Costs of mate-guarding in wild male long-tailed macaques (*Macaca fascicularis*)

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I hereby declare that I have written this thesis independently and with no other aids or sources than quoted

_____ Göttingen,

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This thesis is dedicated to my grandfather, François.

Table of Contents

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Summary

In promiscuous mating systems, several males compete with each other for access to fertile females, and males have evolved a variety of mating tactics to outcompete their rivals. Mate-guarding is a mating tactic used by males of several vertebrate and invertebrate taxa to exclude other males from accessing the guarded female, and hence secure their paternity. In multi-male mammal groups, high-ranking males are often the ones mate-guarding females the most, since they gain priority of access to females and are the only one capable of efficiently monopolising females. Whereas mate-guarding has been proven to increase male reproductive success, this mating tactic may also entail some costs associated with life-history trade-offs between current and future reproduction, body condition maintenance and survival. In turn, these costs may limit the ability of top-ranking males to monopolise females and hence affect male reproductive skew (i.e. the partitioning of reproduction among males). Costs of mating tactics may also promote the evolution of male mate-choice by forcing the males to concentrate their reproductive effort on the females with the highest fitness value. Quantifying the costs of mate-guarding may therefore shed light on the factors driving the evolution of male mating decisions and ultimately contributes to our understanding of variation in male reproductive skew.

Primates are an interesting taxa to study this question since several species live in stable multi-male groups and mate-guarding is a highly beneficial mating tactic commonly employed by high-ranking males. However, studies investigating the costs of mate-guarding in primates are mainly limited to the quantification of feeding costs and yielded, so far, inconsistent results. Our understanding of these costs is also impaired by the lack of a reliable non-invasive physiological marker of energetic condition in non-hominid primates.

The overall aim of this thesis was therefore to quantify the costs of mate-guarding for males in a primate species living in multi-male groups, the long-tailed macaques (*Macaca fascicularis*). In this species, alpha males mate-guard females to a lower extent than predicted by the Priority of Access model, suggesting that costs of mate-guarding may limit males' ability to monopolise females. In *study 1*, I evaluated the suitability of urinary C-peptide (UCP, a by-product of insulin production) as a marker of male energetic status in macaques. In *study 2* and *3,* I quantified the energetic, physiological and physical (i.e. aggression) costs of mate-guarding. Finally, in *study 4*, I investigated the influence of female value on the costs of mate-guarding and the investment of males into this behaviour.

Summmary

To carry out the validation of UCP as a reliable marker of energetic status in non-hominid primates (*study 1*), I first investigated the relationship between UCP measures and indexes of body condition in free-ranging and captive macaques. UCP levels were positively correlated with body-mass index and skinfold-fatness across individuals. In addition, a food reduction experiment revealed that UCP levels co-varied with changes in both body mass and dietary intake. UCP is therefore a useful marker to track non-invasively intra- and inter-individual variations in body condition and nutritional status.

Subsequently, I studied, during two mating periods, three groups of wild long-tailed macaques living in the Ketambe research area, Gunung Leuser National Park, Indonesia. To provide a comprehensive picture of the potential costs of mate-guarding, while controlling for environmental factors, I combined 1) focal behavioural observations on males' activity, height in the canopy, and socio-sexual interactions, 2) GPS records of distance travelled, 3) non-invasive measurements of physiological stress levels (faecal glucocorticoids, fGC) and energetic status (UCP) and 4) assessments of fruit availability. In total 2088 hours of focal data, 331 urine samples and 771 faecal samples were collected and analysed and 360 fruit trees were surveyed monthly.

In *study 2*, I found that mate-guarding reduced parameters of both energy intake and expenditure but had no significant overall effect on a male's energetic status (UCP levels). These results suggest that energy intake and expenditure were balanced during mate-guarding in the study males.

Study 3 revealed that during mate-guarding, males had, in general, higher fGC levels but this effect was modulated by a male's vigilance time. Mate-guarding also increased a male's vigilance time and male-male aggression rates. In addition, alpha males were more stressed than other males year round, independently of mating competition. I suggest that elevated glucocorticoid levels during mate-guarding may help males to maintain their energetic homeostasis but may constitute a long-term cost inherent to the risk of exposure to chronic stress. The combination of this physiological cost and the risk of injury associated with aggression may limit the ability of alpha males to mate-guard females and hence affect male reproductive skew.

In *study 4*, I showed that male long-tailed macaques may reduce some costs of mate-guarding by selectively monopolising females with high reproductive value since males had lower fGC when mate-guarding high-ranking parous females. Furthermore, males adjusted their mate-guarding investment to female quality by being more vigilant and more aggressive when mate-guarding high ranking females or females with whom they had stronger bonds. This later result shows that males make mate-guarding choices not only by mate-guarding highly valuable females longer, but also by monopolising them better.

In this thesis I identified clear costs of mate-guarding in a primate species and highlight how these costs may influence male reproductive skew. I suggest that male long-tailed macaques may have evolved an "incomplete female monopolisation strategy" whereby males limit the costs of mate-guarding by selectively mate-guarding only certain females and by monopolising females of low value less thoroughly. This incomplete female monopoly may be a crucial component of a top-ranking male's overall energy management strategy allowing him to respond to rank challenges year round and hence enhance the alpha tenure length and associated fitness benefits.

By comparing my results with other mammalian taxa, I discuss in this thesis how the relationship between costs of male reproductive effort and reproductive skew might be modulated by 1) reproductive seasonality, 2) male energy management strategy, 3) males' top dominance rank achievement process and 4) social structure.

Future studies on the cost of male mating tactics should consider the complexity of male reproductive effort, which is not limited solely to the reproductive periods and may be distributed over the whole year and expressed as male-male competition for dominance status or social interactions.

Zusammenfassung

In vielen promisken Paarungssystemen konkurrieren die Männchen einer Gruppe um den Zugang zu fertilen Weibchen. Um es Rivalen zu erschweren, haben sie verschiedene Paarungstaktiken entwickelt. Eine von Vertebraten und Invertebraten oft genutzte Strategie ist das *„mate-guarding“.* Hier bewacht ein hochrangiges Männchen das fertile Weibchen indem es ständig in dessen Nähe bleibt, wodurch es den Zugang der anderen Männchen stark minimiert. Durch diese Monopolisierung des Weibchens erhöht ein Männchen seinen Reproduktionserfolg und damit zusätzlich die Wahrscheinlichkeit der Vaterschaft. Diese für das Männchen gewinnbringende Strategie birgt jedoch auch energetische Kosten. Solche negativen Verknüpfungen zwischen Kosten und Gewinn, sogenannte *„trade-offs“*, beeinflussen den Fortpflanzungserfolg ebenso wie die Körperkondition und die Überlebenschance eines Männchens. Haben solche Kosten beispielsweise eine Verschlechterung der körperlichen Verfassung zur Folge, kann sich das negativ auf die Fähigkeiten der Männchen, ein Weibchen zu monopolisieren, auswirken und damit den Vaterschaftserfolg der Männchen mindern. Die mit solch einer Paarungstaktik wie dem *„mate-guarding“* einhergehenden Kosten könnten sich auch auf die Entstehung von Strategien zur Partnerwahl bei den Männchen auswirken: Männchen sollten ihre Energie vor allem auf die Reproduktion mit den fittesten Weibchen aufwenden. Um die grundlegenden Faktoren der Partnerwahl sowie die Verteilung des Reproduktionserfolges unter den Männchen (*„reproductive skew“*) besser zu verstehen, müssen die bei der Monopolisierung des Weibchens entstehenden Kosten quantifiziert werden.

Primaten sind ein interessantes Taxa um diese Fragen zu untersuchen, da viele Arten in stabilen Mehr-Männchen-Gruppen leben und *„mate-guarding“* eine vorteilhafte Taktik ist, die oft von hochrangigen Männchen angewandt wird. Allerdings haben sich bisherige Studien an Primaten auf die Quantifizierung der Futterkosten beschränkt und die Ergebnisse sind bisher sehr widersprüchlich. Unser Verständnis dieser Kosten wird weiterhin durch das Fehlen eines zuverlässigen, nicht-invasiven physiologischen Markers, der den energetischen Zustand von Nicht-Menschenaffen misst, beeinträchtigt.

Das Hauptziel dieser Arbeit war es daher, die Kosten des *„mate-guarding“* in einer Primatenart, die in Mehr-Männchen-Gruppen lebt wie die Javaneraffen (*Macaca fascicularis*), zu quantifizieren. Bisherige Ergebnisse zeigen, dass die Alpha-Männchen dieser Primatenart ihre Weibchen weniger monopolisieren als das *„Priority of Access-Model“* vorhersagt. Der Monopolisierungserfolg scheint demnach durch die Kosten, die den Männchen durch das *„mate-guarding“* entstehen, limitiert zu sein. In Studie 1 der vorliegenden Arbeit habe ich die Eignung von Urin C-Peptiden (UCP, ein

Nebenprodukt der Insulinproduktion), als Marker für den Energiestatus von Makakenmännchen, evaluiert. In Studie 2 und 3 quantifizierte ich die energetischen, physiologischen und physischen (z.B. Aggression) Kosten des „*mate-guardings*“. In der vierten Studie untersuchte ich den Einfluss der Qualität der Weibchen auf die Kosten der des „*mate-guardings*“ und die Investition der Männchen in dieses Verhalten.

Als erstes betrachtete ich den Zusammenhang zwischen den UCP Werten und Indikatoren für den Zustand der körperlichen Verfassung bei frei- und in Gefangenschaft lebender Makaken, um UCP als zuverlässigen Marker für Energiestatus (Studie 1) zu validieren. Die UCP Level waren positiv korreliert mit dem BMI (*Body-Mass-Index*) sowie mit dem Fettgehalt einer Hautfalte. In einem Experiment, bei dem die Futterzufuhr reduziert wurde, stellte sich heraus, dass UCP Level mit Änderungen des BMI und der geminderten Futterzufuhr kovariiert. Demzufolge ist UCP ein nützlicher Marker um nicht-invasiv intra- und interindividuelle Veränderungen der Körperkondition und des Ernährungszustandes zu ermitteln.

Für die weitere Fragestellung beobachtete ich drei freilebende Javaneraffengruppen während zwei Paarungsperioden, in Ketambe, Gunung Leuser National Park in Indonesien. Um ein möglichst umfassendes Bild der potentiellen Kosten des „*mate-guarding*“ bereitzustellen, kombinierte ich zum einen meine durchgeführten Verhaltensbeobachtungen der Männchen, den Aufenthalt der Männchen in den Bäumen und sexuelle Interaktionen der Männchen mit den Weibchen. Zum anderen ermittelte ich GPS-Daten der Wanderungsdistanz, non-invasive Indikatoren für physiologischen Stress (*faecal glucocorticoid,* fGC), den Energiestatus (UCP) und bewertete die Verfügbarkeit von Früchten. Insgesamt konnten 2088 Fokusstunden, 331 Urin- und 771 Kotproben gesammelt und analysiert werden. Zudem wurden jeden Monat 360 Fruchtbäume begutachtet.

In Studie 2 konnte ich zeigen, dass „*mate-guarding*“ die Parameter der Energieaufnahme und des Energieverbrauches reduziert. Dies hatte jedoch keine signifikanten Auswirkungen auf den gesamten Energiestatus (UCP Level) eines Männchens. Dieses Ergebnis weist auf ein ausbalanciertes Verhältnis von Energieaufnahme und Energieverbrauch der Männchen während des „*mate-guardings*“ hin.

In Studie 3 konnte ich nachweisen, dass die Männchen während des „*mate-guardings*“, höhere fGC Werte aufwiesen. Dieser Wert wurde jedoch durch die Zeit, die Männchen in Vigilanz investieren, moduliert. „*Mate-guarding*” erhöhte einerseits die Vigilanzzeit eines Männchens und andererseits auch die Aggressionsrate der Männchen. Alpha-Männchen waren das ganze Jahr über gestresster als andere Männchen, unabhängig von Paarungskonkurrenz. Dies suggeriert, dass erhöhte Glucocorticoidlevel während des „*mate-guarding*“ den Männchen helfen ihre energetische Homöostase aufrechtzuerhalten, jedoch könnte dies Langzeitkosten darstellen, die bei lang

anhaltender Belastung zu chronischem Stress führen können. Die Kombination dieser physiologischer Kosten und dem Verletzungsrisiko, dass mit Aggressionen einhergeht, könnte die Möglichkeit eines Alphamännchens ein Weibchen zu monopolisieren minimieren und damit auch Einfluss auf die Verteilung des Reproduktionserfolges der Männchen in einer Gruppe haben.

In Studie 4 konnte ich zeigen, dass männliche Javaneraffen einige der Kosten des „*mate-guarding*“ reduzieren können indem sie gezielt Weibchen mit hohem reproduktiven Wert bewachen, da sie dann geringere fGC Werte haben. Darüber hinaus passten Männchen ihre Investition in „*mate-guarding*“ an, indem sie aufmerksamer und aggressiver waren wenn sie hochrangige Weibchen oder Weibchen mit denen sie starke Bindungen formten, bewachten. Diese Ergebnisse bestätigen, dass Männchen nicht nur hochwertige Weibchen auswählen, sondern diese auch länger und besser monopolisieren.

In meiner Arbeit konnte ich die Kosten, die „*mate-guarding*“ für die Männchen einer Primatenart mit sich bringt, aufzeigen und hervorheben wie diese Kosten die Verteilung des Reproduktionserfolges unter den Männchen in der Gruppe beeinflusst. Auf Grundlage meiner Ergebnisse schlage ich vor, dass männliche Javaneraffen eine „unvollständige Weibchenmonopolisierungs-Strategie“ entwickelt haben, bei der sie die Kosten des „*mate-guarding*“ reduzieren indem sie Weibchen selektiv nach deren Reproduktionsqualität wählen und Weibchen mit geringerer Qualität weniger gründlich monopolisieren. Diese unvollständige Weibchenmonopolisierung könnte eine entscheidende Komponente des Energiemanagements von Alphamännchen sein, die ihnen erlaubt ganzjährig adäquat auf versuchte Rangübernahmen zu reagieren und somit ihre Amtszeit zu verlängern und die damit einhergehenden Fitnessvorteile zu erhalten.

Beim Vergleich meiner Ergebnisse mit anderen Säugetier-Taxa, diskutiere ich in meiner Arbeit weiterhin die Beziehung zwischen den Kosten des „*mate-guarding*“ und der Verteilung des Reproduktionserfolges der Männchen in der Gruppe, die durch 1) reproduktive Saisonalität, 2) Energie-Management-Strategien der Männchen, 3) Errungenschaft eines hohen Ranges in der Gruppe und 4) der Sozialstruktur, moduliert sein kann. Zukünftige Studien, die die Kosten der Paarungstaktiken der Männchen untersuchen, sollten die Komplexität des Reproduktionsaufwandes, den Männchen investieren, bedenken. Diese Investitionen scheinen nicht ausschließlich auf die reproduktive Phase im Jahr beschränkt zu sein, sondern können sich über das ganze Jahr verteilen und spiegeln sich in Form der Konkurrenz zwischen Männchen in Bezug auf Rangstatus und sozialen Interaktionen wider.

Résumé

Dans les systèmes de reproduction polygynandre, plusieurs mâles se disputent l'accès aux femelles fertiles et les mâles ont développé des stratégies reproductives pour prendre le dessus sur leurs rivaux. Le *mate-guarding* est une stratégie reproductive utilisée par les mâles dans de nombreux taxa de vertébrés et invertébrés. Elle consiste à monopoliser une femelle en empêchant les autres mâles d'accéder à cette femelle, garantissant ainsi la paternité du mâle. Chez les mammifères formant des groupes multimâles, les mâles de rangs supérieurs sont souvent ceux qui mate-gardent les femelles le plus, puisqu'ils ont un accès prioritaire aux femelles et sont les seuls capables de les monopoliser efficacement. Il a été démontré que le comportement de mate-guarding augmente le succès reproducteur des mâles. Néanmoins, cette stratégie peut également comporter certains coûts, associés aux compromis entre les différents traits d'histoire de vie: reproduction, maintien de bonnes conditions physique et survie. A leur tour, ces coûts peuvent limiter la capacité des mâles de rang supérieur à monopoliser les femelles et ainsi affecter le biais reproducteur des mâles (i.e. la distribution du succès reproducteur entre les mâles). Les coûts des stratégies reproductives peuvent également favoriser l'évolution des choix reproductifs des mâles en les forçant à concentrer leurs efforts sur les femelles aux plus grandes valeurs sélectives (ou *fitness*). Ainsi, quantifier les coûts du mate-guarding peut permettre de faire la lumière sur les facteurs qui sous-tendent l'évolution des décisions reproductives des mâles et contribue au final à une meilleure compréhension des variations dans le biais reproducteur des mâles.

