate (r_s = -0.34, P = 0.28). Mean male UCP levels were not correlated with dominance rank in this dataset either (r_s = 0.434, N=12 males, P = 0.159), but they were significantly and negatively associated with mean daily party size (r_s = -0.601, P = 0.039; Figure 5.14).

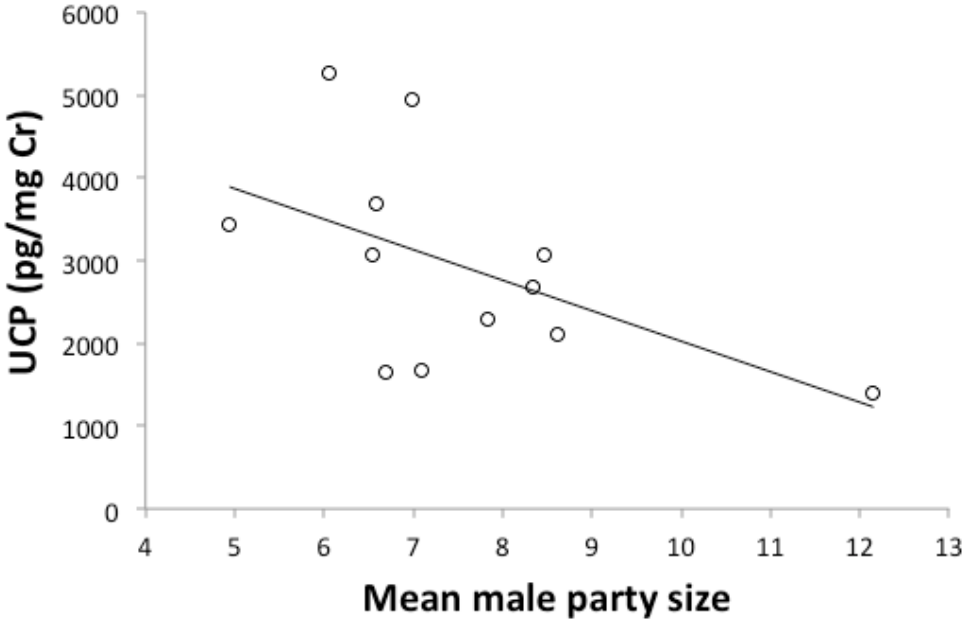


Figure 5.14 Relationship between mean male daily party size (number of independent individuals) and mean male urinary C-peptide (UCP) levels. N = 12 males. Regression line is for illustrative purposes only. See text for statistics.

I re-examined the relationships of the same variables with mean UCP levels in an extended dataset. I used the entire focal set of 85 focal follows to

calculate average male activity profiles. To calculate mean UCP levels, I used all urine samples collected during the study period (690 samples; 321 male-urine days). Within this dataset, there was no association between mean male UCP levels and daily travel time (r_s = -0.413; N = 12 males; P = 0.183), rate of aggression given (r_s = -0.077; P = 0.812), rate of aggression received (r_s = -0.028; P = 0.931) and copulation rate (r_s = -0.476; P = 0.118). However male UCP levels were significantly and positively associated with total feeding time (r_s = 0.657; P = 0.02, Figure 5.15) and ripe fruit feeding time (r_s = 0.608; P = 0.036). While mean daily party size was no longer significantly correlated with mean UCP levels (r_s = -0.448; P = 0.145), male rank was (r_s = 0.692; P = 0.013).

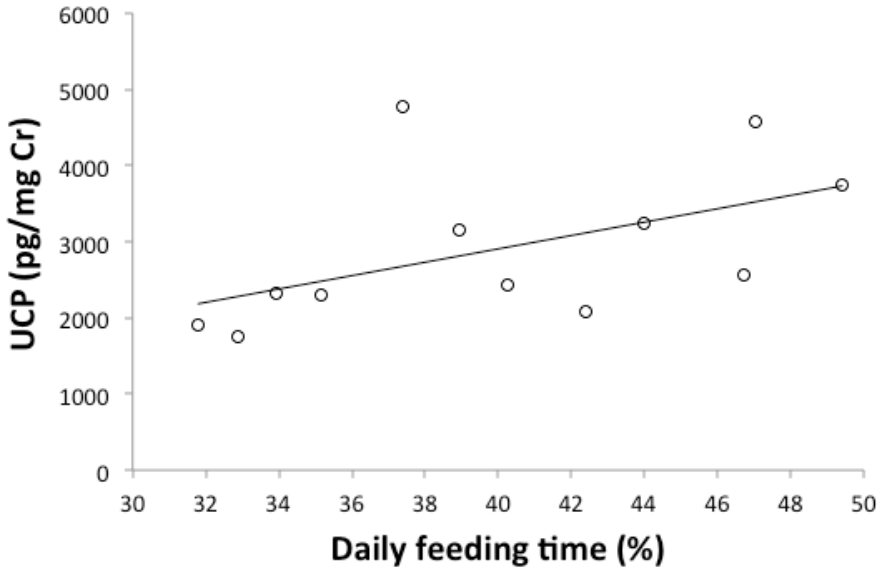


Figure 5.15 Relationship between mean male daily feeding time (%) and mean male urinary C-peptide (UCP) levels. N = 12 males. Regression line is for illustrative purposes only.

Linear mixed model analysis of the influence of behavioral activity factors on male UCP levels: N=81 focal days

I used the full data-set of focal follows, for which at least one urine sample was collected (average sampling intensity: 3.2 +-0.3 samples/follow; days with 27.2% of days with only 1 urine sample). I examined how focal activity affected mean daily UCP levels. The full model was significant when tested against the null (χ^2 =23.31; df = 6; P = 0.0007). Two factors in this full model had a significant negative effect on mean daily UCP levels: rate of aggression given (P = 0.02, Figure 5.16) and time spent traveling (P = 0.03, Figure 5.17). After model simplification (χ^2 = 21.81; df = 3; P = 0.00007) no other factor was shown to affect male UCP levels, apart from aggression given and travel time (Table 5.11), as already indicated by the full model.

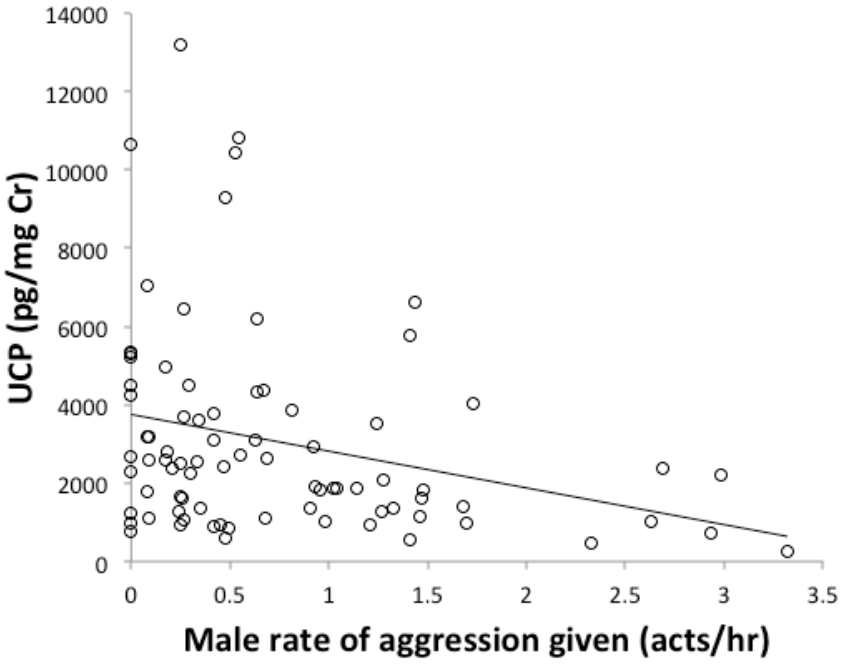


Figure 5.16 Relationship between male rate of aggression given and mean daily urinary C-peptide (UCP) levels. N = 81 focal follows. Regression line is for illustrative purposes. See text for details on statistical analyses.

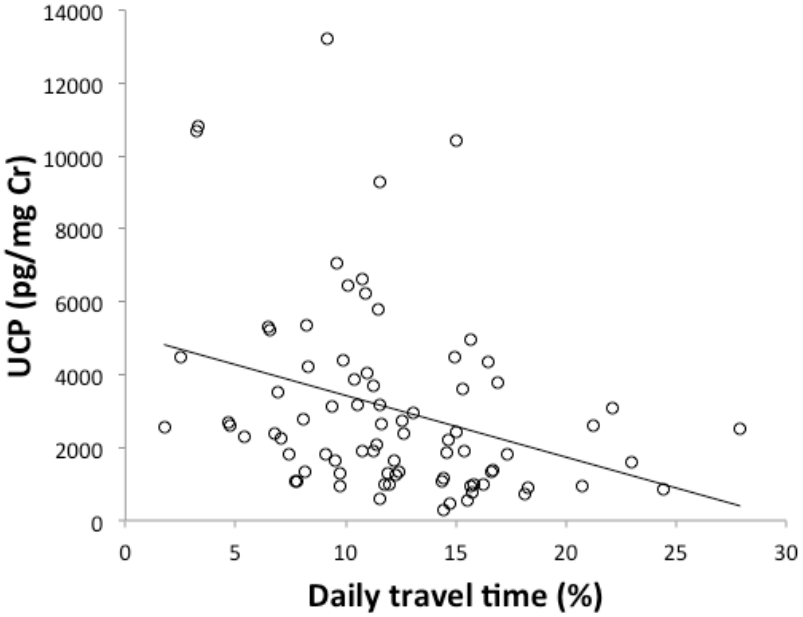


Figure 5.17 Relationship between male travel time and mean daily urinary C-peptide (UCP) levels. Regression line is for illustrative purposes. N = 81 focal follows. See text for details on statistical analyses.

Table 5.11 Summary of LMM analysis of behavioral activity factors (N= 81 focal days).

Final model:	**Estimate**	**SE**	**P_{MCMC}**
Intercept	3.4569	0.186	0.0001
Aggression given	-0.1199	0.05	0.0198
Feeding time	0.0045	0.003	0.1314
Travel time	-0.0158	0.007	0.0272
Autocorrelation term	0.0038	0.034	0.7298
Terms removed from full model:			
Aggression received	-0.1885	0.202	0.4784
Copulation rate	0.113636	0.2537	0.7032
Ripe fruit feeding time	-0.001988	0.003676	0.6284

Linear mixed model analysis of the influence of behavioral activity factors on male UCP levels: N=40 focal days

The full model was significant when tested against the null (χ^2 = 16.5; df = 6; P = 0.011). The only term, which was significant in this model, was time spent traveling on the ground (P = 0.012). The more time males traveled per day, the lower their mean daily UCP levels were (Figure 5.18). After model simplification, the optimal model contained only travel time as a fixed term (Table 5.12). None of the other factors examined explained the variation in male daily UCP levels. It is worth noting, however that the variance in rates of aggression in this subset (N = 40 focal follows) is lower (0 – 1.73 acts/hr; mean = 0.54), relative to the full focal dataset with urine samples (N = 81 focal follows; 0- 3.32 acts/hr; mean = 0.74).

Table 5.12 Summary of LMM analysis of behavioral activity factors. N = 40 focal follows.

Final model:	**Estimate**	**SE**	**P_{MCMC}**
Intercept	3.8189	0.10521	0.0001
Travel time	-0.0306	0.00825	0.0018
Autocorrelation term	0.077	0.04425	0.111
Terms removed from the full model:			
Aggression received	-0.404	0.351004	0.2384
Aggression given	-0.085639	0.09039	0.3612
Ripe fruit feeding time	-0.00295	0.003308	0.3858
Feeding time	-0.0008601	0.0043988	0.8536
Copulation rate	0.0005033	0.3071172	0.9854

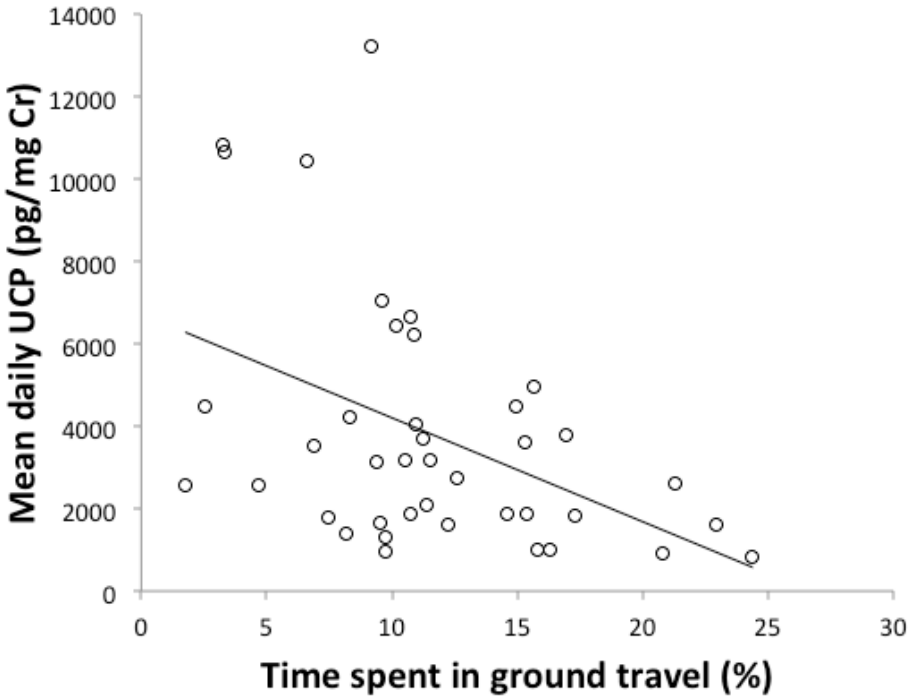


Fig 5.18 Relationship between male terrestrial travel time and mean daily urinary C-peptide (UCP) levels. Regression line is for illustrative purposes. See text for details on LMM analysis. Only days for which at least 3 urine samples were obtained are used (N=40).

What factors affected male travel time?

Because of the important effect of male travel time on male energetic condition, I considered the factors that explain variation in male travel time.

I constructed a full model that contained the following fixed effects: male dominance rank, presence of estrous females (none, nulliparous or parous), mean daily party size, 14-day dietary score (% non-fig fruit score calculated from on the basis of feeding data 2 weeks prior to the day of focal observation

including the day of focal observation itself). This full model was significant when tested against the null (χ^2 = 12.45; df = 5; P = 0.03) and only one of the fixed terms had a significant effect on male daily travel time (logit-transformed): the two-week index of dietary quality and abundance of high-quality fruits (P = 0.04, Figure 5.19). After model simplification, the result did not change and dietary quality in the two weeks preceeding observation was the most influential effect on male travel time – the higher the dietary index was, the more time males spent traveling (Table 5.13).

Table 5.13 Summary of LMM analysis of factors affecting male travel time. N = 85 focal follows.

Final model:	Estimate	SE	P_{MCMC}
Intercept	-1.0344	0.05	0.0001
Dietary quality (14-day NFF score)	0.0037	0.001	0.0016
Autocorrelation term	0.0286	0.03	0.364
Terms removed from the full model:			
Male party size	0.0064	0.007	0.3522
Male rank	-0.01688	0.024697	0.5624
Nulliparous females	0.093522	0.0741	0.2442
Parous females	0.026628	0.062853	0.6768

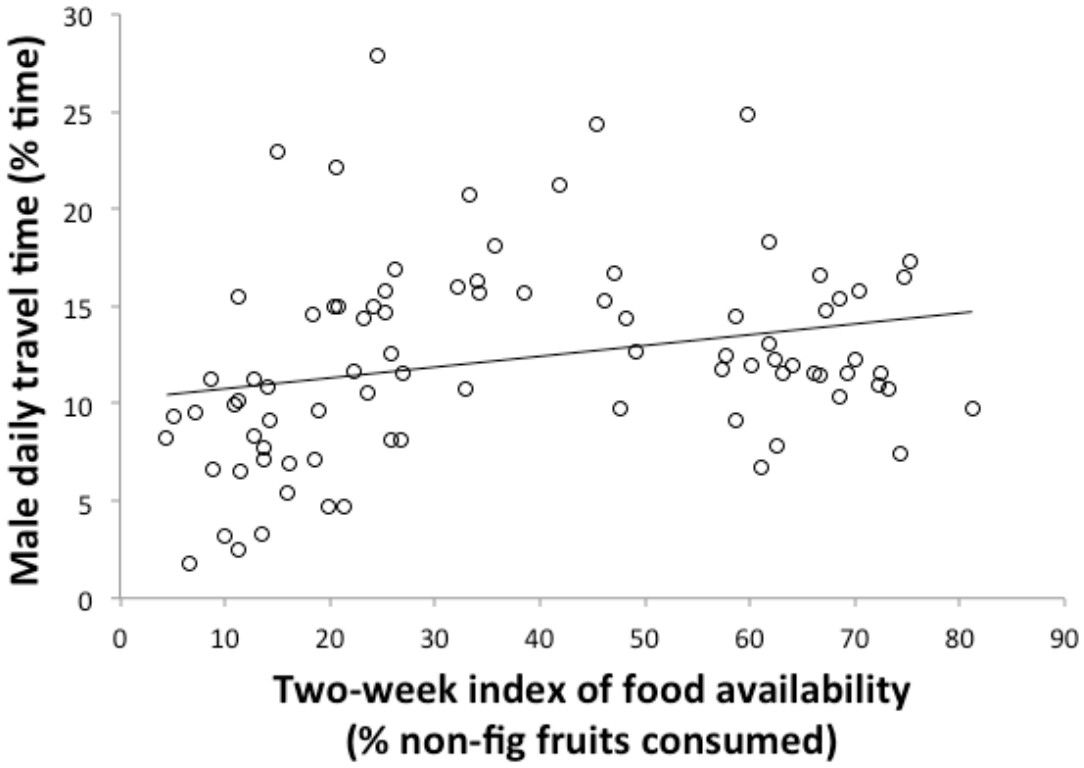


Figure 5.19 Relationship between food availability and male travel time. N = 85 focal follows. Regression line is for illustrative purposes. Analysis was carried out on logit-transformed travel time (see text).

Scarcity of high-quality fruit limited male aggression

Finally, I tested the hypothesis that male competitive effort can be limited by food availability. The full model that I used to examine factors affecting male aggression rates (N = 85 focal follows) was significant when tested against the null (χ^2=32.31, df=6, P<0.0001). The final minimal model was also significant when tested against the null (χ^2=32.14, df=4, P<0.0001). Because male rank and the presence of parous estrous females were involved in significant interaction I do not report their independent effects (Table 5.14).

Table 5.14 Summary of LMM analysis for male rates of aggression

Final model:	**Estimate**	**SEM**	**P_{MCMC}**
Intercept	0.211	0.167	0.138
Dietary quality (14-day NFF score)	0.011	0.003	0.001
Autocorrelation term	0.066	0.066	0.253
Male rank	-	-	-
Presence of parous estrous females	-	-	-
Interaction Rank * Parous females	-0.612	0.183	0.001
Non-significant terms (removed):			
Party size	0.004	0.024	0.972
Interaction Parous females * Dietary quality	-0.003	0.008	0.703

Male dominance rank and the presence of parous estrous females interacted significantly so that the highest-ranking males were more aggressive when parous females were in estrus (interaction rank*parous females: estimate= -0.6±0.183; P=0.001, Figure 5.20). Mean daily party size did not affect aggression rates but the dietary quality in the forest, measured over 14 days prior and up to the day of behavioral observations had a significant impact. When the abundance of non-fig fruits was high, male rates of aggression were higher (0.012±0.003; P=0.001, Fig 5.21). Importantly the effect of dietary quality was independent of the presence of estrous females, as the interaction between the two terms was not significant (estimate= -0.003±0.008; P=0.7, Figure 5.22). During times of nutritional surplus, males therefore allocated more energetic resources to aggressive competition.

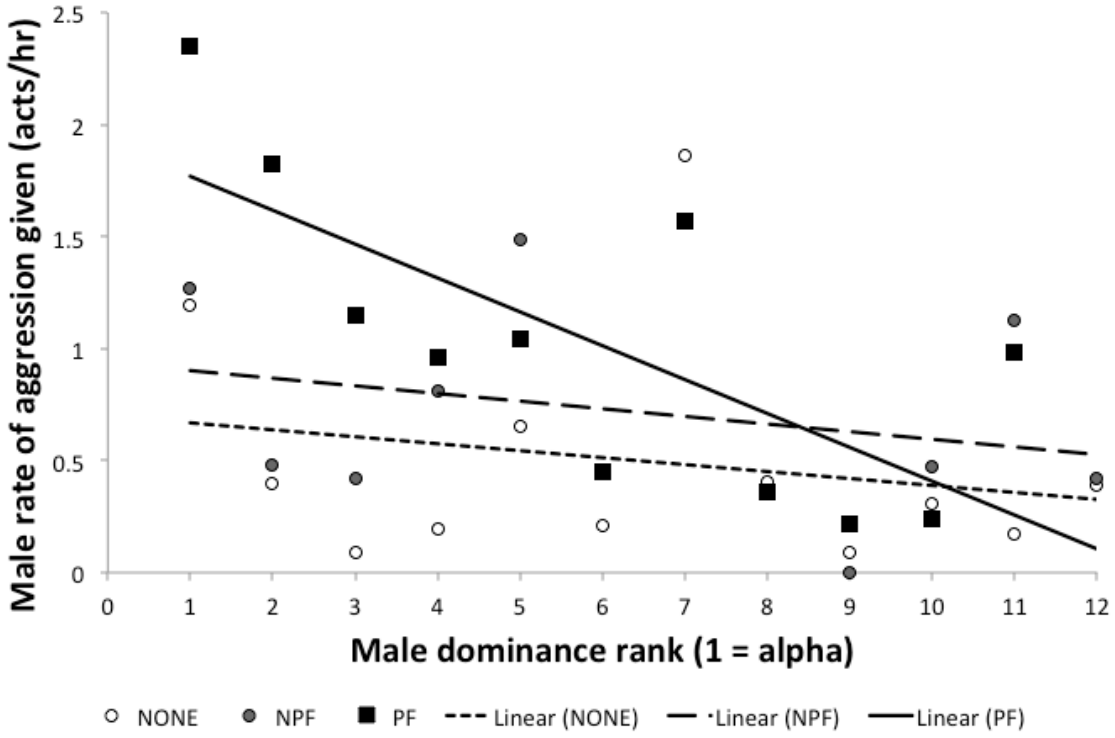


Figure 5.20 A significant interaction between male dominance rank and presence of parous females affected male rates of aggression given. High-ranking males were more aggressive only when parous females were in estrous.

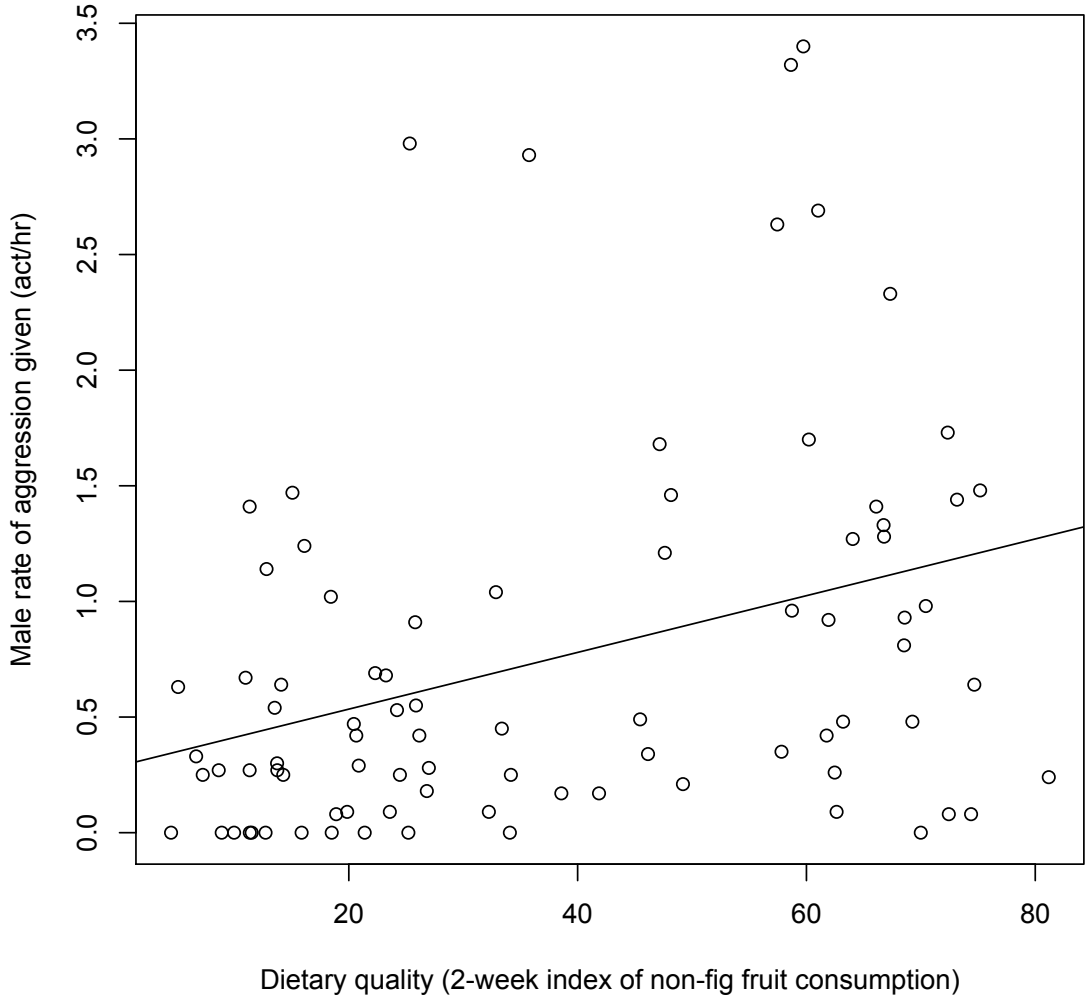


Figure 5.21 Relationship between dietary quality and male rates of aggression given.