Les primates sont un taxa intéressant pour étudier cette question car plusieurs espèces vivent dans des groupes multimâles et le mate-guarding est une stratégie reproductive très bénéfique employée fréquemment par les mâles de rangs supérieurs. Néanmoins, les études examinant les coûts du mate-guarding chez les primates se limitent principalement à la quantification des coûts liés à l'alimentation et ont conduit, jusqu'à présent, à des conclusions contradictoires. Notre compréhension de ces coûts a également été freinée par le manque de marqueurs physiologiques capables de mesurer de façon non-invasive les conditions énergétiques des primates non-hominides.

L'objectif général de cette thèse était donc de quantifier les coûts du mate-guarding pour les mâles chez une espèce de primate vivant en groupes multimâles, le macaque crabier (*Macaca fascicularis*). Chez cette espèce, le mâle alpha mate-garde les femelles dans des proportions moindre que celles prédites par un des modèles théorique sur le biais reproductif, le « Priority of Access Model », ce qui suggère que les coûts du mate-guarding peuvent limiter la capacité des mâles à monopoliser les femelles. Dans *l'étude 1*, j'ai évalué si les dosages urinaires de peptide C (UPC, un sous-produit de la

production d'insuline), étaient un bon marqueur du statut énergétique chez les macaques. Lors des *études 2 et 3*, j'ai quantifié les coûts énergétiques, physiologiques et physiques (i.e. les agressions) du mate-guarding. Finalement, dans l'*étude 4*, j'ai analysé l'influence de la valeur des femelles sur les coûts du mate-guarding et sur l'investissement des mâles dans ce comportement.

Afin de valider l'UPC comme un marqueur fiable du statut énergétique des primates non-hominides (*étude 1*), j'ai tout d'abord examiné la relation entre les mesures d'UPC et certains indices de condition physique et de masse graisseuse. Les niveaux individuels moyens d'UPC étaient corrélés avec les indices de masse corporelle et les mesures de masse graisseuse sous-cutanée individuels. De plus, une expérience de réduction alimentaire a révélé que les niveaux d'UPC covariaient avec les changements intra-individuels en masse corporelle et en ingestion alimentaire. L'UPC est donc un marqueur utile pour détecter des variations dans les conditions physiques mais également dans le statut nutritif à l'échelle intra- mais également interindividuelle et de manière non-invasive.

Par la suite, j'ai étudié, au cours de deux périodes de reproduction, trois groupes de macaques crabiers sauvages vivant au sein de l'aire de recherche de Ketambe, dans le parc national de Gunung Leuser, en Indonésie. Afin de procurer une étude exhaustive des coûts du mate-guarding, tout en contrôlant pour l'influence des facteurs environnementaux, j'ai combiné 1) des observations comportementales *focales* sur les mâles, enregistrant leurs activités, leurs positions et hauteurs dans la canopée et leurs interactions socio-sexuelles, 2) des enregistrement GPS des distances parcourues, 3) des mesures non-invasives des niveaux de stress physiologique (glucocorticoïdes fécaux, GCf) et du statut énergétique (UPC) et 4) une évaluation de la disponibilité en fruits dans l'environnement. Au total, 2088 heures de données *focales*, 331 échantillons urinaires et 771 échantillons fécaux ont été collectés et analysés et 360 arbres ont été surveillés chaque mois.

Dans l'*étude 2*, j'ai montré que le comportement de mate-guarding réduisait plusieurs paramètres d'apport et également de dépense énergétique mais n'avait pas d'effet général sur le statut énergétique des mâles (i.e. leurs niveaux d'UPC). Ces résultats suggèrent que, pendant les périodes de mate-guarding, les dépenses et apports énergétiques étaient équilibrés chez les mâles étudiés.

L'étude 3 a révélé que, pendant les périodes de mate-guarding, les mâles avaient, en général, des niveaux de stress (GCf) plus élevés, mais cet effet était modulé par le temps que les mâles passaient à être vigilants. Le mate-guarding accroissait également le temps de vigilance des mâles et le taux d'agression entre les mâles. De plus, les mâles alphas étaient plus stressés que les autres mâles durant toute l'année, et ce, indépendamment de la période de reproduction. Je suggère donc que les niveaux élevés de glucocorticoïdes pendant le mate-guarding peuvent aider les mâles à maintenir leur statut énergétique équilibré mais peuvent cependant constituer un coût sur le long terme lié au

risque de stress chronique. Les effets conjugués de ces coûts physiologiques et du risque de blessure associé aux agressions, peuvent limiter les capacités des mâles alphas à monopoliser les femelles et ainsi affecter la structure du biais reproducteur entre les mâles.

Dans l'*étude 4*, je montre que les mâles macaques crabier peuvent réduire certains coûts du mate-guarding en monopolisant sélectivement les femelles de valeur reproductive supérieure. Les mâles avaient des niveaux de GCf plus bas quand ils mate-gardaient des femelles multipares et de rang supérieurs. De plus, les mâles ajustaient leur investissement dans le mate-guarding en fonction de la qualité des femelles en étant plus vigilants et plus agressifs lorsqu'ils monopolisaient des femelles de rangs supérieurs ou avec lesquelles ils avaient de forts liens sociaux. Ce dernier résultat montre que les mâles exercent des choix afférents au mate-guarding non seulement en mate-gardant les femelles les plus importantes plus longtemps mais également en les monopolisant de manière plus optimale.

Dans cette thèse j'identifie clairement certains coûts du mate-guarding chez une espèce de primate et souligne comment ces coûts peuvent influencer le biais reproducteur entre les mâles. Je suggère que les mâles macaques crabiers ont possiblement développé une stratégie de monopolisation incomplète des femelles par laquelle les mâles limitent les coûts du mate-guarding en mate-gardant seulement certaines femelles et en monopolisant les femelles les plus importantes plus scrupuleusement. Cette monopolisation incomplète est possiblement un composant principal de la stratégie générale de gestion énergétique des mâles qui leur permet de répondre à des challenges de dominance tout au long de l'année et ainsi de maintenir leur position alpha et les avantages reproductifs associés avec ce rang plus longtemps.

En comparant mes résultats avec ceux d'autres taxa de mammifères, je discute dans cette thèse comment la relation entre les coûts de l'effort reproducteur des mâles et le biais reproducteur entre les mâles peut être modulée par 1) la saisonnalité de reproduction, 2) la stratégie de gestion énergétique des mâles, 3) le processus d'accès à la dominance et 4) la structure sociale.

Les études futures portant sur les coûts des stratégies reproductives des mâles devraient considérer la complexité de l'effort reproducteur des mâles qui ne se limite pas à la seule période reproductive mais peut-être dilué tout au long de l'année et exprimé sous la forme d'interaction sociales ou de conflits directs entre les mâles pour le statut de dominance.

Chapter 1

General Introduction

Sexual selection theory posits that in sexually reproducing animals the fitness of males is limited by access to receptive females and males intensively compete with each other for access to this limited resource (Trivers 1972; Andersson 1994). The costs of this competition mediate the life-history trade-offs between current reproduction, future reproductive prospects and survival (Stearns 1989). Comparative studies across different taxa and mating systems highlight how the magnitude of these trade-offs is tightly linked to the intensity of male-male competition (Clutton-Brock 1988; Promislow 1992; Bronikowski et al. 2011). In monogamous species, males and females have usually a similar reproductive tenure and survival. In contrast, in species with more intense male-male competition (polygynous and promiscuous mating systems) males die younger than females and are reproductively active over a shorter period. To understand the costs of reproduction in different mating systems, as well as intra-species variation between males and to identify the constraints limiting male reproductive decisions, it is important to quantify the costs of the singular behavioural tactic. To this end, this thesis investigates the costs of mate-guarding, a mating tactic with high fitness benefits but also potentially high costs. In this thesis I define mate-guarding following Alberts et al. (1996, page 1270) as "close, persistent following of a female by a male that involves exclusion of other males from access to the female" (but see *section 1.1* for a range of definitions). While mate-guarding significantly increases mating and/or reproductive success of males (Censky 1995; del Castillo 2003; Setchell et al. 2005; Engelhardt et al. 2006), this mating tactic is potentially costly since the time and energy allocated to mate-guarding has to be traded-off against that required for body condition maintenance and survival (Parker 1974). Since the costs of mate-guarding ultimately limit the monopolisation potential of the highest-ranking male in a group (e.g. Hirotani 1994), understanding and quantifying these costs may shed light on the high variability in male reproductive skew (i.e. the partitioning of reproduction among males) observed across taxa (Clutton-Brock 1988, 1998; Hager & Jones 2009; Port & Kappeler 2010).

In the following introduction I will firstly discuss the interplay between sperm competition and male reproductive strategies with a specific emphasise on mate-guarding in multi-male animal groups (*section 1.1*). Here I will also emphasise the relevance of female reproductive strategies for male reproductive potential. In *section 1.2* I will introduce briefly the theoretical basis of recent reproductive skew models and their link to the verbal primate-focused Priority of Access model (Altmann 1962). In this section I will highlight why quantifying the costs of female monopolisation is an important parameter to consider in the framework of reproductive skew theories. Subsequently, I will outline the importance of energy in the modulation of male life-history trade-offs (*section 1.3*) and detail the behavioural and physiological components of an animal's energetic status (*section 1.4*). I will then review our current knowledge on the energetic costs of male reproductive effort in

vertebrates with a specific emphasis on mate-guarding (*section 1.5*). Here I will highlight the lack of comprehensive studies on the costs of mate-guarding in mammals in general and in non-human primates in particular. Further, I will discuss the usefulness of urinary C-peptide (a non-invasive marker of energetic status) in the quantification of these costs in wild primates (*section 1.6*). I will then show the importance of the stress response and the associated glucocorticoid release as a physiological tool providing males with the readily available energy required to succeed during reproductive competition (*section 1.7*). Since the costs of reproductive effort is likely to drive the evolution of mate-choice, in *section 1.8* I will briefly describe the evidence of male mate and mate-guarding choices in primates towards females of high reproductive and/or social value. Finally, I will introduce the study species (the long-tailed macaques, *Macaca fascicularis*) and briefly review the current knowledge about their reproduction, ecology and social system (*section 1.9*) before describing the specific aims of the thesis (*section 1.10*).

1.1 Male mating tactics in multi-male groups

In promiscuous mating systems several males compete with each other to fertilise a female during the short period when conception can occur (the fertile period) (Andersson 1994). Subsequently, if a female mates with more than one male during her fertile period, sperm competition arises whereby sperm from different males compete to fertilise the ova (Parker 1970; Birkhead & Moller 1998). Males have evolved diverse mating tactics to outcompete rivals in sperm competition (Gross 1996; Neff & Svensson 2013). Within a same species, drastically different tactics can arise and the ability for an individual male to adopt a given tactic is generally contingent on his physical condition and competitive abilities (Gross 1996; Oliveira et al. 2008; Neff & Svensson 2013). For example, in many species, the largest and/or high-ranking males engage in female mate-guarding whereas other males avoid direct male-male competition and access females using alternative mating tactics such as opportunistic sneaky copulations (Andersson 1994; Gross 1996; Setchell & Kappeler 2003; Neff & Svensson 2013).

Mate-guarding is a common mating tactic in a broad range of taxa, including insects, reptiles, crustaceans, birds and mammals (Alcock 1994; Censky 1995; Sparkes et al. 1996; Manson 1997; Low 2006; Willis & Dill 2007). Although mate-guarding always serves the function of limiting sperm competition by excluding rivals from accessing the guarded female, its duration varies considerably across taxa from few minutes in dragonflies (*Sympetrum obtrusum*, Singer 1987) to several weeks in long-tailed macaques (van Noordwijk 1985a). Mate-guarding can take various forms such as: 1) prolonged copulation beyond the time required for fertilisation (Carroll 1991), 2) maintenance of permanent physical contact with the female (female-grasping) or continuous monitoring of the

female after mating (Alberts et al. 1996; Sparkes et al. 1996) and 3) formation of mating plugs sealing the access to the female genital track (Alcock 1994). Interestingly, the adoption of different mating tactics (e.g. mate-guarding or sneaking) may modulate male allocation of resources into sperm production. Sneakers, facing a higher risk of sperm competition than mate-guarding males have bigger testes and produce sperm of better quality, in larger quantity and/or with different morphology (Simmons et al. 2007; Sarasa et al. 2010; Iwata et al. 2011). Mate-guarding males may also modulate sperm production and/or mate-guarding intensity depending on the risk of sperm competition (number of male rivals) and on female quality (Komdeur 2001; Setchell & Wickings 2006; Simmons et al. 2007; Ancona et al. 2010, see also *section 1.8*). In fact, the higher the number of males in a group the lower is the monopolisation potential of females by the highest-ranking male (e.g. in primates Kutsukake & Nunn 2006; Ostner et al. 2008b; Gogarten & Koenig 2013).

Beyond intra-sexual competition, the ability of high-ranking males to efficiently monopolise females may also be impaired by female counter-monopolisation strategies arising from male and female diverging interests (Trivers 1972; Setchell & Kappeler 2003). The ultimate goal for mate-guarding males is to be the only mating partner of each female. In contrast, females benefit from mating with multiple partners by increasing the extent of sperm competition and hence the chance to be fertilised by sperm of high quality males or males with compatible genes (Yasui 1997; Zeh & Zeh 2001). By mating polyandrously, females can also better confuse paternity as a strategy against future infanticide by males (van Schaik 2000). Females have evolved various counter-strategies to break down the monopolisation potential of males. In primates, for example, females initiate sneaky copulations away from the vigilance of the guarding males (de Ruiter et al. 1994; Berard et al. 1994) and can extend their period of sexual proceptivity and receptivity beyond the fertile window i.e. the period during which fertilisation can actually occur (Engelhardt et al. 2007; Fürtbauer et al. 2011b; Young et al. 2013a). The interplay between male and female reproductive strategies determines female monopolisation potential which in turn modulates the degree of reproductive skew across males.

1.2 Reproductive skew and the Priority of Access model

In group living animals, the partitioning of reproduction among same-sexed individuals – i.e. *reproductive skew* - differs greatly within and among species (Clutton-Brock 1988; Keller & Reeve 1994; Hager & Jones 2009). Several theoretical models have been developed to predict how social and ecological factors affect the degree of reproductive skew in group living animals (reviewed in Clutton-Brock 1998; Johnstone 2000; Buston & Zink 2009; Port & Kappeler 2010). These models propose that the degree of skew within a group is strongly influenced by dispersal patterns,

cooperation between group members and ecological constraints (Vehrencamp 1983a, 1983b; Keller & Reeve 1994; Clutton-Brock 1998). Reproductive skew theory is thus a potential candidate for a general theory of social evolution (Keller & Reeve 1994; Sherman et al. 1995; Johnstone 2000). Despite this common ground, models of reproductive skew can be classified into two categories: transactional and compromise models (reviewed in Clutton-Brock 1998; Johnstone 2000; Buston & Zink 2009; Port & Kappeler 2010). One such transactional model, the *concession model*, assumes that a dominant individual has complete control over the reproductive output in the group but concedes a certain share of reproduction to other individuals as an incentive to stay in the group (Vehrencamp 1983a; Clutton-Brock 1998; Reeve & Emlen 2000). Here the reproductive share that subordinates can claim within the group depends on their degree of genetic relatedness with the dominant and their reproductive potential outside the group (Reeve & Emlen 2000; Buston & Zink 2009). In contrast, compromise (or limited control) models assume that the reproduction cannot be controlled by a single individual and is partitioned among group members solely based on their competitive abilities (Reeve et al. 1998). The resulting degree of skew therefore depends on the degree of control by the dominant individual (Reeve et al. 1998).