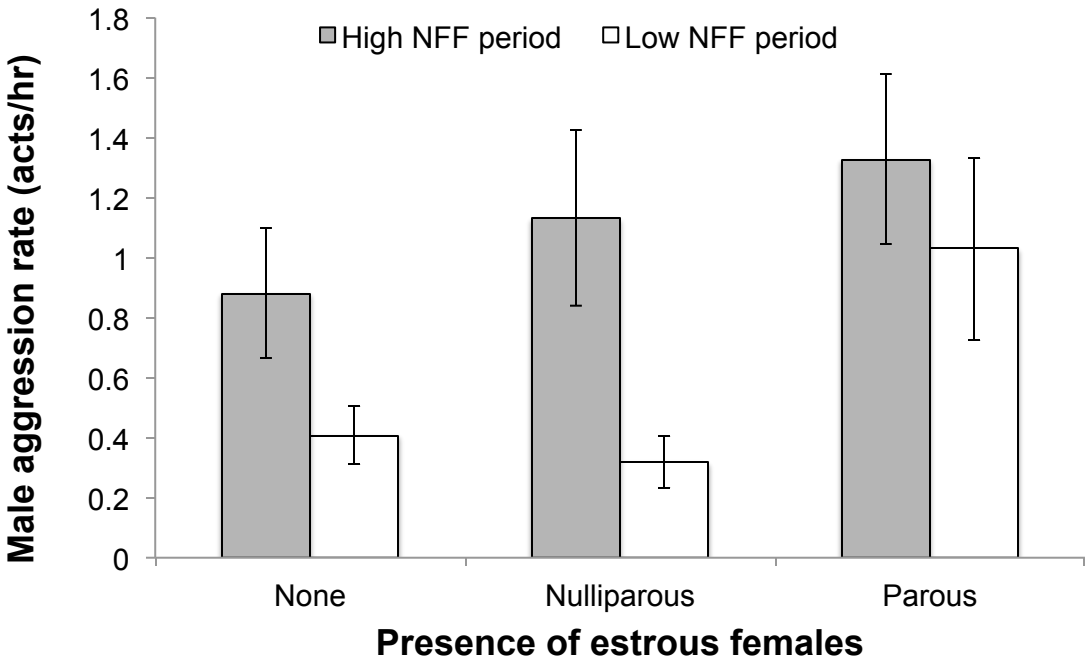


Figure 5.22 Non-significant interaction between fruit availability and presence of estrous females on male aggression rates. The bar charts were calculated on the basis of monthly non-fig fruit consumption rather than the 14-day index used in analysis. High NFF (non-fig fruit) period are months with NFF contributing more than 40% to dietary intake, following Gilby and Wrangham (2007).

DISCUSSION

Dominance status confers significant fitness benefits to male primates (Majolo et al. 2012), including male chimpanzee (Boesch et al. 2006; Wroblewski et al. 2009; Newton-Fisher et al. 2010). In this study I show that male-male competition in chimpanzees, which is essential for obtaining high dominance rank and for improving access to reproductive opportunities, also carries significant energetic costs.

This study extends the findings that mating effort constrains male foraging in chimpanzees (Chapter 4) by showing that males also had lower levels of UCP on days when parous females were in estrus. Males had lower UCP levels on days on which they were more aggressive as well. Multivariate analyses of the causes of variation in male energetic status revealed several important predictors. Among socio-ecological factors, party size had a significant negative impact on UCP production. Among behavioral activity factors, increased travel time and increased rates of aggression also had a negative effect on UCP. Rate of copulation did not affect male energetic status, probably because of the short duration of chimpanzee intromission (mean = 7 seconds; KCP long-term data) in comparison to the prolonged mating behaviors in multiple-mount ejaculators such as rhesus macaques, where ejaculatory copulations have a significant negative impact on male UCP levels, even after controlling for male dominance rank (Higham et al. 2011).

The effect of presence of estrous females in itself was more difficult to interpret in multivariate analyses. Because parous females were the main factor that accounted for the formation of large parties at Kanyawara, it was difficult to assess its independent effect on male energetic condition (i.e. parties containing parous estrous females were never small). When I examined the effects of male party size and the presence of parous estrous females in the parties of focal males in separate linear mixed models and in a multiple regression using residuals of party size scores I showed that although the models containing parous estrous females as a predictor variable had a lower explanatory power

than those incorporating party size as a predictor, the negative effect of parous females on male UCP was also significant. In summary, on days when parous estrous females were present, males traveled in larger parties, were more aggressive and fed less. These inter-related factors together contributed to the significant reduction of UCP levels that males experienced during periods of mating competition (Table 5.15).

Table 5.15 Summary of results

Analysis/model	Unit of analysis	Sample size	Response	No effect	Positive effect	Negative effect
LMM behavioral activity	Male-day	81	Mean daily UCP	Feeding t; RF feeding t; Agro received; Copulation rate		Agro given; Travel
LMM socio-ecology A	Male-day	81	Mean daily UCP	Rank; NPF; PF; RF score; Rank*NPF; Rank*PF		Party size
LMM socio-ecology B	Male-day	81	Mean daily UCP	Rank; RF score; Rank*NPF; Rank*PF		PF; NPF
LMM behavioral activity	Male-day	40	Mean daily UCP	Agro received; Agro given; RF feeding t; Feeding t; Copulation rate		Travel
LMM socio-ecology	Male-day	40	Mean daily UCP	RF score; Rank; PF; Rank*PF		Party size
Bivariate linear regression	Month	11	Mean monthly UCP	PF days; Estrous days (trend); NFF score; RF score	Fig score	Party size
Multiple linear regression	Month	11	Mean monthly UCP	PF days, Estrous days, NFF score, RF score, Fig score		Party size
LMM socio-ecology	Male-month	92	Male monthly UCP	PF-days; Rank*PF-days	RF score; Rank	Party size

Table 5.15 Summary of results (continued)

Analysis/model	Unit of analysis	Sample size	Response	No effect	Positive effect	Negative effect
Friedman test	Males	10	Mean UCP on days with none/NPF/PF in estrous	NPF		PF
Wilcoxon test	Males	9	Mean UCP on days with high vs. low agro given			High aggression
Friedman test	Males	9	Mean UCP on days with no/low/high agro received	Low aggression; high aggression received		
Spearman correlation (within 81 focal follows)	Males	12	Mean male UCP	Travel; Feeding t; RF feeding t; Aggression given; Aggression received; Copulation rate; Rank		Party size
Spearman correlation (within 85 focal follows and the entire urine set)	Males	12	Mean male UCP	Travel; Aggression given; Aggression received; Party size	Feeding time; RF feeding time; Rank	

Table 5.15 Summary of results (continued)

Analysis/model	Unit of analysis	Sample size	Response	No effect	Positive effect	Negative effect
Wilcoxon test	Males	11	Male behavior in small vs. large parties	No difference: RF feeding time	**Larger parties**: Higher rate of aggression given and received; more travel	**Larger parties**: less feeding t; lower UCP levels
Wilcoxon test	Males	8	Male UCP in large parties only with/without PF	PF presence		
Wilcoxon test	Males	8	Male UCP in parties without estrous FF	Large party size (trend P=0.05) tend to have lower UCP but N.S.		Party size (0.05)
UCP = urinary C-peptide; NPF = nulliparous females; PF = parous females; RF = ripe fruit; NFF = non-fig fruit (drupes); LMM = linear mixed model						

How reliable are the urinary C-peptide (UCP) measures as indicators of the costs of male-male competition?

Considering the fact that UCP is the key measure of energetic costs in this study, how confident can we be that these findings are meaningful in regard to the hypotheses and are not confounded by the significant inter- and intra-individual variation in the production of this marker (e.g. Chapter 3)? To evaluate the proximate factors that account for variation in male UCP levels I made several predictions. First, I expected that UCP would be negatively correlated with behavioral measures of energy expenditure such as time spent traveling, rates of aggression given and copulation rates. Second, I also predicted that feeding time and ripe fruit feeding time should be positively associated with UCP levels. Crucially, I tested each of these predictions at the level of single-day focal follows by using linear mixed effects model (LMM) analyses that account for repeated observations of the same individuals and for temporal autocorrelation between successive observations. Because of the significant diurnal variation in UCP levels identified in Chapter 3, I conducted these analyses at two levels. The first LMM used all focal 81 follows, for which at least one urine sample per day was available. It supported the prediction for male aggression rates and travel time. Despite the fact that 27.2% of the focal observations in this dataset relied on a single urine sample to estimate 'average daily energetic balance' the data revealed a meaningful relationship between two of the predictors and UCP levels. To confirm that these findings were robust I also carried out the same analysis in a smaller dataset comprising of only 40 focal follows for which at least

3 urine samples per day were available. This dataset was expected to provide a more robust measure of 'average daily energetic balance' because each focal follow relied on multiple urine samples to derive an average UCP value for subsequent analysis. Travel time retained a significant negative effect on male UCP levels but aggression given did not. This can be explained by the reduced by half sample size of follows in the latter analysis, thus omitting much of the inter-follow variation in aggressive behavior that was present in the full 81-day focal dataset.

The general concordance between these two LMM suggests that despite much variation in UCP output with time of day and other factors, given a large sample of observation days, even single-spot urine measures of UCP can prove valuable in evaluating inter- and intra-individual differences in energetic balance. These results show also that UCP is not simply a measure of energy intake but also correlates with behavioral measures of energy expenditure. This supports the role of UCP as an integrated measure of energy balance (Sherry and Ellison 2007; Emery Thompson and Knott 2008; Emery Thompson et al. 2009).

In addition, I analyzed UCP levels at the level of individual males. Average male UCP levels measured across all urine samples collected for each of those males during focal observations did not correlate with any of the behavioral factors examined. This discrepancy in results between the outcome of LMM analysis and correlation analyses indicates that averaging all urine samples within males masked important intra-individual variation that is accounted for by differences in male activity.

Taken together, these findings represent an important advance in the application of UCP to field studies of primate energetics. I demonstrated for the first time that UCP can be successfully used to quantify individual variance in energetic costs and benefits under wild conditions not only on the scale of weeks, months, or seasons as shown by previous research (Emery Thompson and Knott 2008; Emery Thompson et al. 2009; Georgiev et al. 2011; Higham et al. 2011) but also on a day-to-day basis. While one previous study was also successful in relating daily UCP measures obtained from single urine samples to daily food availability (Harris et al. 2010), the findings presented in this chapter expand significantly our understanding of UCP dynamics in relation to both socio-ecological factors and physical activity. Logistical constraints on obtaining sufficient and frequent sampling coverage are likely to be the main limitation in the future use of UCP to monitor short-term changes in the energy balance in wild animals.

Is male competitive ability in chimpanzees energetically constrained?

For female chimpanzees, as for many other mammals, high-quality diet is essential for their reproductive function (Emery Thompson and Wrangham 2008a). Females that feed better and maintain higher energy reserves are more fecund, produce offspring more frequently and their offspring have improved survivorship (Pusey et al. 1997; Emery Thompson et al. 2007b). Conversely, male fecundity is not constrained by energy availability (Muller and Wrangham

2005) and thus access to energy is expected to have a minor role for male reproductive strategies. Nevertheless, high-ranking males prefer to feed higher in the trees where fruit density and quality are greater (Kahlenberg 2006; Houle et al. 2007). Although males range more widely than females (Wrangham and Smuts 1980; Bates and Byrne 2009), when traveling alone they retain the ranging habits of their mothers throughout adulthood regardless of changes in their own social status (Murray et al. 2008). This long-term ranging area fidelity suggests that the foraging benefits that familiarity with the terrain may bring are of some importance to males, too (Murray et al. 2008).

This study provides an explanation for the reasons males may benefit from increased foraging efficiency and identifies a mechanism, through which energy acquisition may improve their success in the competition for status and sex. The finding that male mating effort and aggressive behavior have a measureable and negative impact on male energetic balance, as assessed via UCP levels, indicates that male chimpanzee competitive ability is energy dependent. Those males who are successful in obtaining more energy quicker than their competitors are likely to become dominant and sire more offspring because they will be able to allocate more energy to aggressive competition. The positive association between non-fig ripe fruit abundance and male rates of aggression further supports the claim that when males have surplus energy, they are more likely to increase their competitiveness.

The role of ecological variation in food availability for the expression of competitive behavior has additional implications beyond the level of individual

reproductive strategies. The aggressive inter-group interactions in chimpanzees and the expansion of their home ranges that may result from such interactions have important fitness consequences. Enlarging of the home range improves food availability in their territory and with that females increase their reproductive rate (Williams et al. 2004). If energy availability has a positive impact on male competitive ability not just at the individual but also at the community level, we could predict outcomes of inter-group contests among chimpanzees and territorial primates and mammals. Groups that inhabit territories richer in food may be able to invest more energy in aggressive dominance and eventually expand their territory at the expense of their neighbors. This is indeed happening with the Ngogo chimpanzees in Kibale (Mitani et al. 2010), which are not only the largest known community but also have access to particularly rich feeding resources throughout the year (Potts et al. 2011; Watts et al. 2012). The idea that nutrient access plays an important role in aggressive competition has found firm support in experiments with Argentine ants (Grover et al. 2007) but for species that are difficult to manipulate in laboratory conditions such as chimpanzees, testing such hypotheses may require further decades of behavioral and ecological observations at multiple field sites.

Energetic costs of dominance: why do high-ranking males have lower UCP levels?

The analyses in this study replicate the finding of Emery Thompson et al. (2009) that high-ranking males have lower UCP levels. Despite the different temporal scale on which urine sampling was carried out (over 11 months during

this study; over 8 years in the earlier study), the two studies concur: male dominance rank is associated with low energy balance. If we compare the activity profiles of males of different rank, there is only one behavior that is significantly associated with dominance – agonism. Neither male travel time nor mean party size were affected by the rank of the focal male. In Chapter 4 I also showed that while there was a trend for higher-ranking males to spend less time feeding, this effect was not significant. Ripe fruit intake was not related to rank, either. The only major difference in behaviors related to energy intake or expenditure was the rate of aggression that males engaged in – high-ranking males are the most aggressive. Given the effect of aggression rates on male UCP levels identified in one of the multivariate analyses, low energetic balance of dominant male chimpanzees is best explained as a result of the high metabolic costs of aggression.

The effect of seasonal reproduction and fission-fusion grouping on the energetics of male-male competition in primates

The costs of male-male competition in chimpanzees and their distribution across the dominance hierarchy reveal similarities but also important differences to what we know about the energetics of male reproduction in other primates. Like rhesus macaque males (Higham et al. 2011), male chimpanzees incur significant energetic costs when estrous females are available. Unlike in rhesus, however, the negative association between rank and UCP levels among male chimpanzees did not hold during mating periods. While dominant rhesus have higher UCP during the birth season, and lower UCP during the mating season

(Higham et al. 2011), in male chimpanzees this relationship was partially reversed. The negative association between rank and UCP levels was significant only when we considered urine samples collected on days without parous estrous females. When parous females were in estrus, the association between male rank and UCP was no longer significant.

These results show that high-ranking male chimpanzees do not or cannot improve their energetic condition in the way that dominant rhesus are able to outsides periods of mating competition. On the other hand, while all males experience reduced energy balance during periods of mating competition, high-ranking males did not incur greater costs than lower-ranking individuals. Yet overall, high ranking male chimpanzees have lower UCP levels than subordinates. In comparison to rhesus macaques which invest in mating and aggressive competition primarily during the breeding season and use the rest of the year to gain fat, high-ranking male chimpanzees exist in a perpetual state of 'endurance rivalry'. When estrous females are not available, aggression still has an important role in maintaining the dominance hierarchy and high-ranking males must constantly channel energetic resources towards agonistic behavior. This interpretation fits hormonal data showing that high-ranking male chimpanzees maintain higher levels of testosterone production, relative to subordinates at all times – a result of the inherent social instability of the fission-fusion organization of chimpanzee society (Muller and Wrangham 2004a). This pattern of mating effort suggests important differences in allocation of energy towards reproduction among primates with seasonal breeding, relative to those without a distinct

mating period. The lack of seasonal mating periods in chimpanzees, together with the fluid hierarchy in the context of fission-fusion grouping, creates unique pressure on males who strive to obtain and sustain high position within their communities. For chimpanzees therefore condition or 'individual quality' seems no less important than for seasonal breeders engaging in 'endurance rivalry' such as rhesus macaques. Only males that are able to maintain energetic investment towards reproductive effort year-round would be able to retain high rank for long periods of time.

Reproductive energetics and life history theory

Inter-species differences in the relative costs of mating effort and their temporal distribution may have important consequences for male life history trajectories. A central concept of life history theory is the trade-off between current reproduction and future survival (Stearns 1992). This trade-off is used to explain both sex-specific and inter-individual differences in survivorship. Costs incurred during reproductive effort are expected to contribute to increasing mortality among individuals at a later time. The magnitude of such costs thus may determine an individual's lifetime fitness. Comparing species that exhibit differences in the temporal pattern of reproductive costs may also help explain differences in life-history parameters. For example, male rhesus macaques, suffer increased mortality during the breeding season (Hoffman et al. 2008) but whether this mortality is higher for those who allocated most to their reproductive effort is not known. Among other species there is evidence that trade-offs may only apply to those individuals who are not in prime condition. Often the most

reproductively successful males do not suffer decreased survival, despite their greater investment in current reproduction (McElligott and Hayden 2000; Galimberti et al. 2007). Such observations further underline the importance of male 'quality' for reproductive success. I suggest that among male chimpanzees the temporal distribution of costs of mating effort provides conditions under which the trade-off between current reproductive investment and future survival should not apply. This suggestion is supported by the fact that some males manage to maintain their alpha position in the dominance hierarchy for over a decade (e.g. Imoso at Kanyawara, 1997-2009) despite the potentially detrimental high levels of cortisol and testosterone that they maintain (Muller and Wrangham 2004a; 2004b). Preliminary analysis of male chimpanzee longevity suggest that alpha males do indeed have longer life-span than lower-ranking individuals (McCarthy et al. 2011). An approach to the study of reproductive effort in male primates that unites the measurement of costs over the short term with data on survivorship over the long-term is an exciting prospect for future research on sexual selection.

CHAPTER 6
Conclusions

Aggressive behavior plays an important role in the life of male chimpanzees. My thesis research focused on one aspect of male aggression – the competition for dominance and mating opportunities and, specifically, the energetics costs that they incur. To understand why a certain behavior evolves and is maintained in a population or a species we must appreciate not only the benefits that it brings in terms of reproductive fitness but also understand the costs that are associated with it. Identifying those costs, in the case of my study system, will allow us to distinguish the factors that contribute to a male chimpanzee's achieving high social rank and siring many offspring in his lifetime. Conversely, these costs will also help identify the reasons why some males never reach the alpha position and father fewer or no infants.

To this end I used a combination of behavioral observations and non-invasive measures of urinary C-peptide (UCP) of wild chimpanzees at Kanyawara, Kibale National Park, Uganda to assess the impact that male competition has on energy intake and expenditure. I specifically aimed to identify if the males who are generally more reproductively successful (high-ranking males and the alpha in particular) suffer energetic costs of higher magnitude than others in their group.

The results of my study support the prediction that male competition for status and mating opportunities incurs tangible energetic costs. The data in support of the second prediction (that high-ranking males incur greater costs) is

less easy to interpret. The lack of significant interaction between dominance rank and presence of parous estrous females in the analyses of costs indicates that all males incur similar costs of mating effort. In that sense, high-ranking males do not incur greater costs during periods of mating competition than lower-ranking individuals. However, this conclusion changes when we consider the costs of male-male competition outside the mating context.

From a previous study on the relationship between male rank and UCP levels in the Kanyawara community we know that high-ranking males have lower UCP (Emery Thompson et al. 2009). The data presented in this dissertation replicate this finding (Chapter 5) but also identify an important detail. Male UCP levels were negatively associated with high rank only when we considered urinary data collected outside the mating context. On days when parous estrous females were present the relationship between rank and UCP was no longer significant (Chapter 5). Taken together these results indicate a clear difference between the strategies that high-ranking and low-ranking males pursue. The high-ranking males (the alpha, in particular) continuously allocate significant energetic resources to aggressive competition both in the absence and in the presence of mating opportunities. Conversely, lower-ranking individuals allocate energetic resources to mating competition only during periods when estrous females are available.

An analysis of the aggression rates of males of different rank in the presence and absence of parous estrous females shows an additional characteristic of the strategies that males of different rank pursue. While the four

highest-ranking males invested heavily in aggression (see Figure 5.20 in Chapter 5), most lower-ranking individuals did not increase, or even decreased, their rate of aggression. Their approach to mating competition might thus be characterized as a 'sit-and-wait' game, whereby they follow the estrous female as she moves from one feeding patch to another as close to her as the higher-ranking males would tolerate. They would often sit inactive under the feeding trees, where the estrous female was feeding, usually with some of the highest-ranking males feeding in close proximity to her and wait for an opportunity to mate. Lower-ranking individuals therefore trade-off feeding time for opportunistic waiting (for a copulation) and high-ranking males pursue a more active strategy by increasing their aggression towards males that follow the female and monitor her from an even closer distance (which may also allow them to feed while doing so).

While there is certainly a great deal of variation in the contexts, in which mating occurs in chimpanzees, this scenario fits some of my observations at Kanyawara and also suggests an explanation for the similar energetic costs of mating effort observed among males of different rank. More detailed data on spatial proximity between estrous females and males, as well as concurrent focal observations on the feeding activity on all males in sight would be required to test this prediction. Unfortunately these data are not available from my study because I focused observation on one male at a time.

The ability of alpha males to continuously invest in aggressive displays and competition sets them apart from all other males in the community who only expend energy when immediate sexual benefits are available. This pattern of

year-round energetic investment into status competition also sets dominant male chimpanzees apart from males in some other species where dominance hierarchies are important for reproductive success. Dominant rhesus macaques on Cayo Santiago Island feed better and have higher UCP levels than all other males outside the breeding season (Higham et al. 2011). They accumulate energy stores that then allow them to outcompete other males in endurance rivalries during a 6-month breeding season. Thus among rhesus macaque males, high rank and UCP are positively associated during the non-breeding (birthing) season and negatively associated at the end of the breeding season (Higham et al. 2011). These observations suggest that mating competition is more intense among rhesus macaque males than among chimpanzees. Alternatively, regular provisioning with monkey chow on Cayo Santiago Island may be responsible for creating unusual energetic conditions that allow males to build fat reserves in the birthing season to a level not possible in the wild. The difference in UCP dynamics at the species level suggested here might partly be accounted for different levels of food availability between Cayo Santiago and Kanyawara. More studies on wild populations would provide important context for interpreting these differences.

Less intense mating competition among chimpanzees than among rhesus macaques is supported by data on sexual dimorphism in canine size (Plavcan 2004) but unfortunately comparative data on energetic costs on mating effort in other primates are currently unavailable to carry out a wider test the prediction that intensity of sexual selection on male aggressive behavior would be positively

associated with male costs of mating effort. I suggest that using a behavioral rather than a morphological measure of the intensity of sexual selection (e.g. relative male mating costs) could provide higher-resolution in studies examining factors that affect the intensity of sexual selection. Behavioral traits in general are less phylogenetically conserved than morphological traits (Bloomberg et al. 2003) and thus can also be more usefully applied at the intra-species level.

Using the costs of mating effort as a proxy for the intensity of sexual selection for male competitive ability can also help interpret inter-species differences in male reproductive strategies as well as to evaluate the relative contribution of the different mechanisms of sexual selection (i.e. mate choice, inter-sexual and intra-sexual competition; contests, scrambles and endurance rivalries) in shaping male reproductive success.

Sexual selection theory provides a strong explanation for sex-differences in aggression among modern humans (Archer 2009; Puts 2010). It also explains why bonobo males are less aggressive than chimpanzee males. Hare *et al*. (2012) recently presented evidence for the 'self-domestication hypothesis' according to which a suite of juvenilized morphological, behavioral, cognitive and developmental traits of bonobos (relative to chimpanzees) are due to selection against aggression, similar to what occurs during the domestication of animals. Their argument essentially is a sexual selection one even though Hare *et al.* do not explicitly acknowledge that. The conclusion, which follows from their argument, is that bonobo male reproductive strategies have been shaped by a reduction in sexual selection for aggressive male-male competition, relative to

chimpanzees. That this is the case can be inferred from the generally lower rates of male aggression in bonobos. However to discriminate from other explanations for a reduction in male aggression in this species we must show that bonobos males have lower costs of mating effort than chimpanzees do.