Whether complete (transactional models) or incomplete (compromise models), the degree of control over reproduction by dominants is a fundamental aspect of reproductive skew theory. For males, this control can be strongly influenced by the temporal overlap of female fertile periods since males are usually physically unable to monopolise access to more than one female at one time. Based on this reasoning, the Priority of Access model (hereafter PoA model, Altmann 1962) has been developed to explain the variation in male reproductive skew in primates. This model posits that if females are completely asynchronous in the timing of their fertile periods, the highest-ranking male will be able to fully monopolise access to all the females. If more than one female is fertile at the same time, other males will gain reproductive access to these additionally fertile females following a hierarchical order (beta male first, then gamma and so on). In line with the PoA model, high-ranking male primates are often the individuals mate-guarding females most extensively (de Ruiter et al. 1994; Berard et al. 1994; Bercovitch 1997; Matsubara 2003; Setchell et al. 2005; Engelhardt et al. 2006) and they achieve higher reproductive success than low-ranking males (reviewed in Ellis 1995; Majolo et al. 2012). Meta-analyses further support the general concept of the PoA model since female reproductive synchrony predicts the degree of reproductive skew across primate species (Kutsukake & Nunn 2006; Ostner et al. 2008b; Gogarten & Koenig 2013). However, in some species, the alpha male's mating and/or reproductive success is lower than predicted by the model (e.g. savannah baboons, *Papio cynocephalus*, Alberts et al. 2003; rhesus macaques, *M. mulatta*, Dubuc et al. 2011; long-tailed macaques, Engelhardt et al. 2006; and Barbary macaques, *M. sylvanus* Young et al.

2013b). This indicates that additional factors other than temporal overlap of female fertility periods may further limit the alpha male monopolisation potential such as 1) males' ability to accurately assess the timing of female fertile phase, 2) male-male coalition formation, and 3) energetic and physiological costs of mate-guarding (reviewed in Alberts 2012). The ability of males to discern the female fertile phase has been empirically tested in several species (chimpanzees, *Pan troglodytes*, Deschner et al. 2004; long-tailed, rhesus and Barbary macaques, Engelhardt et al. 2004, Dubuc et al. 2012, Young et al. 2013a; and Hanuman langurs, *Semnopithecus entellus*, Heistermann et al. 2001). Further, the formation of male-male coalitions to disrupt mate-guarding behaviour of other males and subsequently gain access to the guarded female has been documented in baboons and macaques (Packer 1979; Bercovitch 1988; Noe & Slujiter 1995; Bissonnette et al. 2011). In contrast, the costs of mate-guarding remain largely unclear for primates (*section 1.5*). A test of the PoA model in a non-primate species, the reindeer (*Rangifer tarandus*), shows that the energetic costs of mate-guarding may directly impair the capacity of the alpha male to monopolise females (Hirotani 1994). Due to reduced food intake, the alpha male became exhausted in the middle of the mating season and his ratio of observed to expected reproductive success dropped from 0.98 at the beginning of the mating season to 0.57 at the end. This example illustrates why assessing the energetic costs of male reproductive effort in general (and of mate-guarding in particular) is crucial to better comprehend the factors limiting male monopolisation potential in multi-male groups and ultimately affecting male reproductive skew (Clutton-Brock 1998; Port & Kappeler 2010).

1.3 Energetic status and life-history trade-offs

An organism's energetic status derives from its capacities to respond to its metabolic needs and to balance energy intake and expenditure (McEwen & Wingfield 2010). A number of predictable cyclic life-history events (e.g. breeding/birth seasons, raining/dry seasons) but also of unpredictable events (e.g. predation, diseases, female fertility periods in aseasonal breeders) affect an individual's energy intake and expenditure (McEwen & Wingfield 2010, *Figure 1.1*). Energetic status is important for male life-history trade-offs since it strongly impacts a male's lifetime reproductive output and longevity (Lindström 1999). It underlies an individual's fighting abilities (Briffa & Elwood 2004) and hence the capacity to engage in contest competition (reviewed in Briffa & Sneddon 2007). Furthermore, energetic status may determine which mating tactic a male employs. For example, in seals, only males with above average body fatness and energy engaged in mate-guarding (grey seals, *Halichoerus grypus*, Lidgard et al. 2005). Additionally, heavier males were able to sustain reproductive effort for longer periods and achieve higher reproductive success than other males (Northern elephant seals, *Mirounga angustirostris,* Crocker et al. 2012). An individual's energetic

condition is also important for the maintenance of a functional immune system (Lochmiller & Deerenberg 2000). To comprehend the mechanisms underlying a male's life-history trade-offs, it is thus important to quantify a males' energetic status.

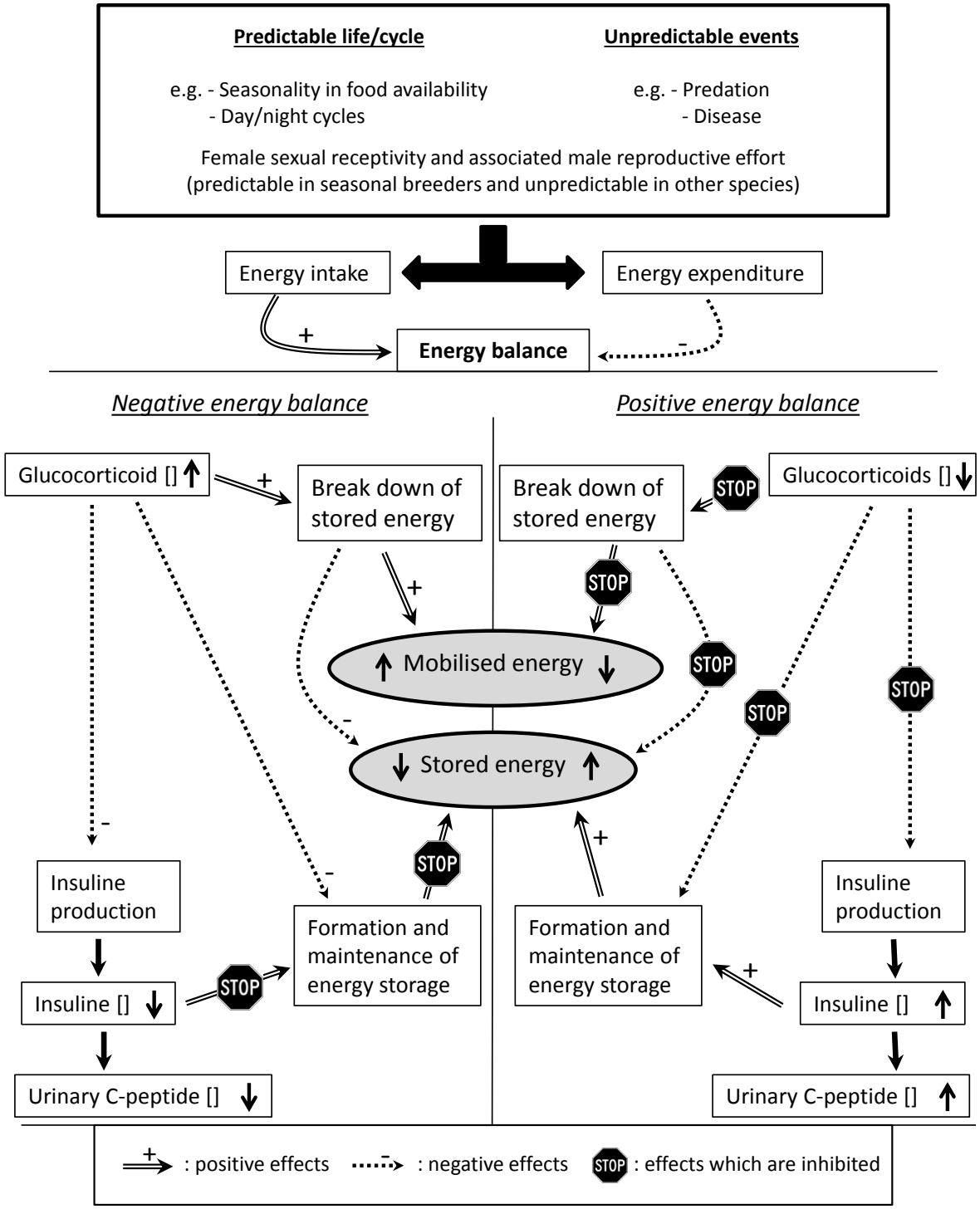

Figure 1.1: Illustrative representation of the link between energy balance, glucocorticoid, insulin and UCP production and energy mobilisation and storage.

Situations of negative (left) and positive (right) energy balance are depicted. Hormone concentrations are indicated by []. Inspired from Sapolsky 2002; McEwen & Wingfield 2010.

1.4 Components of energetic status

The fundamental components of an individual's energetic status are his energy intake and expenditure. When demand in energy expenditure surpasses the energy acquired through feeding, males face a state of negative energy balance (McEwen & Wingfield 2010, *Figure 1.1*). The body responds to this energetic stress through a chain of hormonal reactions, involving (among others) glucocorticoids (hereafter GCs) and insulin, which modulate energy storage and mobilisation and insure that vital energetic needs are fulfilled (Sapolsky 2002, *Figure 1.1*). In response to a stressor (such as negative energy balance), GCs are released as the outcome of a cascade of reactions (Sapolsky 2002; Tsigos & Chrousos 2002). Following the perception of the stressor by the brain, corticotropin-releasing hormones (CRH) are produced by the hypothalamus. Subsequently, CRH stimulates the production of adrenocortiocotropic hormones (ACTH) by the pituitary gland, which in turn activates the release of GCs by the adrenal cortex. Subsequently, GCs inhibit the storage of energy and stimulate the breakdown of stored energy via gluconeogenesis (*Figure 1.1*). GCs also inhibit insulin production and block its stimulating action on the formation and maintenance of energy storage (*Figure 1.1*). As a general consequence of the action of GCs and the inhibition of insulin the stored energy stock diminishes whereas the amount of readily available energy increases (*Figure 1.1*). In a reversed case of positive energy balance, GCs levels are lower and their inhibiting actions on insulin production and fat storage are suppressed (*Figure 1.1*). This leads to a rise in insulin concentration which stimulates the formation and maintenance of energy storage (*Figure 1.1*). The stored energy stock increases and the requirement for energy expenditure are fulfilled by dietary intake (*Figure 1.1*).

In order to gather a comprehensive picture of the effects of a male reproductive effort on his energetic status it is important to consider both behavioural and physiological components of this status.

1.5 Energetics of male reproductive effort

Many studies quantified the effect of male mating and mate-guarding effort on one of the two principle components of males' energetic status, energy intake (i.e. feeding or foraging time, ingestion rate and diet quality, see *Table 1.1* for mate-guarding). Similar to reindeer (*section 1.2*), males in other mammalian species commonly reduce their food intake during periods of intense male-male competition (e.g. the mating season, Poole 1989; Bercovitch 1997; Pelletier 2005; Galimberti et al. 2007; Ancona et al. 2010). In some ungulates, this effect is extreme since males stop feeding completely during the rut (Miquelle 1990; Mysterud et al. 2008).

Table 1.1: A summary of the various costs of mate-guarding in animal species.

species	Class	costs	effect(s) of mate-guarding on males	reference
Yellow baboons (*Papio cynocephalus)*	Mammalia	energetic	decreased feeding time and feeding bout duration [(a)]	Rasmussen 1985; Alberts et al. 1996
Chacma baboons *(Papio hamadryas)*	Mammalia	energetic stress	decreased foraging time [(a) 1] increased glucocorticoid levels [(a)]	Weingrill et al. 2003; Bergman et al. 2005
Olive baboons *(Papio anubis)*	Mammalia	aggression energetic	increased the time spent in agonistic interactions [(a)] decrease feeding time [(a)]	Packer 1979; Bercovitch 1983
Moustached tamarins *(Saguinus mystax)*	Mammalia	predation	increased conspicuousness [(a)]	Huck et al. 2004
Japanese Macaques *(*Macaca fuscata*)*	Mammalia	energetic	decreased feeding time [(b)]	Matsubara 2003
Round-eared sengis *(Macroscelides proboscideus)*	Mammalia	energetic	body mass loss [(a)]	Schubert et al. 2009
Whiptail lizards *(Aspidoscelis costata)*	Reptilia	energetic aggression	decreased prey size and capture rate [(a)] increased rate of initiated agonistic interactions [(a)]	Ancona et al. 2010
Caribbean ameivas (Ameiva plei)	Reptilia	energetic	decreased foraging time [(b)]	Censky 1995
Horseshoe crabs *(Limulus polyphemus)*	Malacostraca	energetic	emptier digestive tract and higher $\delta^{15}N$ (a marker of nutritional stress) [(b)]	Smith et al. 2013
Common Eiders (*Somateria mollissima*)	Aves	aggression	increased the time spent in agonistic interactions [(b)]	Steele et al. 2007
Seychelles warblers (*Acrocephalus sechellensis*)	Aves	energetic	decreased feeding time [(a)] body mass loss [(a)]	Komdeur 2001
Stitchbirds (*Notiomystis cincta)*	Aves	energetic	body mass loss [(b)]	Low 2006
Japanese beetles *(Popillia japonica)*	Insecta	thermo-regulation	increased thoracic temperature [(b)]	Saeki et al. 2005

[(a)] Comparisons within the same individuals between mate-guarding and non-mate-guarding periods e.g. for yellow baboons: males have shorter feeding bouts when mate-guarding than when not. [(b)] Comparisons between mate-guarding and non-mate-guarding individuals – e.g. for Caribbean ameiva: mate-guarding males forage less time than non-mate-guarding males. [1] The effect was found only in one of the two studied groups.

Males also reduce their feeding time and/or efficiency and the quality of their diet while mate-guarding females in mammalian, reptilian and avian species (*Table 1.1*). Nevertheless, intra-taxa and even intra-species variations exist regarding this effect. In primates, for example, feeding costs of mate-guarding have been identified in some species (*Table 1.1*), but evidence is sometimes equivocal. For instance, these costs have been documented in one study of olive baboons (*P. Anubis,* Packer 1979) but were not found in another study of the same species (Bercovitch 1983). Furthermore, feeding costs of mate-guarding were identified only in one of the two studied groups in chacma baboons (*P. hamadryas ursinus*, Weingrill et al. 2003) and were completely absent in moustached tamarins (*Saguinus mystax*, Huck et al. 2004).

The decrease in feeding time associated with male reproductive effort in vertebrates may be related to a trade-off between vigilance and feeding time arising from the need to monitor conspecifics more intensively during periods when male-male competition is high. Males are more vigilant during the reproductive season in birds and mammals (Reboreda & Fernandez 1997; Li et al. 2012) and particularly when they are guarding females (Guillemain et al. 2003). Vigilance is important for male energetic status since this activity not only reduces energy intake (through the trade-off with feeding time), but is also in itself a source of energy expenditure (Warm et al. 2008). To my knowledge, however, only one study investigated the effect of mating competition on the trade-off between vigilance and feeding in mammals (Przewalski's gazelle, *Procapra przewalskii*, Li et al. 2012) and, in primates, no study quantified the vigilance costs of male mating effort in general and mate-guarding in particular.

Male-male agonistic interactions associated with male mating competition may also result in substantial increase in male energy expenditure (reviewed in Briffa & Sneddon 2007). Male-male competition for access to mates usually leads to an increase of aggression between males during the breeding season (Shepard 2004; Franceschini et al. 2007; Gould & Ziegler 2007; Mass et al. 2009). In addition, within the breeding season, mate-guarding behaviour further increases the rate of male-male aggression or the time spent in agonistic interactions in mammals, reptiles and birds (*Table 1.1*). Mate-guarding thus affects male energy intake and expenditure and hence leads, at least in some species, to a degradation of male overall energetic condition (e.g. body mass loss, *Table 1.1*).

However, no study so far quantified the overall effect of mate-guarding on primate males' energy balance using a physiological tool or direct body mass measurements, which prevents the drawing of clear conclusions on the effect of mate-guarding on a male's energetic in this taxa. This lack of study is principally due to the absence of a validated non-invasive physiological marker to assess accurately non-hominid primate energetic status in the wild.