A recent study of testosterone levels in wild bonobos suggests that this might be the case. Even though bonobo males establish and maintain a dominance hierarchy, and high rank has some a role in increasing male fitness (Gerloff et al. 1999), there is a crucial difference between the reproductive physiology of bonobo and chimpanzee males that indicates a lower metabolic costs of mating effort for bonobos. High-ranking male chimpanzees maintain high testosterone levels year-round and they are elevated in the presence of estrous parous females (Muller and Wrangham 2004a). In other words, testosterone is positively correlated both with dominance rank and with male rates of aggression. In bonobos, however, there is no association between testosterone and rank or testosterone and rates of aggression (Surbeck et al. 2012). It was also among the lowest-ranking males that testosterone was elevated most when estrous females were present (Surbeck et al. 2012). Because testosterone increases metabolic rates and thus energy consumption, even in a resting state (Tsai and Sapolsky 1996) these data suggest that the overall energetic costs of mating effort would be lower in bonobos, in comparison to what my study demonstrated for chimpanzees.

In summary, I suggest that studies of sexual selection in primates have much to gain from adopting an approach that explicitly considers not only the

benefits but also the costs associated with the diverse male reproductive strategies that are seen in this mammalian order. Charting how the variation in the costs of mating competition is reflected or not in morphological measures of sexual dimorphism across primates and more generally mammals will further aid the interpretation of the hominin fossil record, if only to confirm previous conclusions (Plavcan and van Schaik 1997) that some questions in the study of human evolution may never be resolved beyond a reasonable doubt.

BIBLIOGRAPHY

Abbott D, Keverne E, Bercovitch F, Shively C, Mendoza S, Saltzman W, Snowdon C, Ziegler T, Banjevic M, Garland T, Sapolsky R (2003) Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior 43:67-82

Ahmed M, Gannon MG, Nuttall FQ (1976) Postprandial plasma glucose, insulin, glucagon and triglyceride responses to a standard diet in normal subjects. Diabetologica 12:61-67

Aiello L, Wells J (2002) Energetics and the evolution of the genus *Homo*. Annual Review of Anthropology 31:323-338

Aiello L, Wheeler P (1995) The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. Current Anthropology 36:199-221

Aiello LC, Key C (2002) Energetic consequences of being a Homo erectus female. American Journal of Human Biology 14:551-565

Alberts SC, Altmann J, Wilson ML (1996) Mate guarding constrains foraging activity of male baboons. Animal Behaviour 51:1269-1277

Alberts SC, Buchan JC, Altmann J (2006) Sexual selection in wild baboons: from mating opportunities to paternity success. Animal Behaviour 72:1177-1196

Altmann J (1974) Observational study of behavior - sampling methods. Behaviour 49:227-267

Altmann J, Muruthi P (1988) Differences in daily life between semiprovisioned and wild-feeding baboons. American Journal of Primatology 15:213-221

Amsler SJ (2010) Energetic costs of territorial boundary patrols by wild chimpanzees. American Journal of Primatology 72:93-103

Ancona S, Drummond H, Zalvidar-Rae J (2010) Male whiptail lizards adjust energetically costly mate guarding to male-male competition and female reproductive value. Animal Behaviour 79:75-82

Andersson M (1994) Sexual Selection. Princeton University Press, Princeton, NJ

Anestis S, Bribiescas R (2004) Rapid changes in chimpanzee (*Pan troglodytes*) urinary cortisol excretion. Hormones and Behavior 45:209-213

Archer J (2009) Does sexual selection explain human sex differences in aggression? Behavioral and Brain Sciences 32:249-311

Asensio N, Korstjens AH, Schaffner CM, Aureli F (2008) Intragroup aggression, fission-fusion dynamics and feeding competition in spider monkeys. Behaviour 145:983-1001

Baayen RH (2008) Analyzing Linguistic Data. Cambridge University Press, Cambridge

Baayen RH (2011) languageR: Data Sets and Functions with "Analyzing Linguistic Data: A practical introduction to statistics" R package version 1.2. R foundation for Statistical Computing. http://r-project.org, Vienna

Barelli C, Reichard UH, Mundry R (2011) Is grooming used as a commodity in wild white-handed gibbons, *Hylobates lar*? Animal Behaviour 82:801-809

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349-368

Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. In. R Foundation for Statistical Computing. http://r-project.org, Vienna

Bates LA, Byrne RW (2009) Sex differences in the movement patterns of free-ranging chimpanzees (*Pan troglodytes schweinfurthii*): foraging and border checking. Behavioral Ecology and Sociobiology 64:247-255

Bercovitch FB (1997) Reproductive strategies of rhesus macaques. Primates 38:247-263

Bercovitch FB, Nurnberg P (1996) Socioendocrine and morphological correlates of paternity in rhesus macaques (*Macaca mulatta*). Journal of Reproduction and Fertility 107:59-68

Besser REJ, Ludvigsson J, Jones AG, McDonald TJ, Shields BM, Knight BA, Hattersley AT (2011) Urine C-peptide creatinine ratio is a noninvasive alternative to the mixed-meal tolerance test in children and adults with type 1 diabetes. Diabetes Care 34:607-609

Bliege Bird R, Smith EA, Bird DW (2001) The hunting handicap: costly signaling in human foraging strategies. Behavioral Ecology and Sociobiology 50:9-19

Bloomberg SP, Garland JR T, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:715-745

Boesch C (1996) Social grouping in Tai chimpanzees. In: McGrew W, Marchant L, Nishida T (eds) Great Ape Societies. Cambridge University Press, Cambridge, pp 101-113

Boesch C, Kohou G, Nene H, Vigilant L (2006) Male competition and paternity in wild chimpanzees of the Tai forest. American Journal of Physical Anthropology 130:103-115

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-S (2008) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127-135

Bowman P, McDonald TJ, Shields BM, Knight BA, Hattersley AT (2011) Validation of a single-sample urinary C-peptide creatinine ratio as a reproductible alternative to serum C-peptide in patients with Type 2 diabetes. Diabetic Medicine DOI: 10.1111/j.1464-5491.2011.03428.x

Bradley BJ (2008) Reconstructing phylogenies and phenotypes: a molecular view of human evolution. Journal of Anatomy 212:337-353

Brandenburg D (2008) History and diagnostic significance of C-peptide. Experimental Diabetic Research 2008

Bribiescas RG (2001) Reproductive ecology and life history of the human male. Yearbook of Physical Anthropology, Vol 44 44:148-176

Bribiescas RG (2006) Men: Evolutionary and Life History. Harvard University Press, Cambridge, MA

Bribiescas RG, Ellison PT, Gray PB (in press) On the evolution of male life history variability and the emergence of the genus *Homo*: New directions and perspectives. Current Anthropology

Briffa M, Sneddon LU (2007) Physiological constraints on contest behaviour. Functional Ecology 21:627-637

Brivio F, Grignolio S, Apollonio M (2010) To feed or not to feed? Testing different hypotheses on rut-induced hypophagia in a mountain ungulate. Ethology 116:406-415

Bronikowski AM, Altmann J, Brockman DK, Cords M, Fedigan LM, Pusey A, Stoinski T, Morris WF, Strier KB, Alberts SC (2011) Aging in the natural world: comparative data reveal similar mortality patterns across primates. Science 331:1325-1328

Buyken AE, Kellerhoff Y, Hahn S, Kroke A, Remer T (2006) Urinary C-peptide excretion in free-living healthy children is related to dietary carbohydrate intake but not to the dietary glycemic index. THe Journal of Nutrition 136:1828-1833

Bygott D (1974) Agonistic behavior and dominance in wild chimpanzees. Ph.D. thesis. Cambridge University, UK.

Bygott J (1979) Agonistic behavior, dominance and social structure in wild chimpanzees of the Gombe National Park. In: Hamburg D, McCown E (eds) The Great Apes. Benjamin Cummings, Menlo Park, CA

Carmody RN, Weintraub GS, Wrangham RW (2011) Energetic consequences of thermal and nonthermal food processing. Proceedings of the National Academy of Sciences of the United States of America 108:19199-19203

Chapman CA, White FJ, Wrangham RW (1994) Party size in chimpanzees and bonobos: a reevaluation of theory based on two similarly forested sites. In: Wrangham RW, McGrew WC, de Waal FBM, Heltne PG (eds) Chimpanzee Cultures. Harvard University Press, Cambridge, MA

Chapman CA, Wrangham RW (1993) Range use of the forest chimpanzees of Kibale: Implications of the understanding of chimpanzee social organization. American Journal of Primatology 31:263-273

Chen FC, Li WH (2001) Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees. American Journal of Human Genetics 68:444-456

Chuang-Dobbs HC, Webster MS, Holmes RT (2001) The effectiveness of mate guarding by male black-throated blue warblers. Behavioral Ecology 12:541-546

Clauss M, Streich WJ, Nunn CL, Ortmann S, Hohmann G, Schwarm A, Hummel J (2008) The influence of natural diet composition, food intake level, and body size on ingesta passage in primates. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 150:274-281

Clutton-Brock TH (1998) Reproductive skew, concessions and limited control. Trends in Ecology and Evolution 13:288-292

Clutton-Brock TH (2004) What is sexual selection? In: Kappeler PM, van Schaik CP (eds) Sexual Selection in Primates: New and Comparative Perspecitves. Cambridge University Press, Cambridge

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE (1979) The logical stag: adaptive aspects of fighting in red deer (*Cervus celaphus* L.). Animal Behaviour 27:211-225

Clutton-Brock TH, Guiness FE, Albon SD (1982) Red Deer: Behavior and Ecology of Two Sexes. University of Chicago Press, Chicago

Clutton-Brock TH, Isvaran K (2007) Sex differences in ageing in natural populations of vertebrates. Proceedings of the Royal Society B: Biological Sciences 274:3097-3104

Codding BF, Bird RB, Bird DW (2011) Provisioning offspring and others: risk-energy trade-offs and gender differences in hunter-gatherer foraging strategies. Proceedings of the Royal Society B-Biological Sciences 278:2502-2509

Collier JF, Rosaldo MZ (1981) Politics and gender in simple societies. In: Ortner SB, Whitehead H (eds) Sexual Meanings: The Cultural Construction of Gender and Sexuality. Cambridge University Press, Cambridge, pp 275-329

Conklin-Brittain N, Knott C, Wrangham RW (2006) Energy intake by wild chimpanzees and orangutans: Methodological considerations and a preliminary comparison. In: Hohmann G, Robbins M, Boesch C (eds) Feeding ecology in apes and other primates. Cambridge University Press, Cambridge, pp 445-472

Conklin-Brittain NL, Wrangham RW, Hunt KD (1998) Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. II. Macronutrients. International Journal of Primatology 19:971-998

Creel S, Sands J (2003) Is social stress a consequence of subordination or a cost of dominance? In: de Waal F, Tyack P (eds) Animal social complexity. Harvard University Press, Cambridge, MA, pp 153-169

Crocker DE, Houser DS, Webb PM (2012) Impact of body reserves on energy expenditure, water flux, and mating success in breeding male northern elephant seals. Physiological and Biochemical Zoology 85:11-20

Daly ME, Vale C, Walker M, Littlefield A, Alberti KGMM, Mathers JC (1998) Acute effects on insulin sensitivity and diurnal metabolic profiles of a high-sucrose compared with a high-starch diet. American Journal of Clinical Nutrition 67:1186-96

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

de Vries H (1998) Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. Animal Behaviour 55:827-843

de Vries H, Appleby MC (2000) Finding an appropriate order for a hierarchy: a comparison of the I&SI and the BBS methods. Animal Behaviour 59:239-245

de Vries H, Netto WJ, Hanegraaf PLH (1993) Matman: a program for the analysis of sociometric matrices and behavioural transition matrices. Behaviour 125:159-175

de Waal FBM (1986) The brutal elimination of a rival among captive male chimpanzees. Ethology and Sociobiology 7:237-251

Deschner T, Heistermann M, Hodges J, Boesch C (2004) Female sexual swelling size, timing of ovulation, and male behavior in wild West African chimpanzees. Hormones and Behavior 46:204-215

Deschner T, Kratzsch J, Hohmann G (2008) Urinary C-peptide as a method for monitoring body mass changes in captive bonobos (*Pan paniscus*). Hormones and Behavior 54:620-6

Dubuc C, Muniz L, Heistermann M, Engelhardt A, Widdig A (2011) Testing the priority-of-access model in a seasonally breeding primate species. Behavioral Ecology and Sociobiology 65:1615-1627

Ducey PK, Heuer J (1991) Effects of food availability on intraspecific aggression in salamanders of the genus *Ambystoma*. Canadian Journal of Zoology 69:288-290

Duffy KG, Wrangham RW, Silk JB (2007) Male chimpanzees exchange political support for mating opportunities. Current Biology 17:R586-R587

Ellison P (2003) Energetics and reproductive effort. American Journal of Human Biology 15:342-351

Ellison PT (2008) Energetics, reproductive ecology, and human evolution. PaleoAnthropology:172-200

Ellison PT, Valeggia CR (2003) C-peptide levels and the duration of lactational amenorrhea. Fertility and Sterility 80:1279-1280

Emery M, Whitten P (2003) Size of sexual swellings reflects ovarian function in chimpanzees (*Pan troglodytes*). Behavioral Ecology and Sociobiology 54:340-351