1.6 Urinary C-peptide to measure primates' energetic status in the wild

Several methods have been applied to assess primate energetic condition non-invasively in the wild but several drawbacks of these methods make them unrecommandable for energetic studies. First primatologists implemented techniques to measure an animals' body mass without trapping by baiting the animal on a scale with food (Mori 1979; Altmann & Alberts 1987; Cooper et al. 2004). Yet this approach may interfere with an animal's nutritional status. Later, in order to assess primate nutritional status completely non-invasively, indirect methods have been developed such as 1) visual estimation of animal body condition (Berman & Schwartz 1988; Koenig et al. 1997; Heesen et al. 2013), 2) estimation of energy intake and expenditure based on behavioural observations (Altmann & Samuels 1992; Tsuji et al. 2008; Heesen et al. 2013) and 3) semi-quantitative measurement of urinary ketone metabolites (a marker of fat metabolism, Robinson & Williamson 1980) using urinalysis strip (Knott 1998). Some of these methods are, however, extremely labour intensive and were criticised for often leading to imprecise assessments of an animal nutritional status (Leonard & Robertson 1997; Chivers 1998; Schülke et al. 2006, see details **Chapter 2**). Therefore, primatologists recently advocated the need for a reliable non-invasive physiological tool capable of detecting fine-tuned variation in wild primates' nutritional status and proposed urinary C-peptide (hereafter UCP) as a potential candidate (Sherry & Ellison 2007). UCP excretion is directly linked to insulin production and insulin concentrations (through the action of GCs, *Figure 1.1*) and therefore relates to an individual's energy balance. During insulin production, C-peptide is co-secreted into the blood stream from the islet beta cells in equimolar amounts with insulin (Rubenstein et al. 1969), and a constant fraction of the C-peptide produced is excreted in the urine (Kruszynska et al. 1987). In humans, UCP measures are correlated with insulin concentration in the blood (Kruszynska et al. 1987) and increased caloric intake lead to a concurrent increase in 24 hour UCP excretion (Hoogwerf et al. 1986). UCP also negatively correlates to measures of energy expenditure (Reiches 2011). In humans this marker thus captures both components of energetic status (i.e. energy intake and expenditure). Recent studies on wild and captive great apes extended the applicability of UCP to non-human primates. In wild orangutans (*Pongo pygmaeus*) and chimpanzees, UCP measures positively correlated with estimates of food availability and/or caloric intake in the wild (Sherry & Ellison 2007; Emery Thompson & Knott 2008; Emery Thompson et al. 2009). Wild chimpanzees had on average lower UCP levels than captive ones under better energetic conditions (Emery Thompson et al. 2009). Finally, a food-reduction experiment in captive bonobos (*Pan paniscus*) showed that UCP levels co-vary with daily changes in individual food intake and body mass (Deschner et al. 2008).

UCP has been recently used to quantify the energetic costs of male reproductive effort in free-ranging rhesus macaques (Higham et al. 2011a). During the reproductive season energetic condition

(i.e. UCP measures) declined significantly more for high- than for low-ranking males. Furthermore, individual copulation rates were negatively correlated with mean UCP levels. High-ranking male rhesus macaques mate-guarded females more and copulated at higher rates than low-ranking males (Higham et al. 2011a). Therefore, the greater degradation in energetic condition in high-ranking males may stem from higher mating and/or mate-guarding effort. Yet, the energetic costs of mate-guarding have never been quantified in wild primates using physiological measures.

1.7 Male reproductive effort and physiological stress

UCP is a relatively new marker in field studies. In contrast, non-invasive measurement of GCs from animals' faeces have been implemented earlier (Whitten et al. 1998) and many studies used this marker to investigate how males respond physiologically to the energetic challenge of male-male competition (reviewed in Romero 2002).

Male vertebrates often show substantial increases in their GC levels during the breeding season (mammals, Barrett et al. 2002; Mooring et al. 2006, reptiles and amphibians, Tokarz et al. 1998; Moore & Jessop 2003, reviewed in Romero 2002). GCs play a prominent role in an animal's energetics (Sapolsky 2002, *Figure 1.1*, *section 1.5*) and the "energy mobilisation hypothesis" posits that males' GC levels are the highest during the breeding season because it is the most energetically costly time of the year (Romero 2002). In fact, males commonly face increases in energy expenditure and decreases in energy intake during the breeding season (*section 1.5*). Therefore, GCs function as a physiological tool insuring energy delivery (*Figure 1.1*) and priming a male's body for competition.

Even though males exhibit a general increase in GC levels associated with reproductive competition, pronounced inter-individual differences, often linked to dominance rank, can be found between males in the same group (Creel 2001). Different GC levels between high- and low-ranking males can derive from the use of different mating tactics. For example, in baboons, the difference in GC levels between the alpha and the beta male matched differences in time spent mate-guarding females (Gesquiere et al. 2011) and an intra-individual comparison revealed that males have higher GCs levels when mate-guarding females than when not (Bergman et al. 2005, *Table 1.1*). Whereas elevated GCs insure an adaptive proximate function by providing males with the energy required to perform a specific mating tactic and outcompete their rivals, prolonged exposure to high GC levels may lead to chronic stress and negatively impact a male's fitness (Sapolsky 2002). Chronic stress may alter sperm production (Sapolsky 1985; Hardy et al. 2005), suppress the immune system (Grossman 1985; Setchell et al. 2010) and have an overall negative effect on an animal's health (Sapolsky 2002).

In the long run physiological stress and the negative energy balance associated with male reproductive effort may limit the ability of a male to engage fully in certain mating tactics (e.g. mate-guarding). In turn these costs of reproducing combined with variation in the fitness value of females may favour the evolution of male mating and mate-guarding choices (Kokko & Monaghan 2001; Edward & Chapman 2011).

1.8 Male mate-choice in primates

In primates, as in many other taxa (see Edward & Chapman 2011), males have evolved a certain degree of mate-choice to reduce the costs of reproducing (see *sections 1.5* and *1.7*) by concentrating their reproductive effort towards the most valuable females (reviewed in Keddy-Hector 1992; Setchell & Kappeler 2003; Kappeler 2012). From a male's perspective, females can have two non-mutually exclusive types of value: 1) a reproductive value inherent to a female's capacity to produce high quality offspring, capable of siring offspring themselves (Setchell & Kappeler 2003), and 2) a social value via strong male-female social-bonds leading in some species to female support during conflicts or cooperation during mate-guarding (e.g. yellow baboons, Rasmussen 1980). In several primate species, high-ranking and/or parous females are of higher reproductive value than low-ranking and/or nulliparous females since they produce offspring of better quality, surviving until adulthood and achieving a high rank position in the future (long-tailed macaques, van Noordwijk & van Schaik 1999, 2001; mandrills, *Mandrillus sphinx*, Setchell et al. 2002; mountain gorillas, *Gorilla beringei beringei*, Robbins et al. 2011; reviewed in Majolo et al. 2012). Furthermore, strong male-female bonds directly increase the reproductive success of males independently of male dominance status (e.g. in rhesus macaques, Kulik et al. 2012; Massen et al. 2012) showing the fitness relevance of female social value, at least in some species. Males therefore often concentrate their mating and/or mate-guarding efforts on high-ranking and parous females and/or on females with whom they have strong social bonds (mandrills, Setchell & Wickings 2006; macaques,Takahata 1982; Chapais 1983; de Ruiter et al. 1994; Kuester & Paul 1996; Engelhardt et al. 2006; baboons, Berenstain & Wade 1983; Smuts 1985). Mate-guarding choices in particular were quantified as the number of days a given female was guarded or as whether a female was preferably guarded over another one when two females were fertile at the same time (Engelhardt et al. 2006; Setchell & Wickings 2006). However, to my knowledge, no study ever investigated whether the mate-guarding behaviour of the male itself varies with female quality (i.e. if they mate-guard highly valuable females more thoroughly or aggressively) and whether the potential costs of mate-guarding vary with female quality (i.e. if mate-guarding choices are also driven by cost-related factors).

In this thesis, I investigated if and how mate-guarding tactic and guarded female value influence high-ranking males' energetic and physiological stress parameters in long-tailed macaques. In this species, male reproductive skew has been quantified and male mating tactics described in details (van Noordwijk 1985b; de Ruiter et al. 1994; Engelhardt 2004). However, the extent to which mate-guarding is costly for males and whether these costs (if any) may influence mate-guarding choices in this species (Engelhardt et al. 2006) remains unknown.

1.9 Study site and species

Long-tailed macaques are the smallest and lightest of all the macaques (Ashmore Declue 1992). This species exhibits a pronounced sexual dimorphism with males being 50-80% heavier and larger than females (reviewed in Ashmore Declue 1992). Long-tailed macaques are widely distributed in south-east Asia ranging from south east Bangladesh to the Philippines and central and west Indonesia (Fooden 1982; Thierry 2007). They inhabit a vast diversity of habitats (reviewed in Gumert 2011) comprising many different types of primary and secondary forests (shrub, swamp, coastal, tropical, deciduous, evergreen, riverine), mangroves, rocky shorelines and a variety of anthropogenic environments (temples, agricultural lands, recreational parks). To adapt to these various ecosystems long-tailed macaques have developed an extraordinary plasticity in their diet and feed on hundreds of different items ranging from fruits to molluscs or anthropogenic food (Richard et al. 1989; Wheatley et al. 1996; Fuentes et al. 2011; Gumert 2011; Gumert & Malaivijitnond 2012). The social organisation of *M. fascicularis* is similar to other macaque species (Thierry 2007). They live in multi-male multi-female groups, females are philopatric and males disperse (van Noordwijk & van Schaik 2001). Males and females organise themselves in a linear dominance hierarchy in which females usually inherit the rank of their mother and males achieve their dominance rank mainly through contest competition (van Noordwijk & van Schaik 1987, 2001; de Ruiter et al. 1994).

For this thesis, I studied wild long-tailed macaques in the primary lowland rainforest surrounding the Ketambe research station (3º41'N, 97º39'E) within the Gunung Leuser National Park, province of Aceh, Sumatra, Indonesia. The long-tailed macaques in this area have been studied since 1979 (van Schaik & van Noordwijk 1985; de Ruiter et al. 1994; Engelhardt et al. 2004). Even though the ecology of long-tailed macaques can vary greatly between different habitats, in primary rainforests they are mainly frugivorous (Ungar 1995; Yeager 1996) and highly arboreal (95-98%, reviewed in Richter et al. 2013). Similar to many south-east Asian primate species, long-tailed macaques inhabiting primary forests face extreme resource unpredictability (Brockman & van Schaik 2005). They adopt therefore a capital breeder strategy - i.e. the timing of reproduction is triggered by internal cues and contingent on resource availability (Brockman & van Schaik 2005). In Ketambe, female long-tailed

macaques can conceive year round. Yet periods of 4 to 7 months with increased female receptivity and subsequent birth peaks frequently occur, the timing of which depends on inter-annual variation in seasonality of fruit abundance (van Schaik & van Noordwijk 1985).

Long-tailed macaques are a good model species for studying the link between the costs of reproductive tactics and reproductive skew. In this species, high-ranking males use mate-guarding as their primary mating tactic and the reproductive success is highly skewed towards the alpha male (52-92% alpha male paternity, de Ruiter et al. 1994). Yet it was found that the alpha male did not mate-guard all females and did not achieve 100% of reproductive success despite the absence of an overlap in the timing of female fertile phases (Engelhardt et al. 2006). This deviation from the predictions of the PoA model could be explained by the costs associated with female monopolisation.

1.10 Aims of the thesis

The overall aim of this thesis was to quantify the behavioural and physiological costs of mate-guarding in male long-tailed macaques and to identify the factors modulating these costs.

As discussed in *section 1.6*, accurately quantifying the energetic costs of reproductive tactics in wild primates requires non-invasive tools allowing the detection of fine-tuned variation in an animal's energetic condition. Urinary C-peptide (UCP) is a promising marker in this respect but its applicability in non-great-ape-primates (such as macaques) has never been thoroughly validated and thus remains uncertain. Accordingly, I first aimed to validate UCP as a reliable marker of energetic status in macaques (**chapter 2**). I used free ranging and captive adult individuals of two macaque species (*M. fascicularis* and *M. mulatta*) to assess the reliability of UCP to track intra- and inter-individual variation in body mass, skinfold fatness, body-mass-index and plasma C-peptide levels.

In **chapter 3,** I focused on the energetic costs of mate-guarding by quantifying behavioural components of energy intake and expenditure, but also by using the newly validated UCP marker to assess the effect of female monopolisation on a male's energetic status. Males reduced their feeding time while mate-guarding females, but I could not detect any effect of this behaviour on male energetic status. I aimed thus to investigate other potential costs of mate-guarding such as physiological stress levels, vigilance and aggressions (**chapter 4**).

Finally, in **chapter 5,** I aimed to determine whether males can be constrained in their mate-guarding decisions by costs associated with the identity of the guarded-female. I detailed how females' reproductive value (dominance rank and parity status) and social value (male-female social bond)

affect the costs of mate-guarding measured in **chapters 4** and **5**. I also examined if and to what extent males' investment into mate-guarding depends on females' value.

The ultimate aim of this thesis was to better comprehend the factors driving the evolution of male reproductive decisions and the related variation in the pattern of male reproductive skew within but also across species by comprehensively quantifying the costs of mating tactic providing high fitness benefits to males, mate-guarding.

Chapter 2

Urinary C-peptide measurement as a marker of nutritional status in macaques

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Abstract

Studies of the nutritional status of wild animals are important in a wide range of research areas such as ecology, behavioural ecology and reproductive biology. However, they have so far been strongly limited by the indirect nature of the available non-invasive tools for the measurement of individual energetic status. The measurement of urinary C-peptide (UCP), which in humans and great apes shows a close link to individual nutritional status, may be a more direct, non-invasive tool for such studies in other primates as well and possibly even in non-primate mammals. Here, we test the suitability of UCPs as markers of nutritional status in non-hominid primates, investigating relationships between UCPs and body-mass-index (BMI), skinfold fatness, and plasma C-peptide levels in captive and free-ranging macaques. We also conducted a food reduction experiment, with daily monitoring of body weight and UCP levels. UCP levels showed significant positive correlations with BMI and skinfold fatness in both captive and free-ranging animals and with plasma C-peptide levels in captive ones. In the feeding experiment, UCP levels were positively correlated with changes in body mass and were significantly lower during food reduction than during re-feeding and the pre-experimental control condition. We conclude that UCPs may be used as reliable biomarkers of body condition and nutritional status in studies of free-ranging catarrhines. Our results open exciting opportunities for energetic studies on free-ranging primates and possibly also other mammals.

Introduction

Nutritional status significantly influences an individual's daily activities, ranging from the maintenance of basal body functions to the behavioural strategies that ensure survival and successful reproduction. In the long-term, it influences all aspects of animal life-history from growth, life-time reproductive output to longevity (Lindström 1999). Studies on individual energetic status therefore form an important part of biological research areas such as ecology, behavioural ecology and reproductive biology (e.g. Karasov 1986; Bronson 1989; Jürgens & Prothero 1991; Krebs & Davies 1993; Cuthill & Houston 1997). Direct measurement of energetic status has, however, been limited so far for wild and/or free-ranging animals by the lack of suitable non-invasive tools.

In non-human primates, for example, most studies have so far used very indirect or invasive methods for the measure of individual nutritional status such as: 1) the visual estimation of body fat and/or condition (e.g. Berman & Schwartz 1988; Koenig et al. 1997), which is subjective, susceptible to inter-observer inconsistencies, and only possible when differences in nutritional status are extreme enough to recognise them visually; 2) the weighing of animals, which is usually difficult in the wild, either requires trapping or darting of animals (inducing stress and potentially altering natural behaviour and metabolism; Puri et al. 1981; Sapolsky 1982, 1985, 1986; Goldizen et al. 1988; Altmann et al. 1993) or includes baiting scales with food (Mori 1979; Altmann & Alberts 1987), which interferes with studies of nutritional status. These problems are particularly problematic when measurements need to be systematically repeated, and for arboreal animals; 3) behavioural observation, in which calorific intake is estimated (e.g. Altmann 1998; Chivers 1998) or feeding and travelling activity budgets used (e.g. Altmann & Samuels 1992), methods that are labour intensive, imprecise, and have produced results that have proven difficult to interpret (Barton et al. 1993; Chivers 1998; Schülke et al. 2006); 4) the assessment of urinary ketones, a measure of fat metabolism with a semiquantitative strip test (e.g. Knott 1998) which allows only very rough quantification of nutritional status and is often not sensitive enough to detect intra- or inter-individual variation (Kelly et al. 2004; Wich et al. 2006); and 5) the measure of nutritional status through the use of X-rays (Blanc et al. 2005) or doubly-labelled water (Lifson & McClinto 1966), which cannot be applied to free-ranging or wild animals without capture (see above).

The analysis of urinary C-peptide, which has recently been shown to be closely linked to individual nutritional status in humans and great apes, may offer a method to allow energetic status to be measured more directly and with a higher degree of sensitivity in free-ranging/wild mammals. C-peptide is produced in an equimolar ratio to insulin when the body converts proinsulin to insulin during insulin biosynthesis (Rubenstein et al. 1969). In humans, C-peptide is excreted in urine

(urinary C-peptide; UCP), with excretion levels correlated with internal C-peptide secretion and insulin production (Meistas et al. 1982; Kruszynska et al. 1987) under normal circumstances, making UCP a useful marker of insulin production in many clinical studies (e.g. Yoshida et al. 2006; Hoffman et al. 2008). Increased calorific intake over 24h periods leads to increases in 24h UCP excretion (Hoogwerf et al. 1986), and levels of 24h UCP excretion are positively correlated with body weight (Hoogwerf et al. 1986) and BMI (Kruszynska et al. 1987). Recent studies of great apes have demonstrated similar relationships, with UCP levels shown to correlate with serum C-peptide levels and ripe fruit consumption in chimpanzees (*Pan troglodytes*; Sherry & Ellison 2007; Emery Thompson et al. 2009), energetic intake estimates and fruit availability in orangutans (*Pongo pygmaeus*; Emery Thompson & Knott 2008), and body mass in bonobos (*Pan paniscus*; Deschner et al. 2008) (see *Table 2.1*). In addition, feeding experiments have shown that UCP levels respond to dietary changes, with low levels excreted during fasting, and higher levels during re-feeding (Deschner et al. 2008).

UCPs have also been measured in non-great ape primate species. A previous study investigating behavioural and physiological correlates of obesity in captive rhesus macaques (*Macaca mulatta*) using 12 hour urine collections for UCP measurement found that obese animals had significantly higher UCPs than non-obese ones, and that urinary and serum C-peptide levels were correlated (Wolden-Hanson et al. 1993). In a more recent study on colobus monkeys (*Colobus guereza*), scarcity of top folivorous was associated with a decline in UCPs in two lactating females (Harris et al. 2010). Collectively, these studies give reason to believe that UCPs are a valuable biomarker of energetic status also in non-hominid primates, if not even in mammals in general. However, a thorough validation of urinary C-peptides as a measure of energetic condition based on analysis of single urine voidings, usually the only type of sample available in the wild, has not yet been performed for non-hominid primate species.

To undertake such a validation, we conducted a study on macaques in order to investigate systematically the general value of UCPs as biomarkers of nutritional status using a dual approach. First we investigated inter-individual relationships between UCP levels and body mass index (BMI), body fatness, and levels of plasma C-peptide in captive rhesus and long-tailed macaques housed at the German Primate Centre (DPZ). In order to test the robustness of the data obtained for these captive animals and thus see whether C-peptide measurements would provide similar results also under field conditions, we repeated this investigation for all the same variables using free-ranging rhesus macaques on Cayo Santiago (CS), Puerto Rico. Second, we carried out a 4-week feeding experiment on DPZ animals to explore the interrelationships between changes in food supply (and thus energy intake), body mass and UCP levels in a more fine-tuned way. We predicted that: 1) UCPs

Table 2.1: Summary of the known relationship between urinary C-peptide levels and different measures of nutritional status for different primate species under different settings.

Species	Setting	Parameters							
		Serum insulin levels	Serum/plasma C-peptide levels	Body			Food Intake	Natural food availability	Ketone bodies
				Mass	Fatness	Mass Index			
Human	NA	(+) [1,2]	(+) [1.2]	(+) [3]		(+) [1]	(+) [3]		
Bonobo	captive			(+) [7]			(+) [7]		
Orangutan	wild						(+) [5]	(+) [5,6]	(+) [5]
Chimpanzee	captive		(+) [5]						
Chimpanzee	wild							(+) [4,5]	
Rhesus macaque	captive	(+) [8]	(+) [§]	(+) [§]	(+) [8]		(+) [8 §]		
Rhesus macaque	free-ranging				(+) [§]	(+) [§]			
Long-tailed macaque	captive		(+) [§]	(+) [§]	(+) [§]	(+) [§]	(+) [§]		

(+): positive relationship between urinary C-peptide levels and the parameter under study; [§] present results

NA: Non applicable. bibliography: [1]Kruszynska et al. 1987; [2]Meistas et al. 1982; [3]Hoogwerf et al. 1986; [4]Emery Thompson et al. 2009; [5]Sherry & Ellison 2007; [6]Emery Thompson & Knott 2008; [7]Deschner et al. 2008; [8]Wolden-Hanson et al. 1993

would correlate positively with plasma C-peptides, BMI and body fat in both captive and free-ranging animals; 2) UCPs would covary with body mass in response to dietary changes, decreasing during food-reduction, and increasing during re-feeding (as in Bonobos, Deschner et al. 2008). These analyses represent the first comprehensive test of the likely utility of urinary C-peptide for assessing energy intake and body fatness in studies of non-hominid primates.

Methods

Ethics Statement

The protocol for this study was approved by the government of Lower Saxony, Germany for DPZ animals (permit number: 33.14-42502-04-106/09) and by the Institutional Animal Care and Use

Committee, University of Puerto Rico, for CS animals (protocol number: A0100108). All research undertaken adhered to all animal care, legal and ethical requirements of Germany, the United States and Puerto Rico, as well as the Animal Behavior Society (ABS) and Association for the Study of Animal Behaviour (ASAB) "Guidelines for the Use of Animals in Research", and the American Society of Primatologists (ASP) "Principles for the Ethical Treatment of Non-Human Primates". CS animals are free-ranging, while all DPZ animals are housed either as pairs or groups, allowing for social interactions. The vast majority of samples were collected non-invasively (urine samples). Blood samples were collected only once from each individual during the study under sedation by trained veterinarians.

Study Sites and subjects

The study was conducted between 29th September 2009 and 17th February 2010 on 11 adult (≥ 5 years of age) captive macaques: 6 males and one female long-tailed (*Macaca fascicularis*) and two males and two female rhesus (*M. mulatta*) macaques housed at the German Primate Centre (DPZ) and, in addition, between 3rd November 2008 and 24th February 2009 on 13 adult free-ranging male rhesus macaques living on the island of Cayo Santiago (CS), 1 km off the coast of Puerto Rico (Rawlins & Kessler 1986). All captive animals were housed either as same-sex pairs or small same-sex groups in indoor cages, or as single-male-multi-female groups in outdoor enclosures with access to an indoor cage (see *Table 2.2*). DPZ macaques are fed twice a day (early morning and noon) with commercial monkey chow supplemented with fruits. The CS macaques are free-ranging and feed on natural vegetation on the island, but are also provisioned daily in the early morning with commercial monkey chow, made available in several feeding corrals. Though food composition varies between individuals and seasons, on average, Cayo rhesus spend 50.2% of their feeding time ingesting monkey chow, and 49.8% on ingesting a variety of natural vegetation (Marriott et al. 1989).

Collection of morphometric data and plasma sample collection

Collection of morphometric data and blood samples of DPZ animals took place during the annual health check of the macaque colony during which animals are anesthetised in the early morning with an intra-muscular injection of ketamine hydrochloride (10 mg/kg) applied by darting the animal. CS animals were captured during the annual trapping period (Jan-Mar 09). Trained staff members captured the males during the morning in a 100 m^2 feeding corral provisioned with monkey chow, netting or capturing the monkeys by hand and transferring them subsequently to a field laboratory where they remained overnight. The following morning, animals were anaesthetised as described above.

All body measurements were collected within one hour of sedation. Body weight was determined to the nearest 0.1 kg using a platform scale (DPZ animals) or standard hanging scale (CS animals). For morphometric measurements, all animals were laid onto their left side in a standardised position with arms and legs perpendicular to the vertebral axis and the back fully straight (Muehlenbein et al. 2002). We measured crown-rump length of each animal using a flexible tape measure with 1mm gradations (DPZ animals) or 1m ruler with 1mm gradations (CS animals). For DPZ animals, we used a skinfold sliding calliper with 1mm gradations to measure skinfold thickness (a measure of skinfold fat) in 5 body parts (Hamada et al. 1999): caudal aspect of upper arm (triceps), cranial aspect of thigh (quadriceps), belly lateral to the umbilicus, subscapular, and suprailiac. All skinfold measures were taken for the right side only and to the nearest millimetre. For CS animals, monkeys were placed on their side and measurements of skinfold thickness were taken from the belly approximately 2 cm above the umbilicus, using a Lange calliper accurate to 1 mm. For all measurements, we collected each measure three times and used a mean in analysis. Using morphometric data, we calculated the body mass index (BMI), by dividing body mass (kg) by crown-rump length squared (m^2) (Campbell & Gerald 2004). In addition to the collection of morphometric data, a blood sample (2-4ml) was collected from the femoral vein of each animal into a heparinised tube. Samples were stored on ice, centrifuged at 3000 rpm for 10 min and the plasma subsequently recovered and stored at -20°C until measurement. Samples from CS animals were shipped frozen to the endocrine laboratory of the Reproductive Biology Unit, DPZ, where all laboratory analyses were performed.

Urine sample collection

For the majority of DPZ animals, urine samples could often not be collected on the day morphometric data were taken as animals usually emptied their bladder during capture. Instead, urine samples were collected from DPZ animals within three weeks following or preceding the collection of morphometric measures. Samples from DPZ animals living in outside enclosures were collected between 11:00 and 14:00, following a training period during which animals received a food reward in response to cognitive tests. For sample collection, plastic mats were placed below the tunnel used by the animals to travel from the inside to the outside compartment. Urine was collected from the mats directly following urination, placed on ice and stored within 3 hours at -20°C until C-peptide analysis. Samples from DPZ animals living in indoor cages were collected between 8:00 and 13:30 on plastic mats placed under the wire cage; these were stored within 6 hours at -20°C as described above.

During the 2-3 months before CS animals were trapped and measured, we collected 68 urine samples from the study males while they were free-ranging (see below for final sample sizes by male). Urine

***Table 2.2**:* Species, sex, age, body weight, and housing condition of animals.

Animal	Species	Sex	Age (years)	Body weight (kg)	Housing condition		Used in experiment
					Group	Space	
2136	*M. mulatta*	Female	8	8.3	Unisex pair	DPZ-Indoor	Yes
2146	*M. mulatta*	Female	8	6.75	Unisex pair	DPZ-Indoor	Yes
14227	*M. mulatta*	Male	9	17.1	Unisex pair	DPZ-Indoor	Yes
12225	*M. mulatta*	Male	9	12.9	Unisex pair	DPZ-Indoor	Yes
12466	*M. fascicularis*	Male	5	4.4	Unisex group of 4	DPZ-Indoor	Yes
12331	*M. fascicularis*	Male	5	4.95	Unisex group of 4	DPZ-Indoor	Yes
12401	*M. fascicularis*	Male	5	5.6	Unisex group of 4	DPZ-Indoor	No
12400	*M. fascicularis*	Male	5	5.1	Unisex group of 4	DPZ-Indoor	No
10778	*M. fascicularis*	Male	9	5.25	Singly caged	DPZ-Indoor	No
10786	*M. fascicularis*	Male	12	12	Single male multi-female group	DPZ-Outside	No
10587	*M. fascicularis*	Female	9	5.1	Single male multi-female group	DPZ-Outside	No
39L	*M. mulatta*	Male	9	10.0	Multi-male multi-female groups	CS-Free-ranging	No
83L	*M. mulatta*	Male	9	12.9	Multi-male multi-female groups	CS-Free-ranging	No
17K	*M. mulatta*	Male	10	10.2	Multi-male multi-female groups	CS-Free-ranging	No
44H	*M. mulatta*	Male	11	13.3	Multi-male multi-female groups	CS-Free-ranging	No
61G	*M. mulatta*	Male	12	8.9	Multi-male multi-female groups	CS-Free-ranging	No
42F	*M. mulatta*	Male	13	13.5	Multi-male multi-female groups	CS-Free-ranging	No
57D	*M. mulatta*	Male	14	10.5	Multi-male multi-female groups	CS-Free-ranging	No
03D	*M. mulatta*	Male	14	13.1	Multi-male multi-female groups	CS-Free-ranging	No
50B	*M. mulatta*	Male	16	8.8	Multi-male multi-female groups	CS-Free-ranging	No
14A	*M. mulatta*	Male	17	9.8	Multi-male multi-female groups	CS-Free-ranging	No
T82	*M. mulatta*	Male	19	8.8	Multi-male multi-female groups	CS-Free-ranging	No
O15	*M. mulatta*	Male	21	8.3	Multi-male multi-female groups	CS-Free-ranging	No
K85	*M. mulatta*	Male	22	13.5	Multi-male multi-female groups	CS-Free-ranging	No

samples from CS animals were pipetted off the ground or other substrate (e.g. leaves, rocks) directly after a male was observed to urinate. Samples were collected between 7:20 and 13:40, but 78% of samples were collected between 7:20 and 10:20. Urine was placed into 2ml Eppendorf safe-lock microcentrifugue tubes (VWR, West Chester, PA, USA) and placed on ice. The sample was checked for cleanliness, and if there was any particulate matter in the sample, this was allowed to settle to the bottom. The supernatant urine was then pipetted off into a fresh microcentrifuge tube. This process was repeated until the sample was clean. At the end of fieldwork for the day (either 11:30 or 14:30) samples were returned to the Carribean Primate Research Center (CPRC) field station on Puerto Rico, and frozen at -80°C until transportation on ice (together with the collected plasma samples) to the Reproductive Biology Unit, DPZ.

Food reduction experiment

Using 6 of the indoor-housed captive adult macaques (the first 6 animals listed in *Table 2.2*), we conducted a feeding experiment, during which we controlled the amount of food provided. Prior to the onset of the experiment we determined the average amount of monkey chow being consumed daily by each individual over a period of 20 days. For this, individuals were isolated for feeding twice a day (09.15-10.15; 11.45-12.45) into single compartments of their home cage and we weighed the amount of monkey chow provided and the amount remaining after feeding. We then calculated the mean daily net intake over all 20 days (mean daily consumption). In addition, each individual was given one banana and one apple per day. For three days prior to the onset of the experiment we undertook a control period, in which each animal received their mean daily monkey chow consumption, plus the two fruits, in two feeding sessions. For the following two weeks, the amount of monkey chow given to each animal was restricted to 50% of the amount provided during the control period, and animals received in addition only half an apple and half a banana (diet period). Thereafter, animals were provisioned as during the control period (re-feeding period). Water was available *ad libitum* throughout the whole experiment. Before animals were reunited following feeding sessions, daily body weights were determined for each individual to the nearest of 0.05 kg using a platform scale. The scale was placed on the ground of the cage, and animals were trained to step on it and sit still until their weight measure was stable. During this experiment, urine samples were collected twice a day from each individual as described above. Morning samples were usually collected before animals received their first feed (fasting samples), while the second sample was usually collected 2-3 hours after the first feeding (non-fasting samples). Samples were placed on ice directly after collection and stored frozen at -20°C within 5 hours until analysis.

C-peptide analysis

Prior to routine analysis, we tested on a few rhesus macaque urine samples the ability of two C-peptide ELISA Kits designed to measure C-peptide in human serum and plasma to detect C-peptide levels in macaque urine. One kit (DSL-10-7000) was purchased from DSL Diagnostic Systems Laboratories, Sinsheim, Germany, the other from IBL International GmbH, Hamburg, Germany (Art. No. RE 53011). While both assays were able to detect macaque urinary C-peptides levels well above assay sensitivity and levels measured in the two assays were significantly correlated ($r = 0.72$, $P < 0.05$), concentrations measured with the IBL assay were 2-3 times higher than those measured in the DSL assay. Furthermore, urine sample dilutions ran parallel to the C-peptide standard curve in the IBL assay, while this was not clearly the case in the DSL assay. Thus, given the apparent higher cross-reactivity of the antibody used in the IBL assay with macaque C-peptide and the absence of interfering matrix effects in this assay compared to the one from DSL, we routinely analysed all our urine samples using the IBL assay kit. Prior to assay, urine samples were diluted between 1:2 and 1:12 (depending on the C-peptide level and amount of urine available) with IBL sample diluent (Art. No. RE 53017) to bring the samples into the working range of the assay, and 100 µl of the diluted urine was then assayed using the manufacturer provided protocol. Assay sensitivity was 0.064 ng/ml. Inter-assay coefficients of variation calculated from the measurement of low, middle and high value quality controls run in each assay were 14.5%, 10.5% and 10.6%, respectively while intra-assay coefficients of variation values were 6.5%, 6.7% and 5.1%, respectively.