Emery Thompson M (2005) Endocrinology and ecology of female chimpanzee reproduction. PhD dissertaion. Harvard University, Cambridge, MA

Emery Thompson M, Jones JH, Pusey AE, Brewer-Marsden S, Goodall J, Marsden D, Matsuzawa T, Nishida T, Reynolds V, Sugiyama Y, Wrangham RW (2007a) Aging and fertility patterns in wild chimpanzees provide insights into the evolution of menopause. Current Biology 17:2150-2156

Emery Thompson M, Kahlenberg SM, Gilby IC, Wrangham RW (2007b) Core area quality is associated with variance in reproductive success among female chimpanzees at Kibale National Park. Animal Behaviour 73:501-512

Emery Thompson M, Knott CD (2008) Urinary C-peptide of insulin as a non-invasive marker of energy balance in wild orangutans. Hormones and Behavior 53:526-535

Emery Thompson M, Muller MN, Kahlenberg SM, Wrangham RW (2010) Dynamics of social and energetic stress in wild female chimpanzees. Hormones and Behavior 58:440-449

Emery Thompson M, Muller MN, Wrangham RW, Lwanga JS, Potts KB (2009) Urinary C-peptide tracks seasonal and individual variation in energy balance in wild chimpanzees. Hormones and Behavior 55:299-305

Emery Thompson M, Wrangham R (2008a) Diet and reproductive function in wild female chimpanzees (*Pan troglodytes schweinfurthii*) at Kibale National Park, Uganda. American Journal of Physical Anthropology 135:171-181

Emery Thompson M, Wrangham RW (2008b) Male mating interest varies with female fecundity in *Pan troglodytes schweinfurthii* of Kanyawara, Kibale National Park. International Journal of Primatology 29:885-905

Emlen ST, Oring LW (1977) Ecology, sexual selection and the evolution of mating systems. Science 197:215-223

Fabiani A, Galimberti F, Sanvito S, Hoelzel AR (2004) Extreme polygyny among southern elephant seals on Sea Lion Island, Falkland Islands. Behavioral Ecology 15:961-969

Fichtel C, Kraus C, Ganswindt A, Heistermann M (2007) Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreauxi's sifakas (*Propithecus verreauxi*). Hormones and Behavior 51:640-648

Foley R, Gamble C (2009) The ecology of social transitions in human evolution. Philosophical Transactions of the Royal Society B-Biological Sciences 364:3267-3279

Forstmeier W, Schielzeth H (2011) Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behavioral Ecology and Sociobiology 65:47-55

Foster MW, Gilby IC, Murray CM, Johnson A, Wroblewski EE, Pusey AE (2009) Alpha male chimpanzee grooming patterns: implications for dominance "style". American Journal of Primatology 71:136-144

Furuichi T (2009) Factors underlying party size differences between chimpanzees and bonobos: a review and hypotheses for future study Primates 50:197-209

Galgani JE, de Jonge L, Rood JC, Smith SR, Young AA, Ravussin E (2010) Urinary C-peptide excretion: A novel alternate measure of insulin sensitivity in physiological conditions. Obesity 18:1852-1857

Galimberti F, Sanvito S, Braschi C, Boitani L (2007) The cost of success: reproductive effort in male southern elephant seals (*Mirounga leonina*). Behavioral Ecology and Sociobiology 62:159-171

Georgiev AV, Emery Thompson M, Lotana AL, Wrangham RW (2011) Seed predation by bonobos (*Pan paniscus*) at Kokolopori, Democratic Republic of the Congo. Primates 52:309-314

Georgiev AV, Lokasola AL, Nkanga L, Lokondja A, Nsala J, Likenge J, Ilanga-Bomanga A, Likenge JP (2010) New observations of the terrestrial holoparasite *Chlamydophytum aphyllum* Mildbr. and its consumption by bonobos at Kokolopori, Democratic Republic of Congo. African Journal of Ecology 48:849-852

Gerloff U, Hartung B, Fruth B, Hohmann G, Tautz D (1999) Intracommunity relationships, dispersal pattern and paternity success in a wild living community of Bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. Proceedings of the Royal Society of London Series B-Biological Sciences 266:1189-1195

Gilby IC, Pokempner AA, Wrangham RW (2010) A direct comparison of scan and focal sampling methods for measuring wild chimpanzee feeding behaviour. Folia Primatologica 81:254-264

Gilby IC, Wrangham RW (2007) Risk-prone hunting by chimpanzees (*Pan troglodytes schweinfurthii*) increases during periods of high diet quality. Behavioral Ecology and Sociobiology 61:1771-1779

Gilby IC, Wrangham RW (2008) Association patterns among wild chimpanzees (*Pan troglodytes schweinfurthii*) reflect sex differences in cooperation. Behavioral Ecology and Sociobiology 62:1831-1842

Girard-Buttoz C, Higham JP, Heistermann M, Wedegartner S, Maestripieri D, Engelhardt A (2011) Urinary C-peptide measurement as a marker of nutritional status in macaques. PloS One 6: e18042

Gittleman JL, Thompson SD (1988) Energy allocation in mammalian reproduction. American Zoologist 28:863-875

Golabek KA, Ridley AR, Radford AN (2012) Food availability affects strength of seasonal territorial behaviour in a cooperatively breeding bird. Animal Behaviour 83:613-619

Gomez D, Huchard E, Henry P, Perret M (2012) Mutual mate choice in a female-dominant and sexually monomorphic primate. American Journal of Physical Anthropology 147:370-379

Goodall J (1986) The Chimpanzees of Gombe: Patterns of Behavior. Harvard University Press, Cambridge, MA

Goyman W, Trappschuh M (2011) Seasonal and diel variation of hormone metabolites in European stonechats: on the importance of high signal-to-noise ratios in non-invasive hormone studies. Journal of Biological Rhythms 26:44-54

Grover CD, Kay AD, Monson JA, Marsh TC, Holway DA (2007) Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. Proceedings of the Royal Society B-Biological Sciences 274:2951-2957

Gurven M, Hill K (2009) Why do men hunt? A reevaluation of "Man the Hunter" and the sexual division of labor. Current Anthropology 50:51-74

Hare B (2011) From hominoid to hominid mind: What changed and why? Annual Review of Anthropology 40:293-309

Hare B, Wobber V, Wrangham RW (2012) The self-domestication hypothesis: evolution of bonobo psychology is due to selection against aggression. Animal Behaviour 83:573-585

Harris TR, Chapman CA, Monfort SL (2010) Small folivorous primate groups exhibit behavioral and physiological effects of food scarcity. Behav. Ecol. 21:46-56

Harrison M, Marshall A (2011) Strategies for the use of fallback foods in apes. International Journal of Primatology 32: 531-565

Havel PJ (2001) Peripheral signal conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. Experimental Biology and Medicine 226:963-977

Hawkes K (1991) Showing off: tests of an hypothesis about men's foraging goals. Ethology and Sociobiology 12:29-54

Hawkes K (2010) How grandmother effects plus individual variation in frailty shape fertility and mortality: Guidance from human–chimpanzee comparisons. Proceedings of the National Academy of Sciences of the United States of America:8977-8984

Hawkes K, O'Connell JF, Coxworth JE (2010) Family Provisioning Is Not the Only Reason Men Hunt A Comment on Gurven and Hill. Current Anthropology 51:259-264

Heistermann M (2010) Non-invasive monitoring of endocrine status in laboratory primates: methods, guidelines and applications. Advances in Science and Research 5:1-9

Henriksson J (1995) Influence of exercise on insulin sensitivity. European Journal of Preventive Cardiology 2:303-309

Henzi SP, Clarke PMR, van Schaik CP, Pradhan GR, Barrett L (2010) Infanticide and reproductive restraint in a polygynous social mammal. Proceedings of the National Academy of Sciences of the United States of America 107:2130-2135

Higham JP, Heistermann M, Maestripieri D (2011) The energetics of male-male endurance rivalry in free-ranging rhesus macaques, *Macaca mulatta*. Animal Behaviour 81:1001-1007

Hill K, Boesch C, Goodall J, Pusey A, Williams J, Wrangham R (2001) Mortality rates among wild chimpanzees. Journal of Human Evolution 40:437-450

Hoffman CL, Ruiz-Lambides V, Davila E, Maldonado E, Gerald MS, Maestripieri D (2008) Sex differences in survival costs of reproduction in a promiscuous primate. Behavioral Ecology and Sociobiology 62:1711-1718

Hohmann G, Fowler A, Sommer V, Ortmann S (2006) Frugivory and gregariousness of Salonga bonobos and Gashaka chimpanzees: the influence of abundance and nutritional quality of fruit. In: Hohmann G, Robbins MM, Boesch C (eds) Feeding Ecology in Apes and Other Primates: Ecological, Physical and Behavioural Aspects. Cambridge University Press, Cambridge, pp 123-159

Hoogwerf BJ, Goetz FC (1983) Urinary C-peptide: a simple measure of integrated insulin production with emphasis on the effects of body size, diet, and coricosteroids. Journal of Clinical Endocrinology and Metabolism 56:60-67

Hoogwerf BJ, Laine DC, Greene E (1986) Urine C-peptide and creatinine (Jaffe Method) excretion in healthy young adults on varied diets: sustained effects of varied carbohydrate, protein, and meat content. The American Journal of clinical Nutrition 43:350-360

Houle A, Chapman CA, Vickery WL (2007) Intratree variation in fruit production and implications for primate foraging. International Journal of Primatology 28:1197-1217

Huchard E, Canale CI, Le Gros C, Perret M, Henry P, Kappeler PM (2012) Convenience polyandry or convenience polygyny? Costly sex under female control in a promiscuous primate. Proceedings of the Royal Society B-Biological Sciences 279:1371-1379

Huck M, Lottker P, Heymann EW (2004) Proximate mechanisms of reproductive monopolisation in male moustached tamarins (*Saguinus mystax*). American Journal of Primatology 64:39-56

Idani G (1986) Seed dispersal by pygmy chimpanzees (*Pan paniscus*) - a preliminary report. Primates 27:441-447

Idani G, Kuroda S, Kano T, Asato R (1994) Flora and vegetation of Wamba forest, central Zaire with reference to bonobo (*Pan paniscus*) foods at Wamba. Tropics 3:309-332

Inoue E, Inoue-Murayama M, Vigilant L, Takenaka O, Nishida T (2008) Relatedness in wild chimpanzees: influence of paternity, male philopatry, and demographic factors. American Journal of Physical Anthropology 137:256-262

Isaac JL (2005) Mass loss during the mating season in male common brushtail possums (*Trichosurus vulpecula*): implications for age-specific variation in mating strategy. Journal of Zoology 266:181-185

Isabirye-Basuta G (1988) Food competition among indiviuals in a free-ranging chimpanzee community in Kibale Forest, Uganda. Behaviour 105:135-147

Jameson KA, Appleby MC, Freeman LC (1999) Finding an appropriate order for a hierarchy based on probabilistic dominance. Animal Behaviour 57:991-998

Janson C, Vogel R (2006) Hunger and aggression in capuchin monkeys. In: Hohmann G, Robbins MM, Boesch C (eds) Feeding Ecology in Apes and Other Primates: Ecological, Physical and Behavioral Aspects. Cambridge University Press, Cambridge, UK

Jarrett RJ (1972) Circadian variation in blood glucose levels, in glucose tolerance and in plasma immunoreactive insulin levels. Acta Diabetologica 9:263-275

Jolly CJ (2009) Fifty years of looking at human evolution: Backward, forward, and sideways. Current Anthropology 50:187-199

Kahlenberg SM (2006) Female-female competition and male sexual coercion in Kanyawara chimpanzees. PhD Dissertation. Harvard University, Cambridge, MA

Kahlenberg SM, Thompson ME, Muller MN, Wrangham RW (2008) Immigration costs for female chimpanzees and male protection as an immigrant counterstrategy to intrasexual aggression. Animal Behaviour 76:1497-1509

Kalsbeek A, Strubbe JH (1998) Circadian control of insulin secretion is independent of the temporal distribution of feeding. Physiology & Behavior 63:553-558

Kaplan H, Hill K, Lancaster J, Hurtado AM (2000) A theory of human life history evolution: Diet, intelligence, and longevity. Evolutionary Anthropology 9:156-185

Kelly RC (1993) Constructing Inequality: The Fabrication of a Hierarchy of Virtue among the Etoro. University of Michigan Press, Ann Arbor, MI

Kemnitz JW, Sapolsky RM, Altmann J, Muruthi P, Mott GE, Stefanick ML (2002) Effects of food availability on serum insulin and lipid concentrations in free-ranging baboons. American Journal of Primatology 57:13-19

Key C, Ross C (1999) Sex differences in energy expenditure in non-human primates. Proceedings of the Royal Society B-Biological Sciences 266:2479-2485

King DS, Dalsky GP, Staten MA, Clutter WE, Van Houten DR, Holloszy JO (1987) Insulin action and secretion in endurance-trained and untrained humans. Journal of Applied Physiology 63:2247-2252

Knott C (2005) Energetic responses to food availability in the great apes: Implications for Hominin evolution. In: Brockman D, van Schaik C (eds) Primate Seasonality: Implications for Human Evolution. Cambridge University Press, Cambridge, pp 351-378

Knott CD (1997) Field collection and preservation of urine in orangutans and chimpanzees. Tropical Biodiversity 4:95-102

Komdeur J (2001) Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. Proceedings of the Royal Society of London Series B-Biological Sciences 268:2103-2111

Kovach AI, Powell RA (2003) Effects of body size on male mating tactics and paternity in black bears, *Ursus americanus*. Canadian Journal of Zoology 81:1257-1268

Kramer KL, Ellison PT (2010) Pooled energy budgets: resituating human energy allocation trade-offs. Evolutionary Anthropology 19:136-147

Kriska AM, Hanley AJG, Harris SB, Zinman B (2001) Physical activity, physical fitness, and insulin and glucose concentrations in an isolated native Canadian population experiencing rapid lifestyle change. Diabetes Care 24:1787-1792

Kruszynska YT, Home PD, Hanning I, Alberti KGMM (1987) Basal and 24-hr C-peptide and insulin secretion rate in normal man. Diabetologica 30:16-21

Kumar S, Filipski A, Swarna V, Walker A, Hedges SB (2005) Placing confidence limits on the molecular age of the human-chimpanzee divergence. Proceedings of the National Academy of Sciences of the United States of America 102:18842-18847

Kutsukake N, Nunn CL (2009) The causes and consequences of reproductive skew in male primates. In: Hager R, Jones CB (eds) Reproductive Skew in Vertebrates: Proximate and Ultimate Causes. Cambridge University Press, Cambridge

Lambert JE (2011) Primate seed dispersers as umbrella species: a case study from Kibale National Park, Uganda, with implications for Afrotropical forest conservation. American Journal of Primatology 73:9-24

Lane JE, Boutin S, Speakman JR, Humphries MM (2010) Energetic costs of male reproduction in a scramble competition mating system. Journal of Animal Ecology 79:27-34

Lawes MJ, Henzi P (1995) Inter-group encounters in blue monkeys: how territorial must a territorial species be? Animal Behaviour 49:240-243

Leonard W, Robertson M (1997) Comparative primate energetics and hominid evolution. American Journal of Physical Anthropology 102:265-281

Leonard WR, Robertson ML (1992) Nutritional requirements and human evolution: A bioenergetics model. American Journal of Human Biology 4:179-195

Lieberman DE, Carlo J, Ponce de Leon M, Zollikofer CPE (2007) A geometric morphometric analysis of heterochrony in the cranium of chimpanzees and bonobos. Journal of Human Evolution 52:647-662

Lovejoy CO (2009) Reexamining human origins in light of *Ardipithecus ramidus*. Science 326: 74, 74e1-74e8

Lovejoy CO, Suwa G, White TD (2010) Studying extant species to model our past - a response. Science 327:410-411

Low M (2006) The energetic cost of mate guarding is correlated with territorial intrusions in the New Zealand stitchbird. Behavioral Ecology 17:270-276

Loy J (1970) Behavioral responses of free-ranging rhesus monkeys to food shortage. American Journal of Physical Anthropology 33:263-271

Mace R (1989) Great tits choose between food and proximity to a mate: the effect of time of day. Behavioral Ecology and Sociobiology 24:285-290

Mainguy J, Cote SD, Cardinal E, Houle M (2008) Mating tactics and mate choice in relation to age and social rank in male mountain goats. Journal of Mammalogy 89:626-635

Majolo B, Lehmann J, de Bortoli Vizio A, Schino G (2012) Fitness-related benefits of dominance in primates. American Journal of Physical Anthropology 147:652-660

Malherbe C, de Gasparo M, de Hertogh R, Hoet JJ (1969) Circadian variations of blood sugar and plasma insulin levels in man. Diabetologica 5:397-404

Manson JH (1992) Measuring female mate choice in Cayo Santiago rhesus macaques. Animal Behaviour 44:405-416

Manson JH (1994) Male aggression: a cost of female mate choice in Cayo Santiago rhesus macaques. Animal Behaviour 48:473-475

Marler CA, Moore MC (1991) Supplementary feeding compensates for testosterone-induced costs of aggression in male mountain spiny lizards, Sceloporus jarrovi. Animal Behaviour 42:209-219

Marlowe FW (1999a) Male care and mating effort among Hadza foragers. Behavioral Ecology and Sociobiology 46:57-64

Marlowe FW (1999b) Showoffs or providers? The parenting effort of Hadza men. Evolution and Human Behavior 20:391-404

Marlowe FW (2001) Male contribution to diet and female reproductive success among foragers. Current Anthropology 42:755-760

Marsden HM (1972) The effect of food deprivation on intergroup relations in rhesus monkeys. Behavioral Biology 7:369-374

Martin P, Bateson P (2007) Measuring Behaviour: An Introductory Guide (*3rd Edition)*. Cambridge University Press, Cambridge

Mass V, Heistermann M, Kappeler P (2009) Mate-guarding as a male reproductive tactic in *Propithecus verreauxi*. International Journal of Primatology 30:389-409

Matsubara M (2003) Costs of mate guarding and opportunistic mating among wild male Japanese macaques. International Journal of Primatology 24:1057-1075

Matsumoto-Oda A, Hamai M, Hayaki H, Hosaka K, Hunt K, Kasuya E, Kawanaka K, Mitani J, Takasaki H, Takahata Y (2007) Estrus cycle asynchrony in wild female chimpanzees, *Pan troglodytes schweinfurthii*. Behavioral Ecology and Sociobiology 61:661-668

Matsumoto-Oda A, Ihara Y (2011) Estrous asynchrony causes low birth rates in wild chimpanzees. American Journal of Primatology 73:180-188

McCarthy MS, Finch CE, Stanford CB (2011) Social dominance predicts long life expectancy in male chimpanzees (*Pan troglodytes*). American Journal of Primatology 73 Supplement 1:87

McDonald TJ, Knight BA, Shields BM, Bowman P, Salzmann MB, Hattersley AT (2009) Stability and reproducibility of a single-sample urinary C-peptide/creatinine ratio and its correlation with 24-hr urinary C-peptide. Clinical Chemistry 55:2035-2039

McElligott AG, Hayden TJ (2000) Lifetime mating success, sexual selection and life history of fallow bucks (*Dama dama*). Behavioral Ecology and Sociobiology 48:203-210

McGrew WC (2010) In search of the last common ancestor: new findings on wild chimpanzees. Philosophical Transactions of the Royal Society B-Biological Sciences 365:3267-3276

Meis PJ, Rose JC, Sperling MA (1983) Circadian variation in insulin response in African green monkeys (*Cercopithecus aethiops*). American Journal of Primatology 5:261-266

Mejean L, Bicakova-Rocher A, Kolopp M, Villaume C, Levi F, Derby G, Reinberg A, Drouin P (1988) Circadian and ultradian rhythms in blood glucose and plasma insulin of healthy adults. Chronobiology International 5:227-236

Milton K, Demment MW (1988) Digestion and passage kinetics of chimpanzees fed high and low fiber diets and comparison with human data. Journal of Nutrition 118:1082-1088

Mitani J, Watts D (2005a) Correlates of territorial boundary patrol behaviour in wild chimpanzees. Animal Behaviour 70:1079-1086

Mitani JC, Watts DP, Amsler SJ (2010) Lethal intergroup aggression leads to territorial expansion in wild chimpanzees. Current Biology 20:R507-R508

Muehlenbein MP, Watts DP, Whitten PL (2004) Dominance rank and fecal testosterone levels in adult male chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda. American Journal of Primatology 64:71-82

Muller M, Kahlenberg S, Emery Thompson M, Wrangham R (2007) Male coercion and the costs of promiscuous mating for female chimpanzees. Proceedings of the Royal Society of London: B 274:1009-1014

Muller MN, Lipson S (2003) Diurnal patterns of urinary steroid excretion in wild chimpanzees. American Journal of Primatology 60:161-166

Muller MN, Wrangham RW (2004a) Dominance, aggression and testosterone in wild chimpanzees: a test of the 'challenge hypothesis'. Animal Behaviour 67:113-123

Muller MN, Wrangham RW (2004b) Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). Behavioral Ecology and Sociobiology 55:332-340

Muller MN, Wrangham RW (2005) Testosterone and energetics in wild chimpanzees (Pan troglodytes schweinfurthii). American Journal of Primatology 66:119-130

Muller MN, Emery Thompson M, Wrangham RW (2006) Male chimpanzees prefer mating with old females. Current Biology 16:2234-2238

Muller MN, Kahlenberg SM, Wrangham RW (2009) Male aggression against females and sexual coercion in chimpanzees. In: Muller MN, Wrangham RW (eds) Sexual Coercion in Primates and Humans: An Evolutionary Perspective on Male Aggression against Females. Harvard University Press, Cambridge, MA

Muller MN, Mitani JC (2005) Conflict and cooperation in wild chimpanzees. Advances in the Study of Behavior 35:275-331

Muller MN, Thompson ME, Kahlenberg SM, Wrangham RW (2011) Sexual coercion by male chimpanzees shows that female choice may be more apparent than real. Behavioral Ecology and Sociobiology 65:921-933

Mundry R, Nunn CL (2009) Stepwise model fitting and statistical inference: turning noise into signal pollution. The American naturalist 173:119-23

Murray CM, Gilby IC, Mane SV, Pusey AE (2008) Adult male chimpanzees inherit maternal ranging patterns. Current Biology 18:20-24

Mysterud A, Langvatn R, Stenseth NC (2004) Patterns of reproductive effort in male ungulates. Journal of Zoology 264:209-215

Nelson R (2000) An Introduction to Behavioral Endocrinology. Sinauer Associates, Inc., Sunderland, Massachusetts

Newton-Fisher NE, Thompson ME, Reynolds V, Boesch C, Vigilant L (2010) Paternity and social rank in wild chimpanzees (*Pan troglodytes*) from the Budongo Forest, Uganda. American Journal of Physical Anthropology 142:417-428

Nishida T (1968) The social group of wild chimpanzees in the Mahali mountains. Primates 9:167-224

Nishida T (1983) Alpha status and agonistic alliance in wild chimpanzees (*Pan troglodytes schweinfurthii)*. Primates 24:318-336

Nishida T (2012) Chimpanzees of the Lakeshore. Cambridge University Press, Cambridge

Norconk MA, Grafton BW, Conklin-Brittain NL (1998) Seed dispersal by neotropical seed predators. American Journal of Primatology 45:103-126

Pandolfi SS (2004) Ecological Sex Differences in Gombe Chimpanzees (*Pan troglodytes*). PhD Dissertation. Duke University, Durham, NC

Panter-Brick C (2002) Sexual division of labor: Energetic and evolutionary scenarios. American Journal of Human Biology 14:627-640

Parga JA (2006) Male mate choice in *Lemur catta*. International Journal of Primatology 27:107-131

Pate D (1997) Bone chemistry and paleodiet: reconstructing prehistoric subsistence-settlement systems in Australia. Journal of Anthropological Archaeology 16:103-120

Pate D (2006) Hunter-gatherer social complexity at Roonka, South Australia. In: David B, Barker B, McNiven I (eds) Social Archaeology of Australian Indigenous Societies. Aboriginal Studies Press, Canberra, pp 226-241

Patterson N, Richter DJ, Gnerre S, Lander ES, Reich D (2006) Genetic evidence for complex speciation of humans and chimpanzees. Nature 441:1103-1108

Pelletier F, Hogg J, Festa-Bianchet M (2006) Male mating effort in a polygynous ungulate. Behavioral Ecology and Sociobiology 60:645-654

Pelletier F, Mainguy J, Côté SD (2009) Rut-induced hypophagia in male bighorn sheep and mountain goats: foraging under time budget constraints. 115:141-151

Peter R, Dunseath G, Luzio SD, Chudleigh R, Roy Choudhurry S, Owens DR (2010) Daytime variability of postprandial glucose tolerance and pancreatic B-cell function using 12-h profiles in persons with Type 2 diabetes. Diabetic Medicine 27:266-273

Pilbeam D (1996) Genetic and morphological records of the Hominoidea and hominid origins: A synthesis. Molecular Phylogenetics and Evolution 5:155-168

Pilbeam D, Young N (2004) Hominoid evolution: synthesizing disparate data. Comptes Rendus Palevol 3:305-321

Plavcan JM (2004) Sexual selection, measures of sexual selection, and sexual dimorphism in primates. In: Kappeler PM, van Schaik CP (eds) Sexual Selection in Primates: New and Comparative Perspectives. Cambridge University Press, Cambridge

Plavcan JM, van Schaik CP (1992) Intrasexual competition and canine dimorphism in anthropoid primates. American Journal of Physical Anthropology 87:461-477