To adjust for differences in urine concentration, C-peptide values were indexed to the level of urinary creatinine measured according to the method described by Bahr and colleagues (Bahr et al. 2000) and C-peptide concentrations are presented as ng C-peptide/mg creatinine. Means ± SE creatinine concentration (in mg/ml) per individual were: 0.39 ± 0.04 for CS animals, 0.60 ± 0.08 for captive animals outside of the experiment and 0.60 ± 0.03 for DPZ animals used for the experiment (range for all individuals: min: 0.1, max: 3.27). The mean creatinine concentration was not affected by changes in animal diet and did not differ significantly across the different experimental phases (i.e. control, first week of diet, second week of diet, first week of re-feeding and second week of re-feeding) for the fasting samples (Friedman test, $\chi^2 = 5.07$, df = 4, $P = 0.28$) as well as for the non-fasting samples ($\chi^2 = 5.07$, df = 4, $P = 0.81$). Thus, adjusted C-peptide levels during the feeding experiment were not biased as a result of potential changes in creatinine concentrations.

Of the 328 samples collected for the DPZ animals and the 68 samples collected for CS animals, 6 and 32 samples, respectively, had values that were below C-peptide assay sensitivity. In these cases and as done by Deschner and colleagues (2008), we assigned them the maximum possible value they

could have taken, i.e. the value of assay sensitivity (0.064 ng/ml) so as not to artificially exclude samples with low C-peptide levels from analysis. Note that this is a very conservative approach to our data as it means that in samples of low (undetectable) concentration we have slightly overestimated their concentrations, so reducing variation in our dataset. We excluded one sample (from the CS animals) because of a low (<0.1 mg/ml) creatinine concentration (e.g. Muller & Wrangham 2004b). After samples from the same male but from different times on the same day were averaged, we were left with a dataset for CS animals of 64 different 'male days' (unique male day combinations), with a mean ± SE per male of 4.3 ± 0.4 (range 2-12). For DPZ animals, the data set comprised 63 samples from 11 animals with a mean ± SE per individual of 5.7 ± 1.5 (range 2-18). In addition 265 samples (185 fasting, 80 non-fasting) resulted from the feeding experiment on DPZ animals.

Statistics

We examined the relationship between each individual's mean level of UCPs and: 1) BMI; 2) skinfold thickness; and 3) plasma C-peptide levels, using Spearman's correlations. Only animals for which at least two urine samples were collected were used for analysis. Data on captive and free-ranging animals were analysed separately. As UCPs data for CS animals were skewed by two high outliers, we log-transformed all UCPs data for graphing. Note that as we used rank-based statistics, logging has no effect on results of our statistical tests. We used one-tailed probabilities, since we had clear predictions for a positive relationship between UCPs and each of the three variables tested and that the opposite effect would not have been expected.

For the feeding experiment, we examined the relationship between UCPs and body mass during the 28 days of the experiment using Spearman's correlations. Because levels of UCPs in fasting and non-fasting samples were strongly correlated (Spearman´s correlation: $r_s = 0.88$; $P < 0.001$), we restricted the analysis of the effect of food availability and body mass changes on UCPs to fasting samples. For this, mean UCPs from the fasting samples and mean body mass were calculated across all individuals for a given day. In addition, to test the effect of food availability on UCPs, we divided the experimental period into 5 phases: control, diet 1 (first week of food reduction), diet 2 (second week of food reduction), re-feeding 1 (first week of re-feeding) and re-feeding 2 (second week of re-feeding). We tested for general differences in mean UCPs between the 5 experimental phases using the Friedman test. The Wilcoxon signed-ranks test was used post hoc to determine which of the phases differed significantly from each other. Because a study of bonobos (Deschner et al. 2008) suggested that UCPs decrease during food reduction (diet period) and increase during food increase (re-feeding period), and because the opposite effect would not have been expected we used one tailed probabilities. Given the likely difficulties of collecting first morning for wild primates living in

large groups (such as macaques) we tested whether UCPs relate to feeding condition (i.e. whether animals have or have not recently fed) independently of the time of sample collection. Accordingly, we used a Wilcoxon signed rank test to explore for difference between the mean individual UCP levels in fasting samples of the control, re-feeding 1 and re-feeding 2 periods (normal amount of food) and the mean individual UCP levels in non-fasting samples of the diet 1 and diet 2 periods (reduced food). Finally, we used a two-tailed Wilcoxon signed ranks test to explore differences in C-peptide levels between fasting and non-fasting samples.

All statistical tests were conducted with SPSS 15.0 for Windows or R 2.7.0, and we considered $P < 0.05$ significant. For non-parametric tests, exact p-values were computed whenever possible (where no ties occurred). Non-exact p-values are marked as "*" in the result section.

Results

UCPs, BMI, skin fatness and plasma C-peptide levels

UCPs correlated positively with both BMI (DPZ animals: $r_s = 0.664$, $P = 0.015$, *Figure 2.1a*; CS animals: $r_s = 0.599$, $P = 0.017$*, *Figure 2.1b*) and skinfold thickness (DPZ animals: $r_s = 0.569$, $P = 0.034$, *Figure 2.1c*; CS animals: $r_s = 0.609$, $P = 0.014$*, *Figure 2.1d*). We found a strong and highly significant correlation between UCPs and plasma C-peptides for DPZ animals ($r_s = 0.845$, $P < 0.001$, *Figure 2.1e*), but not CS animals ($r_s = 0.390$, $P = 0.093$, *Figure 2.1f*).

UCPs in relation to body mass dynamics and dietary regime

During the diet period of the food reduction experiment, animals lost an average 7.1% (range 4.3% - 9.8%) of body mass (*Figure 2.2a*). All animals gained weight during re-feeding (*Figure 2.2a*). After the two week re-feeding period animals reached on average 98.2% (96.6% - 100.1%) of their pre-experiment weight. As predicted, UCPs in fasting samples co-varied with body mass decreasing during the diet period, and increasing during the re-feeding period with non-fasting samples showing a bigger difference between diet and re-feeding period (in the latter even exceeding control period values) than fasting samples. Accordingly, we found a significant positive correlation between UCPs in fasting samples and body weight ($r_s = 0.536$, $P < 0.002$).

UCPs in fasting samples differed significantly across the 5 different experimental phases ($\chi^2 = 19.6$, df = 4, $P < 0.001$; *Figure 2.3*), decreasing with food restriction (control vs. diet 1: Z = -2.201, $P = 0.016$; diet 1 vs. diet 2: Z = -2.201, $P = 0.016$; *Figure 2.3*) and increasing during re-feeding (diet 2 vs. re-feeding 1: Z = -2.201, $P = 0.016$; diet 2 vs. re-feeding 2: Z = -2.201, $P = 0.016$; *Figure 2.3*). Finally, UCPs

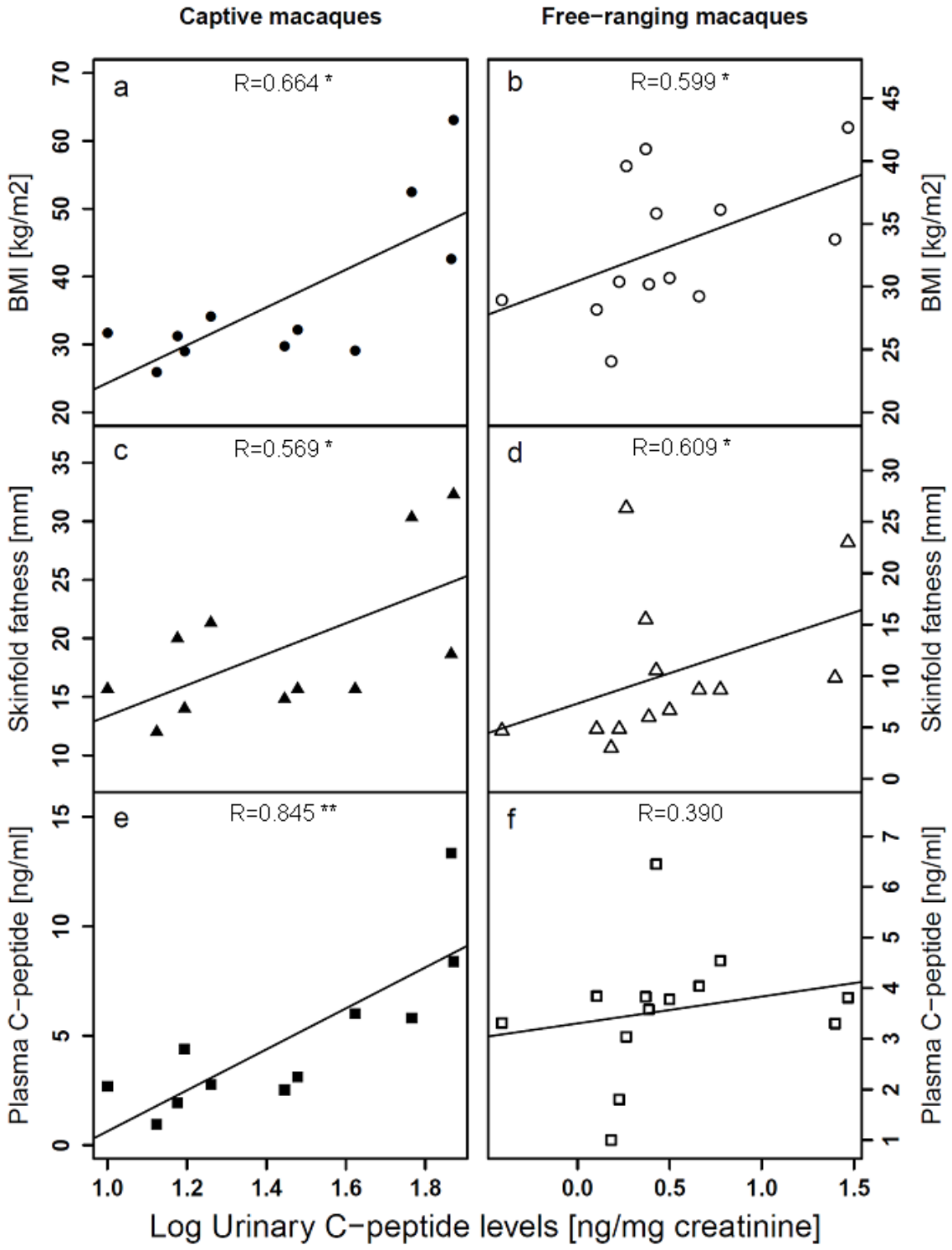

Figure 2.1: Individual UCP levels of animals in relation to BMI, skinfold fatness and plasma C-peptide levels.

Mean individual log10 transformed UCPs of captive DPZ (left boxes; black symbols) and free-ranging Cayo Santiago (right boxes; open symbols) animals in relation to BMI (a,b), skinfold thickness (c,d) and plasma C-peptide levels (e,f). '''' P < 0.05; ''**''P < 0.01. The UCP level values presented in this figure come from samples collected during periods of normal feeding; none come from the experimental period.*

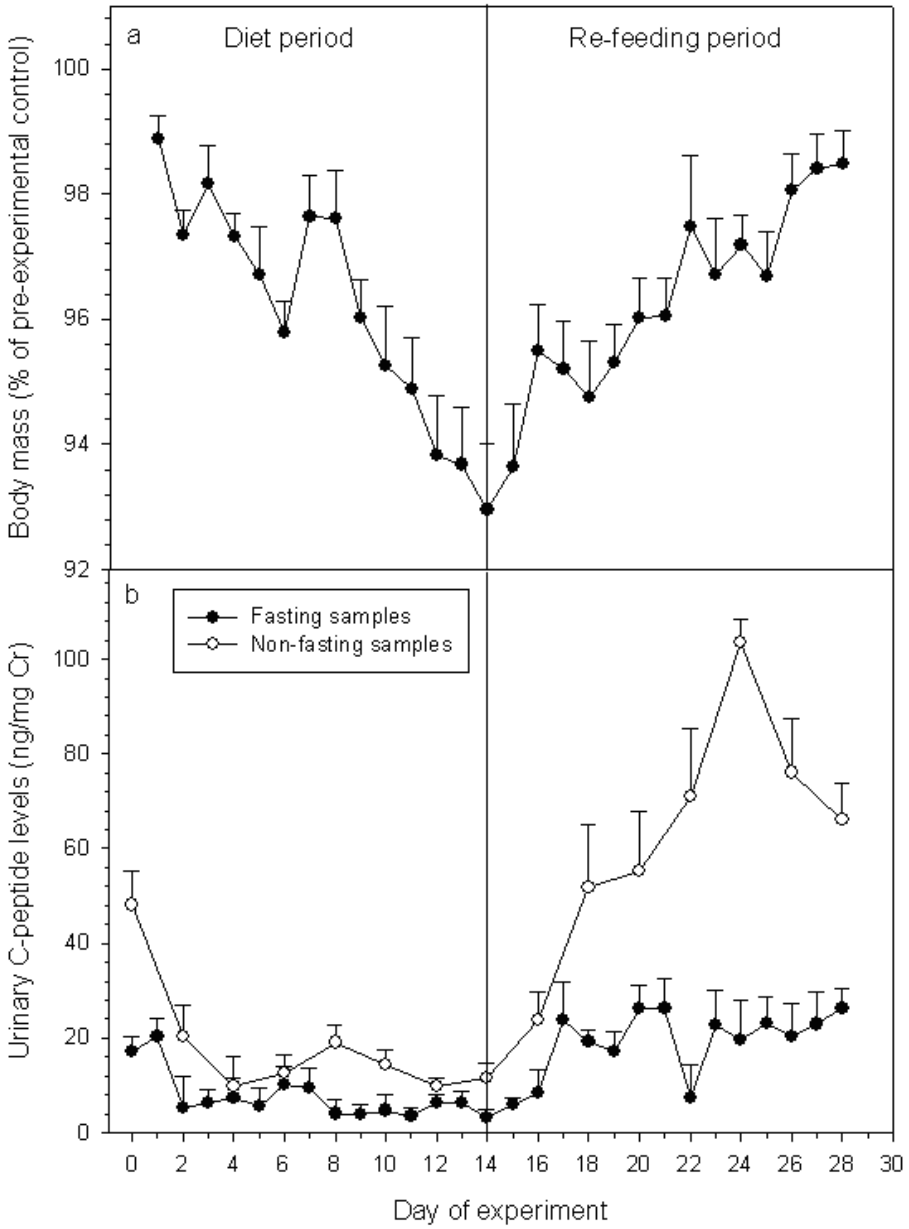

Figure 2.2: Changes in body mass and UCP levels during the feeding experiment.

Changes in body mass (a) and UCP levels (b) for fasting (black circles) and non-fasting (white circles) samples during the diet and re-feeding period of the feeding experiment. For body mass, values represent percentages of the mean weight determined during the pre-experimental control period (=100%). All values represent medians with standard errors. C-peptide values on Day 0 represent individual mean levels during the pre-experimental control period.

in non-fasting samples were significantly higher than in fasting samples (Z = -6.619, *P* < 0.001; *Figure 2.2*). However, UCPs in fasting samples of the control, re-feeding 1 and re-feeding 2 periods were still significantly higher than UCPs in non-fasting samples of the diet 1 and diet 2 periods (Z = -2.201, *P* = 0.031).

Discussion

Our results demonstrate that urinary C-peptide is a useful biomarker of energy intake and body fatness not only in hominids (Hoogwerf et al. 1986; Kruszynska et al. 1987; Deschner et al. 2008) but also in other primates (*Table 2.1*). UCP values were significantly correlated with BMI and body fatness (all animals), as well as plasma C-peptide levels (captive animals), showing that they on one hand reflect insulin production and on the other the animal's nutritional status. In addition to assessing these parameters during a given point in time, results of our feeding experiment show that measurement of UCPs also seems to be a valuable tool for tracking changes of individual diet and nutritional status over time.

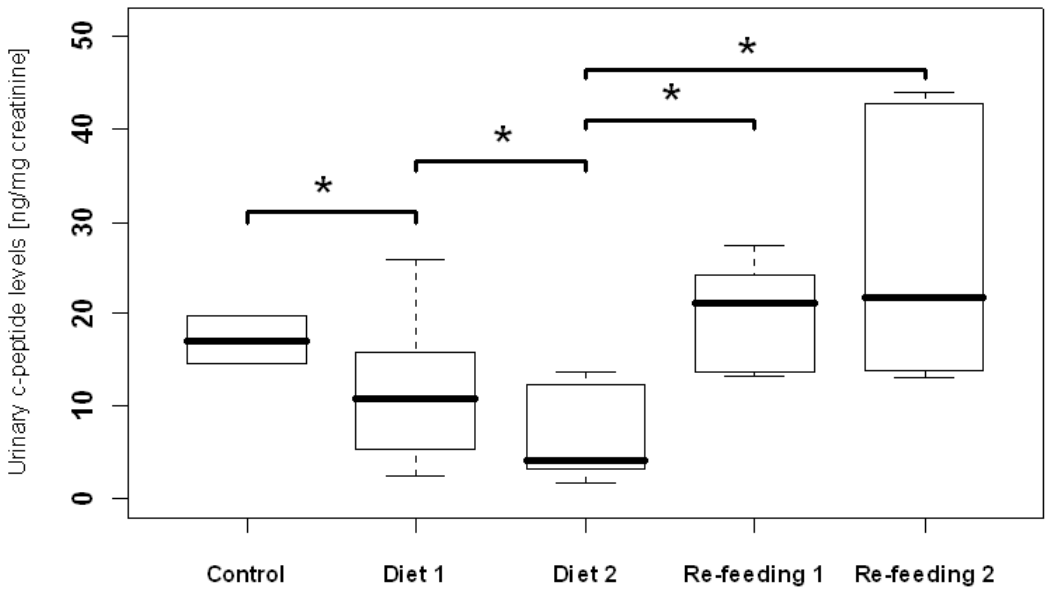

Figure 2.3: Concentration of UCP levels during the five phases of the feeding experiment.

Box plots showing grouped concentrations of UCP levels, for the fasting samples, during the five phases of the food reduction experiment. The boxes indicate medians (line) and first and third quartiles. The whiskers indicate the 90th and 10th percentiles. "" P < 0.05*

A particular strength of our validation is the use of both captive and free-ranging macaques to demonstrate that relationships seen in captive animals can also be extrapolated into field settings, where animals usually have lower BMI and consequently also lower UCP levels. In general, results obtained in captive and free-ranging animals had the same predictive value in both populations. The failure of urinary and plasma C-peptide levels to correlate in the CS animals may be related to sample procedure. For CS animals, plasma C-peptide levels were collected from just one point in time, whereas mean UCP levels were developed from averages over a period of a few months prior to the collection of blood samples. Urine samples of captive animals, in contrast, were collected in a much narrower window around the day on which plasma samples were taken. More critical than this absence of correlation is that UCPs were below assay sensitivity in a portion of samples from several CS (and one DPZ) individuals. Since these problems occurred mainly in individuals at the lower end of the observed BMI variation and since low UCP levels seem less of a problem in studies of great apes which have larger bodies than macaques (e.g. Deschner et al. 2008), body mass may principally be a critical parameter limiting the use of UCPs as biomarker. We were, however, able to measure very low C-peptide values in several individuals with low BMI in both free-ranging and captive animals as well as in juveniles (unpubl. data), suggesting that additional factors may be involved. Dilution of urine (in combination with low BMI) may play an important role here since in our study animals, water was available *ad libitum* and there may have been significant individual differences in water consumption. Applicability of UCP assays to field studies may therefore depend on availability of water and individual differences in drinking behaviour, as well as in the water content of food, and may thus vary between seasons and populations. In addition, environmental pollutants such as soil and faecal matter, may have contaminated urine samples and could potentially cause degradation or absorption of UCPs, particularly in samples taken from the ground (i.e. CS samples; see also Deschner et al. 2008). Hence, further studies investigating the influence of sample contamination on UCP levels are important for optimising sample collection procedures in the wild.

Complementary to our cross-sectional results, we also undertook a food-reduction experiment. The dietary restriction and re-feeding of macaques undertaken during our experiment revealed a close link between changes in C-peptide excretion, food intake and body mass, with UCP levels changing quickly (within two days) in response to changes in food supply and body weight. Given that C-peptide is a by-product of insulin production, changes in C-peptide excretion can be expected to indicate changes in insulin production. A tight relationship between food intake and UCPs has been found in field studies on great apes (i.e. Emery Thompson & Knott 2008; Emery Thompson et al. 2009, see *Table 2.1*). Interestingly however, although food supply (and thus energy intake) remained constant during the food restriction period, UCP levels further decreased significantly as body mass

continued to decline. This indicates that UCP excretion is not just sensitive to caloric intake (i.e. is not just a measurement of dietary input), but also to body mass itself.

In our study, non-fasting samples contained higher UCPs than fasting samples; food consumption via stimulating insulin production thus seems to have a direct effect on UCP levels which in turn may confound their reliability when assessing inter-individual differences in the energetic status of wild animals. The collection of urine at standardised times of the day (e.g. at early morning sleeping sites before animals have moved) should help to control for such effects. For species living in large groups, however, collection of samples at standardised times may be impossible. Nevertheless, we found that average UCP levels after feeding (i.e. non-fasting samples) under reduced food conditions were significantly lower than fasting UCPs under normal feeding, demonstrating the potential of UCPs to assess changes in energy intake regardless of sample collection time. Furthermore, animals feed more continuously in the wild than under most captive conditions, and short term variation in UCPs is likely to be less dramatic in wild animals than in captive ones with fixed feeding times and highly calorific food.

Our results are in line with previous studies on humans and great apes (*Table 2.1*) demonstrating that UCP levels show a positive relationship with measures of individual nutritional status (humans: Hoogwerf et al. 1986; Kruszynska et al. 1987; bonobos: Deschner et al. 2008) and food intake (humans: Hoogwerf et al. 1986; bonobos: Deschner et al. 2008; orang-utans: Emery Thompson & Knott 2008). They are also in line with a previous study on rhesus macaques demonstrating that obese animals have higher levels of UCP excretion than non-obese macaques and that complete food deprivation leads to dramatic decrease in UCP levels (Wolden-Hanson et al. 1993, see also *Table 2.1*). More importantly, they extend this finding considerably by showing that less extreme body mass differences and dietary changes are reflected in C-peptide excretion. In the present study, animals lost on average only 7% of body mass, which was associated with a clear reduction in UCP levels. Macaques regularly demonstrate this type of intra-individual body mass variation; for example, male bonnet macaques lose 6-8% and male rhesus macaques 10-12% of body weight during the mating season due to costly reproductive strategies (Glick 1979; Bernstein et al. 1989; Cooper et al. 2004). We thus show that UCPs are sensitive enough to track the amount of body mass variation typically associated with the behavioural and reproductive strategies seen in macaques. Furthermore, we show that UCP measurements are viable markers of nutritional status from single void urine samples and that it is unnecessary to collect complete 12 hour samples (as done by Wolden-Hanson and colleagues, 1993). This is consistent with results recently presented for non-human great ape species

(Sherry & Ellison 2007; Deschner et al. 2008; Emery Thompson & Knott 2008; Emery Thompson et al. 2009) and makes UCP measurement more applicable to field studies.

In our study, loss of body mass was induced by a reduction of energy intake; energy balance is however usually a product of both energy intake and expenditure. To date, only one study has taken energy expenditure into account when examining UCP levels. In a study on colobus monkeys, Harris and colleagues (Harris & Monfort 2007) showed that UCPs are positively correlated with the distance travelled by females 24h before sample collection. Although it is unclear how reliably UCPs reflect blood C-peptide levels and individual energetic condition in colobus monkeys, the results of our study suggest that the use of UCPs as a marker of energetic condition in this species is likely to be justified. Further studies measuring both energetic expenditure and intake as well as UCP levels from free-ranging primates will improve our understanding of the relationships between the three parameters.

In summary, our study provides the first validation of the use of UCP levels as a non-invasive measure of body fatness and nutritional status in a non-great ape primate species, and suggests that UCPs are a useful biomarker for monitoring changes in nutritional status in studies of primates (and possibly also other mammals) more generally. Potential uses of this biomarker span a broad range of topics such as studies of aging and reproduction, food competition, effects of stress and season, and behavioural strategies. We therefore encourage additional validation, evaluation and adjustment of assays for UCP measurement in other mammalian taxa. This tool opens up exciting new opportunities for field studies in ecology (e.g. on the influence of habitat structure/ home range size/ group size/ availability of specific resources and so on on individual nutritional status), behavioural ecology (e.g. on the costs of food competition and on the relationship between dominance status and age on nutritional status) and reproductive biology (e.g. on the link between nutritional status and reproductive output, and on the costs of specific reproductive strategies). However, before measurement of UCPs is fully applied to field settings, certain issues related to the collection and storage of samples (e.g. effects of sample contamination by soil/faeces, transportation related freeze-thaw and so on), should be systematically investigated. Finally, given that c-peptides are generally produced in equimolar amounts to insulin, and given the positive relationship we found between serum c-peptide levels and UCP levels, UCP measurement may also be a useful non-invasive tool in clinical studies.

Conclusion

Our study shows that C-peptide levels measured non-invasively from urine samples reflect changes in body mass, body fatness and food intake in captive and free-ranging macaques. Our results thus validate for the first time the utility of UCPs as reliable biomarkers of nutritional status in a non-hominid primate species, and suggest that the measurement of UCPs has potential for being useful for a broader range of mammals. This new biomarker opens up exciting opportunities for studies of ecology, behavioural ecology and reproductive biology, and also for biomedical studies under captive, free-ranging and natural settings.

Chapter 3

Mate-guarding constrains feeding activity but not energetic status of wild male long-tailed macaques

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Abstract

Mate-guarding is an important determinant of male reproductive success in a number of species. Little is known however about the constraints of this behaviour, e.g. the associated energetic costs. We investigated these costs in long-tailed macaques where alpha males mate-guard females to a lesser extent than predicted by the Priority of Access model. The study was carried out during two mating periods on three wild groups living in the Gunung Leuser National Park, Indonesia. We combined behavioural observations on males' locomotion and feeding activity, GPS records of distance travelled and non-invasive measurements of urinary C-peptides (UCP), a physiological indicator of male energetic status. Mate-guarding led to a decrease in feeding time and fruit consumption suggesting a reduced intake of energy. At the same time vertical locomotion was reduced which potentially saved energy. These findings, together with the fact that we did not find an effect of mate-guarding on UCP levels, suggest that energy intake and expenditure was balanced during mate-guarding in our study males. Mate-guarding thus seems to not be energetically costly under all circumstances. Given that in strictly seasonal rhesus macaques high-ranking males lose condition over the mating period, we hypothesise that the energetic costs of mate-guarding vary inter-specifically depending on the degree of seasonality and that males of non-strictly seasonal species might be better adapted to maintain balanced energetic condition year round. Finally, our results illustrate the importance of combining behavioural assessments of both energy intake and expenditure with physiological measures when investigating energetic costs of behavioural strategies.

Introduction

In a broad range of taxa (e.g. insects, Alcock 1994; reptiles, Censky 1995, Ancona et al. 2010; crustaceans, Sparkes et al. 1996; birds, Komdeur 2001, Low 2006; mammals, Alberts et al. 1996, Matsubara 2003, Willis & Dill 2007, Schubert et al. 2009) males have evolved mate-guarding behaviour to exclude rivals from mating with the guarded female and hence limit the extent of sperm competition (i.e. sperm from different males competing to fertilise one particular ovum, Birkhead & Moller 1998), thereby increasing their chances to successfully reproduce. Mate-guarding can take different forms such as: 1) prolonged copulation beyond the time required for fertilisation (Carroll 1991), 2) maintenance of permanent physical contact with the female (female-grasping) or continuous monitoring of the female after mating to prevent the female from mating with other males (Alberts et al. 1996; Sparkes et al. 1996) and 3) formation of mating plugs sealing the access to the female genital tract (Alcock 1994). Here, we consider only the second form of mate-guarding and, following Alberts and colleagues (Alberts et al. 1996), define mate-guarding as a "close, persistent following of a female by a male that involves exclusion of other males from access to the female".

While mate-guarding is usually highly beneficial to males as it significantly increases mating and/or reproductive success (Censky 1995; Setchell & Kappeler 2003; del Castillo 2003; Engelhardt et al. 2006), maintaining a permanent physical contact with the female or monitoring female activity after mating has proven to be costly in several taxa. In fact, mate-guarding can affect males' thermoregulation (Singer 1987; Saeki et al. 2005), feeding behaviour (Censky 1995; Alberts et al. 1996; Komdeur 2001; Ancona et al. 2010) and fat reserves (Komdeur 2001; Plaistow et al. 2003; Schubert et al. 2009).

In primates, mate-guarding is a common reproductive strategy in species living in multi-male multi-female groups (Manson 1997). However, the intensity of mate-guarding, i.e. its duration and the diligence with which males engage into this behaviour, varies greatly across species (Manson 1997) most likely due to interspecific differences in costs and benefits of mate-guarding. These differences may derive from variation in demographic and ecological factors with higher degree of male-male competition and female reproductive synchrony leading to lower female monopolisability and an expected lower mate-guarding intensity (Dubuc et al. in prep.) Interestingly, both a higher degree of male-male competition and female reproductive synchrony appear to be also related to a higher degree of reproductive skew (Ostner et al. 2008b; Gogarten & Koenig 2013) suggesting that both parameters, male reproductive skew and the intensity of mate-guarding, might be linked to each

other in primates. Understanding the constraints of male mate-guarding abilities may thus also foster our understanding of what determines male reproductive skew in primates.

A model commonly used to explain the variation in male reproductive skew in primates is the Priority of Access model (PoA model, Altmann 1962). According to this model, the only limiting factor to male mating and reproductive success is female cycle synchrony. Since a male is only able to mate-guard one female at a time, the model predicts that male reproductive skew depends on the number of females cycling at the same time. While data support the PoA model in some species (savannah baboons, *Papio cynocephalus*, Altmann et al. 1996; mandrills, *Mandrillus sphinx,* Setchell et al. 2005; chimpanzees, *Pan troglodytes*, Boesch et al. 2006), in others, mate-guarding behaviour is less intense and reproductive skew is lower than predicted by the model (e.g. long-tailed macaques, *Macaca fascicularis*, Engelhardt et al. 2006; Rhesus macaques, *M. mulatta,* Dubuc et al. 2011; Barbary macaques*, M. sylvanus*, Young et al. 2013b). This indicates that there are additional variables that need to be accounted for when considering the relationship between mate-guarding and priority of access. The most commonly proposed confounding variables are the ability of males to detect the period when the female is fertile and the energetic costs of mate-guarding (Dubuc et al. in prep; Alberts et al. 1996; Engelhardt et al. 2006).

If males are unable to recognise the female fertile phase, the major determinant of male reproductive skew is expected to be sperm competition and female mate choice rather than priority of access (Dubuc et al. in prep; Engelhardt et al. 2006). In this context, males are expected to mate-guard females less intensively since the payoff of mate-guarding is potentially low. Whereas the ability of males to discern the female fertile phase has been tested in a number of primate species (Heistermann et al. 2001; Engelhardt et al. 2004; Deschner et al. 2004; Dubuc et al. 2012) the specific energetic costs of mate-guarding remain largely unclear.

A number of studies on primates have tried to accurately quantify the impact of mate-guarding on key components of male energy intake (feeding duration and ingestion rate) and expenditure (distance travelled) but the results from these studies are inconclusive. Whereas, in a number of studies, mate-guarding led to a reduction in male-feeding time (Packer 1979; Rasmussen 1985; Alberts et al. 1996; Matsubara 2003) evidence is equivocal in chacma baboons (*P. hamadryas ursinus,* Weingrill et al. 2003), and completely absent in olive baboons (*P. Anubis*, Bercovitch 1983) and moustached tamarins (*Saguinus mystax,* Huck et al. 2004). Furthermore, only one study so far investigated energy expenditure in relation to mate-guarding (Alberts et al. 1996) finding that male baboons travelled less distance when mate-guarding females than when not. Finally, only one study investigated potential other than energetic costs to mate-guarding such as elevation of physiological

stress levels. In chacma baboons, males had higher faecal glucocorticoid levels (a marker of physiological stress) when mate-guarding than when not mate-guarding females (Bergman et al. 2005).