Plavcan JM, van Schaik CP (1997) Interpreting hominid behavior on the basis of sexual dimorphism. Journal of Human Evolution:345-374

Pokempner AA (2009) Fission-fusion and foraging: Sex differences in the behavioral ecology of chimpanzees (*Pan troglodytes schweinfurthii*). PhD Dissertation. Stony Brook University, Stony Brook

Poole JH (1987) Rutting behavior in African elephants: the phenomenon of musth. Behaviour 102:283-316

Poole JH (1989) Announcing intent - the aggressive state of musth in African elephants. Animal Behaviour 37:140-152

Poole JH, Lee PC, Njiraini N, Moss SJ (2011) Longevity, competition and musth: a long-term perspective on male reproductive strategies. In: Moss SJ, Croze H, Lee PC (eds) The Amboseli Elephants: A Long-term Perspective on a Long-lived Mammal. Chicago University Press, Chicago, pp 272-286

Post W, Greenlaw JS (1982) Comparative costs of promiscuity and monogramy: A test of reproductive effort theory. Behavioral Ecology and Sociobiology 10:101-107

Potts K, Watts D, Wrangham RW (2011) Comparative feeding ecology of two communities of chimpanzees (*Pan troglodytes*) in Kibale National Park, Uganda. International Journal of Primatology 32:669-690

Powers DR, McKee T (1994) The effect of food availability on time and energy expenditures of territorial and non-territorial hummingbirds. The Condor 96:1064-1075

Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K (2005) Male mate choice influences female promiscuity in Soay sheep. Proceedings of the Royal Society B-Biological Sciences 272:365-373

Pusey AE, Oehlert GW, Williams JM, Goodall J (2005) Influence of ecological and social factors on body mass of wild chimpanzees. International Journal of Primatology 26:3-31

Pusey AE, Williams JM, Goodall J (1997) The influence of dominance rank on the reproductive success of female chimpanzees. Science 277:828-831

Puts DA (2010) Beauty and the beast: mechanisms of sexual selection in humans. Evolution and Human Behavior 31:157-175

R Development Core Team (2011) R: A Language Environment for Statistical Computing. In. R Foundation for Statistical Computing, Vienna

Regensteiner JG, Mayer EJ, Shetterly SM, Eckel RH, Haskell WL, Marshall JA, Baxter J, Hamman RF (1991) Relationship between habitual physical activity and insulin levels among nondiabetic men and women: San Luis Valley Diabetes Study. Diabetes Care 14:1066-1074

Reiches M (2011) Female Adolescent Energy Expenditure and Reproductive Function in The Gambia. PhD Dissertation. Harvard University, Cambridge, MA

Reynolds V (2005) The Chimpanzees of Budongo Forest: Ecology, Behaviour, and Conservation. Oxford University Press, Oxford

Riss D, Goodall J (1977) The recent rise to the alpha-rank in a population of free-living chimpanzees. Folia Primatologica 27:134-151

Robson SL, Wood B (2008) Hominin life history: reconstruction and evolution. Journal of Anatomy 212:394-425

Rogers ME, Maisels F, Williamson EA, Fernandez M, Tutin CEG (1990) Gorilla diet in the Lope Reserve, Gabon: a nutritional analysis. Oecologia 84:326-339

Ruvolo M (1997) Molecular phylogeny of the hominoids: inferences from multiple independent DNA sequence data sets. Molecular Biology and Evolution 14:248-265

Sailer MM, Fietz J (2009) Seasonal differences in the feeding ecology and behavior of male edible dormice (*Glis glis*). Mammalian Biology 74:114-124

Sapolsky R (1994) Why Zebras Don't Get Ulcers. W.H. Freeman and Co., New York

Sayers K, Lovejoy CO (2008) The chimpanzee has no clothes: A critical examination of Pan troglodytes in models of human evolution. Current Anthropology 49:87-114

Schoener TW (1971) Theory of feeding strategies. Annual Review of Ecology and Systematics 2:369-404

Schubert M, Schradin C, Rödel H, Pillay N, Ribble D (2009) Male mate guarding in a socially monogamous mammal, the round-eared sengi: on costs and trade-offs. Behavioral Ecology and Sociobiology 64:257-264

Sherry DS, Ellison PE (2007) Potential applications of urinary C-peptide of insulin for comparative energetics research. American Journal of Physical Anthropology 133:771-778

Simon C, Follenius M, Brandenberger G (1987) Postprandial oscillations of plasma glucose, insulin and C-peptide in man. Diabetologica 30:769-773

Sommer V, Bauer J, Fowler A, Ortmann S (2010) Patriarchal chimpanzees, matriarchal bonobos: potential ecological causes of a *Pan* dichotomy. In: Sommer V, Ross C (eds) Primates of Gashaka: Socioecology and Conservation in Nigeria's Biodiversity Hotspot. Springer, New York

Southwick CH (1967) An experimental study of intragroup agonistic behavior in rhesus monkeys (*Macaca mulatta*). Behaviour 28:182-209

Speth JD (1990) Seasonality, resource stress, and food sharing in so-called "egalitarian" foraging societies. Journal of Anthropological Archaeology 9:148-188

Spielmann KA (1989) A review: dietary restrictions on hunter-gatherer women and the implications for fertility and infant mortality. Human Ecology 17:321-345

Stearns SC (1992) The Evolution of Life Histories. Oxford University Press, Oxford

Struhsaker TT (1997) Ecology of an African Rain Forest: Logging in Kibale and the Conflict between Conservation and Exploitation. University Press of Florida, Gainesville, FL

Sugiyama Y (1968) Social organization of chimpanzees in the Budongo Forest, Uganda. Primates 9:225-258

Surbeck M, Deschner T, Schubert G, Weltring A, Hohmann G (2012) Mate competition, testosterone and intersexual relationships in bonobos, *Pan paniscus*. Animal Behaviour 83:659-669

Suzuki A (1969) An ecological study of chimpanzees in a savanna woodland. Primates 10:103-148

Taussky H (1954) A microcalorimetric determination of creatinine in urine by the Jaffe reaction. Journal of Biological Chemistry 208:853-861

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual Selection and the Descent of Man. Aldine, Chicago, pp 136-179

Tsai LW, Sapolsky RM (1996) Rapid stimulatory effects of testosterone upon myotubule metabolism and sugar transport, as assessed by silicon microphysiometry. Aggressive Behavior 22:357-364

Tsuji Y, Yangozene K, Sakamaki T (2010) Estimation of seed dispersal distance by the bonobo, *Pan paniscus*, in a tropical forest in Democratic Republic of Congo. Journal of Tropical Ecology 26:115-118

Tutin CEG (1979) Mating patterns and reproductive strategies in a community of wild chimpanzees (*Pan troglodytes schweinfurthii*). Behavioral Ecology and Sociobiology 6:29-38

Vehrencamp SL, Bradbury JW, Gibson RM (1989) The energetic cost of display in male sage grouse. Animal Behaviour 38:885-896

Wade GN, Schneider JE (1992) Metabolic fuels and reproduction in female mammals. Neuroscience and Biobehavioral Reviews 16:235-272

Wallace R (2005) Seasonal variations in diet and foraging behavior of *Ateles chamek* in a Southern Amazonian Tropical Forest. International Journal of Primatology 26:1053-1075

Warton DIWDI, Hui FKC (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3-10

Watts D (1998) Coalitionary mate guarding by male chimpanzees at Ngogo, Kibale National Park, Uganda. Behavioral Ecology and Sociobiology 44:43-55

Watts DP (2012) Long-term research on chimpanzee behavioral ecology in Kibale National Park, Uganda. In: Kappeler PM, Watts DP (eds) Long-term Field Studies of Primates. Springer, Berlin

Watts DP, Potts KB, Lwanga JS, Mitani JC (2012) Diet of chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda, 2. temporal variation and fallback foods. American Journal of Primatology 74:130-144

Weingrill T, Lycett JE, Barrett L, Hill RA, Henzi SP (2003) Male consortship behaviour in chacma baboons: The role of demographic factors and female conceptive probabilities. Behaviour 140:405-427

Westneat DF (1994) To guard mates or to forage: conflicting demands affect the paternity of male red-winged blackbirds. American Naturalist 144:343-354

White F, Wrangham RW (1988) Feeding competition and patch size in the chimpanzee species *Pan paniscus* and *Pan troglodytes*. Behaviour 105:148-164

Whiten A, McGrew WC, Aiello LC, Boesch C, Boyd R, Byrne RW, Dunbar RIM, Matsuzawa T, Silk JB, Tomasello M, van Schaik CP, Wrangham R (2010) Studying extant species to model our past. Science 327:410

Whitten P, Brockman D, Stavisky R (1998) Recent advances in noninvasive techniques to monitor hormone-behavior interactions. Yearbook of Physical Anthropology 41:1-23

Williams JM, Oehlert G, Carlis JV, Pusey AE (2004) Why do male chimpanzees defend a group range? Animal Behaviour 68:523-532

Willisch CS, Ingold P (2007) Feeding or Resting? The Strategy of Rutting Male Alpine Chamois. Ethology 113:97-104

Willisch CS, Neuhaus P (2010) Social dominance and conflict reduction in rutting male Alpine ibex, *Capra ibex*. Behavioral Ecology 21:372-380

Wilson AJ, Nussey DH (2010) What is individual quality? An evolutionary perspective. Trends in Ecology & Evolution 25:207-214

Wilson ML, Kahlenberg SM, Wells MT, Wrangham RW (2012) Ecological and social factors affect the occurrence and outcomes of intergroup encounters in chimpanzees. Animal Behaviour 83:277-291

Winter JW (1976) The behaviour and social organisation of the brush-tail possum (*Trichosurus vulpecula* Kerr). In. University of Queensland, Queensland

Wobber V, Hare B, Wrangham RW (2010a) Application of the heterochrony framework to the study of behavior and cognition. Communicative and Integrative Biology 3:337-339

Wobber V, Wrangham RW, Hare B (2010b) Bonobos exhibit delayed development of social behavior and cognition relative to chimpanzees. Current Biology 20:226-230

Wolden-Hanson T, Davis GA, Baum ST, Kemnitz JW (1993) Insulin levels, physical activity, and urinary catecholamine excretion of obese and non-obese rhesus monkeys. Obesity Research 1:5-17

Wolever TMS, Bolognesi C (1996) Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. The Journal of Nutrition 126:2807-2812

Woods SC, Vasselli JR, Kaestner E, Vitiello MV, Milburn P (1977) Conditioned insulin secretion and meal feeding in rats. Journal of Comparative and Physiological Psychology 91:128-133

Wrangham RW (1975) The Behavioural Ecology of Chimpanzees in Gombe National Park, Tanzania. PhD Dissertation. Cambridge University, UK

Wrangham RW (2000) Why are male chimpanzees more gregarious than mothers? A scramble competition hypothesis. In: Kappeler P (ed) Primate Males: Causes and Consequences of Variation in Group Composition. Cambridge University Press, Cambridge, pp 248-258

Wrangham RW (2009) Catching Fire: How Cooking Made Us Human. Basic Books, New York, NY

Wrangham RW, Carmody R (2010) Human adaptation to the control of fire. Evolutionary Anthropology 19:187-199

Wrangham RW, Chapman CA, Clark-Arcadi AP, Isabirye-Basuta G (1996) Social ecology of Kanyawara chimpanzees: Implications for the understanding of great ape groups. In: McGrew WC, Marchant LF, Nishida T (eds) Great Ape Societies. Cambridge University Press, Cambridge, pp 45-57

Wrangham RW, Conklin NL, Chapman CA, Hunt KD (1991) The significance of fibrous foods for Kibale Forest chimpanzees. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 334:171-178

Wrangham RW, Conklin-Brittain NL, Hunt KD (1998) Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. I. Antifeedants. International Journal of Primatology 19:949-970

Wrangham RW, Pilbeam D (2001) African apes as time machines. In: Galdikas B, Briggs N, Sheeran L, Shapiro G, Goodall J (eds) All Apes Great and Small, Volume I: African Apes. Kluwer Academics / Plenum Publishers, New York

Wrangham RW, Smuts B (1980) Sex differences in the behavioral ecology of chimpanzees in the Gombe National Park, Tanzania. Journal of Reproduction and Fertility, Supplement 28:13-31

Wroblewski EE, Murray CM, Keele BF, Schumacher-Stankey JC, Hahn BH, Pusey AE (2009) Male dominance rank and reproductive success in chimpanzees, *Pan troglodytes schweinfurthii*. Animal Behaviour 77:873-885

Zavaroni I, Deferrari G, Lugari R, Bonora E, Garibotto G, Dall'aglio E, Robaudo C, Gnudi A (1987) Renal metabolism of C-peptide in man. The Journal of Clinical Endocrinology and Metabolism 65:494-498

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed Effects Models and Extension in Ecology with R. Springer, New York