None of these aforementioned studies controlled for potential confounding factors although parameters such as variation in food availability are known to influence activity patterns and feeding strategies of primates (Masi et al. 2009; Chaves et al. 2011). More importantly, the lack of physiological measures in these studies prevents clear conclusions about male energetic status since behavioural observations may be rather inaccurate in this regard (Chivers 1998).

A potentially useful tool to assess energetic condition more accurately is the measurement of urinary C-peptide (UCP), a small polypeptide produced in an equimolar ratio when the body converts proinsulin into insulin (Rubenstein et al. 1969). UCP has been validated as a reliable non-invasive measure of energetic status of non-human primates with higher UCP levels reflecting better body condition and positive energy balance (**chapter 2**, Sherry & Ellison 2007; Deschner et al. 2008; Emery Thompson & Knott 2008). Its use to investigate the energetics of reproductive strategies in male primates is restricted to one single study in free-ranging male rhesus macaques. This study found that UCP levels decreased significantly more in high-ranking than in low-ranking males during the mating season (Higham et al. 2011a) and that restlessness (defined as the rate of changes between different activities) was negatively associated with UCP measures in these males suggesting that restlessness is an important parameter of male energetics.

Given the lack of fully conclusive studies on the costs of mate-guarding in primates the aim of our study was to quantify the energetic costs of this male reproductive strategy with a more comprehensive approach using the long-tailed macaque as a model species. Long-tailed macaques are organised in multi-male groups and are non-strictly seasonal breeders (van Schaik & van Noordwijk 1985). Although female long-tailed macaques can conceive year round, periods with increased female sexual receptivity (mating period) and birth peaks frequently occur, the timing of which seems to depend on fruit availability (van Schaik & van Noordwijk 1985). The species is therefore classified as capital breeder on the three grade scale of reproductive seasonality in primates by Brockman and van Schaik (2005). In long-tailed macaques, males are quite well able to discern a female's fertile phase (Engelhardt et al. 2004) and alpha and beta males are the only males to mate-guard females intensively (de Ruiter et al. 1994; Engelhardt et al. 2006). Mate-guarding intensity translates into male reproductive success, which is highly skewed towards high-ranking males (de Ruiter et al. 1994; Engelhardt et al. 2006). However, alpha males do not mate-guard all potentially fertile females even when fertile phases do not overlap (Engelhardt et al. 2006). The later

strongly suggests that alpha male ability to monopolise all females one after the other may be limited due to some costs associated with mate-guarding behaviours among which energetic costs might be the major limiting factor.

In order to better understand the energetic dynamics of mate-guarding in male long-tailed macaques, we quantified potential energetic costs in various ways. More specifically we investigated the effects of mate-guarding on some key components of energy intake and expenditure: 1) feeding time, 2) diet, 3) travelling and climbing distance and 4) restlessness. Further, we used UCP measures to investigate whether mate-guarding intensity affects male energetic status overall. Potential confounding effects of environmental factors (fruit availability and rainfall) were controlled for in all analyses. This study will provide useful insight into male energetic management during reproductive competition. Our results will also provide empirical data which can be used by future mathematical modellers to integrate parameters related to the cost of female monopolisation into new or existing reproductive skew models.

Methods

Animals and study site

The study was carried out from January 2010 to April 2011 on three groups of long-tailed macaques living around the Ketambe Research Station (3º41'N, 97º39'E), Gunung Leuser National Park, North-Sumatra, Indonesia. The study area consists of primary lowland rainforest and has been described by Rijksen (1978) and van Schaik and Mirmanto (1985). The long-tailed macaques in the area have been studied since 1979 (van Schaik & van Noordwijk 1985; de Ruiter et al. 1994; Engelhardt et al. 2004). We focused on three groups: Camp (C), Ketambe Bawa (KB) and Ketambe Atas (KA). All adult individuals were individually known and well habituated to human observers. The total size of a social group varied from 22 to 58 individuals (see *Table 3.1* for group compositions and home range sizes). Between January and April 2010, four males migrated back and forth between the groups KA and KB and associated with the group for periods between a few hours up to 3 weeks before migrating back to their "home" group. The study was conducted completely non-invasively and under the permission of the authorities of Indonesia. We adhered to the Guidelines of the Use of Animals in Research, the legal requirements of Indonesia and the guidelines of the involved institutes.

Behavioural data

Behavioural data were collected by C.G-B and six experienced Indonesian and international field assistants. The observation periods covered two mating periods. Each mating period was defined as the period between the first mate-guarding day and the last mate-guarding day observed in any of the three groups. From March to July 2010, four observers followed groups C and KB every day and from December 2010 until April 2011 all three groups were generally followed every other day and

Table 3.1*:* Composition and home range of the study groups.

Group	N females	N males	N total	Home range size (ha)
Camp	14-15	6-9	54-58	34.3
Ketambe Bawa	9-10	4-8	31-36	20.3
Ketambe Atas	7	4-7	22-25	19.1

frequency of observations increased to every day when alpha and/or beta males were observed mate-guarding. This procedure only affected which male was followed on any given day and full day or half day focal protocol (see below) was completed every day regardless of whether the focal male was mate-guarding females or not. Accordingly, our protocol does not influence the percentage of time during which a male was observed mate-guarding on a given day. Each day, groups were followed from dawn to dusk. We focused our behavioural observation on alpha and beta males because they are the ones who mate-guard female most extensively (Engelhardt et al. 2006). Dominance ranks between males were determined using the 'bared-teeth-face' display, a unidirectional submissive display (van Hooff 1967). Bared-teeth-face giver and receiver were entered into a sociometric matrix and dominance ranks were compiled with Matman 1.1.4 using the I&SI method (de Vries 1998). Alpha and beta males of each group were followed as focal animal for half or entire days depending on the number of observers available. The following focal scan data (Altmann 1974) were recorded every minute: **activity** [resting, being vigilant, feeding (handling and consuming food), drinking, travelling (continuous locomotion during at least one minute with no foraging activity and no social interactions), aggressing, affiliating (including copulation), grooming, self-grooming], **locomotion/position** [lying, sitting, standing, walking, running, jumping, climbing], **canopy height** [six categories: **0**: focal animal on the ground, **1**: 1-5 m; **2**: 5-10 m ; **3**: 10-15 m; **4**: 15-20 m; **5**: 20-25 m; **6**: > 25 m] and, if the animal was feeding, the **food item** was recorded [fruit, leaf,

flower, arthropod, bark, mushroom or others]. Activity states "resting", "being vigilant", "feeding", "drinking", "aggression" and "affiliation" were mutually exclusive. However "grooming", "self-grooming", "feeding" and "travelling" were not (e.g. the animal could be eating and groomed at the same time). Due to the focus of our study being on energetics of mate-guarding, when the focal animal was simultaneously engaged in feeding and another activity (e.g. grooming), we categorised the activity as "feeding". Secondly, all copulations between the focal male and any female or between the guarded-female and any male were recorded. Finally, we recorded every minute if the male was following a female and, if so, the identity of the female and the distance between the focal male and the female. The one-minute scans during which the male was mate-guarding were determined *a posteriori*. A male was considered as "mate-guarding" when he followed a female for more than 5 consecutive minutes and maintained a distance of less than or equal to 10 m between him and the female. If the female moved away from the male and the male did not follow her for more than 2 minutes the mate-guarding activity was considered to have ended. "Extensive mate-guarding days" refers to the days during which the male mate-guarded females more than 50% of the observation time. A mate-guarding period was defined as a period of one to several consecutive extensive mate-guarding days.

Travelling distance

Every day, the position of two of the focal males among the three groups was recorded automatically every minute using the tracking function of a GPS (Garmin© GPSMAP 60csx). Due to canopy density, the precision of GPS locations were only +/- 10 meters (the average measurement error of the GPS was calculated by recording the measurement error displayed on the GPS directly in the field during the first week of the study). We extracted GPS location every 15 minutes +/- 5 minutes and calculated the distance between each 15-minute-location. This approach reduces the average measurement error per hour from 600 m (when recording location every minute) to 40 m (when recording location every 15 minute), while keeping precise information on the focal individual path length. We used only GPS points for which we had focal behaviour on the activity of the animal at the exact minute at which the GPS point were recorded (i.e. when the observer was directly below the animal). For each day, the average horizontal distance travelled per hour was then calculated as $\sum_{i=1}^{n} \frac{4*Di}{n}$ where Di is the distance between two consecutive locations and n the number of distances recorded that day. Di is the distance travelled during each 15 minute interval. The sum of $_{\mathrm{Di}}$ divided by n is the average distance travelled by the animal during 15 minutes. By multiplying this sum by 4 we obtain an average hourly travelling distance.

In order to calculate the vertical distance travelled, we first used the centre of each height category as an estimate for the height at which the male was at each minute-scan-point (e.g. 7.5 m for category 2 or 12.5 m for category 3). Subsequently we calculated the height difference between each minute-scan-height estimate.

Fruit availability

In each of the three studied groups, 40 locations, covering the entire home ranges, were randomly selected (120 locations in total over the three territories). At each location, three trees were randomly selected from three different species among the tree species producing fruit eaten by *M. fascicularis* (Ungar 1995). For each tree, the height and the DBH (diameter at breast height) were recorded using a laser range finder and a flexible tape measure with 1mm gradations (see *Table 3.2*). In total 360 trees, from 87 different species were selected (120 trees for each group's home range). Each tree was surveyed monthly within the last 3 days of every month by an experienced field assistant and fruit abundance was recorded using a logarithmic scale (**0**: absence, **1**: 1-10 items, **2**: 11-100, **3**: 101-1000, **4**: 1001-10000 and **5**: > 10000). The average monthly score of fruit abundance in each territory was highly correlated to the percentage of trees fruiting. For the analyses, we therefore used percentage of trees fruiting as an index of fruit availability.

Table 3.2: Species diversity and morphological characteristics of the 360 trees surveyed for assessing fruit availability.

Group	Camp	Ketambe Atas	Ketambe Bawa
Number of tree species	55	55	59
Mean tree dbh[1] (cm)	41.3	26.9	36.7
Mean tree height (m)	17.4	14.3	16.7

[1] *dbh = diameter at breast height.*

Urine sample collection

Urine samples were generally collected once a week from four males in each group: alpha and beta males and two low-ranking males as "controls" (males which do not mate-guard females extensively). In addition, during mate-guarding periods, we collected urine samples every second day

from the mate-guarding male. A large plastic sheet (100*50 cm) was placed beneath urinating animals. Urine was then pipetted from the plastic sheet and/or from the vegetation directly after urination and placed in a 2ml Eppendorf safe-lock tube. Right after collection, the urine samples were placed on ice in a thermos bottle. At the end of each fieldwork day, the samples were frozen at -20°C in a freezer kept under constant electricity supply. As such, the samples were never stored on ice for more than 12 consecutive hours, a duration which does not significantly affect UCP concentrations (Higham et al. 2011b). In July 2011, all the samples were transported, on ice, to the hormone laboratory of the Bogor Agricultural University (IPB) and then freeze-dried before transportation to the Reproductive Biology Unit of the German Primate Centre for C-peptide analysis. Prior to lyophilisation the volume of urine in each sample was assessed visually to the nearest 0.1 ml.

C-peptide analysis

Since C-peptide levels potentially become unreliable when stored long-term (i.e. > 8 months, see Higham et al. 2011b), all samples that were stored frozen longer than 8 months before lyophilisation were excluded from analyses. Accordingly, only the samples collected during the second mating period (December 2010 to April 2011) were used. C-peptide concentrations were measured using a commercial C-peptide ELISA Kit from IBL International GmbH, Hamburg, Germany (Art. No. RE 53011), which we recently validated for the use in macaques (**chapter 2**). Prior to assay, urine samples were reconstituted into 0.3 ml (samples with a urine volume prior to lyophilisation < 0.5 ml) or 0.5 ml (volume prior to lyophilisation > 0.5 ml) of distilled water by vortexing the samples for 90 seconds. The samples were reconstituted in less volume than the original volume of fresh urine (i.e. prior to lyophilisation) in order to concentrate the sample and increase the chances of UCP measures to be above assay sensitivity (i.e. 0.064 ng/ml). Reconstituting the samples in a different volume from the original ones does not affect UCP measures since these measures are indexed to creatinine concentration (see below). 100 µl of the urine was then assayed using the manufacturer provided protocol. Inter-assay coefficients of variation calculated from the measurement of low, middle and high value quality controls ran in each assay were 9.0%, 7.0% and 8.4%. Intra-assay coefficients of variation provided by the manufacturer are 6.5%, 6.7% and 5.1%.

To adjust for differences in urine concentration, C-peptide values were indexed to urinary creatinine measured according to the method described by Bahr and colleagues (2000). Assay sensitivity was 0.1mg/ml. Inter-assay coefficients of variation calculated from the measurement of low and high value quality controls ran in each assay were 0.6% and 1.4%. C-peptide concentrations are presented as ng C-peptide/mg creatinine.

Statistical analyses

For all analyses, we considered only those days of observation for which at least 1 hour of focal data were recorded. The final data set thus comprised 2088 hours of focal observations over 584 days (see *Table 3.3*).

Table 3.3: Observation time, mate-guarding period length and diversity of females mate-guarded by the study males.

Group	**Camp**		**Ketambe Atas**		**Ketambe Bawa**	
Male rank	α	β	α	β	α	B
Number of mating periods	2	2	1	1	2	2
Focal observation time (hours)	668	455	185	111	388	323
Number of days of observation	147	114	68	48	122	85
Number of urine samples	45	25	27	19	32	22
Number of females mate-guarded	5	3	3	2	8	5
Number of MG days	41	4	30	27	49	10
Mean MG period length (days)	3.9	1	4.9	9	3.7	1.5
Range of MG period length (days)	1-18	1-1	1-13	1-33	1-10	1-4
Overall MG time	27.4%	8.4%	40.2%	53.9%	36.9%	12.0%

"MG" refers to mate-guarding. "MG days" refers to days during which the males were mate-guarding female for more than 50% of observation time. The overall MG time is the percentage of observation time during which the male was mate-guarding females.

Influence of mate-guarding on male behaviour and energetic status

For each day, we calculated the percentage of time spent mate-guarding and feeding, the percentage of fruit in the diet as well as the distance travelled per hour (in meters), the average height difference per minute (in meters) and the rate of changes in locomotion/position (restlessness). We considered fruit as being a relevant item in long-tailed macaque's diet in terms of energy intake. In fact, female long-tailed macaques time their reproduction according to inter-annual variation in fruit abundance seasonality (van Schaik & van Noordwijk 1985) illustrating the importance of this food item to fulfil energetic requirements in this species. Restlessness was derived by generating a binary matrix of

changes in either position or locomotion (locomotion/position was entered in a single column). For each minute of observation, the matrix was filled with a 0 if the locomotion or position was the same as the minute before. Otherwise, it was filled with a 1. For each day we calculated the rate of changes by dividing the total number of "1's" by the sum of 0's and 1's.

We tested whether the percentage of time spent mate-guarding on a given day affected the following parameters in the males: 1) time spent feeding, 2) percentage of fruit in the diet, 3) distance travelled, 4) height climbed, 5) restlessness and 6) energetic status (as assessed by UCP measures). We used a generalised linear mixed model (GLMM, Baayen 2008) for each of the parameters to test whether, on a particular day, this parameter was influenced by the percentage of time spent mate-guarding.

Since ecological conditions are likely to affect these parameters, we included fruit availability as a control factor in each model. Fruit availability on a given day was approximated using the fruit availability measured on the closest monthly record. For example, the percentage of tree fruiting recorded on the 31st of January was used as the fruit availability score for all the days between the 16th of January and the 15th of February.

We also included daily rainfall in each model since, due to thermoregulation constrains, this variable can influence primate locomotion and activity budget (Ganas & Robbins 2005; Majolo et al. 2013) and ultimately their energetic status. Therefore, we included the percentage of time spent mate-guarding, fruit availability, their two-way interaction and rainfall as fixed effects and male ID and group as nested random effects. In all the GLMMs, the interaction between fruit availability and percentage of time spent mate-guarding was never significant (for all tests $P > 0.05$). Therefore, we re-ran all the models without the interaction and all the results presented are for models with no interaction. For the model with UCP levels as response variable we added time of sample collection (to account for possible diurnal variability in our physiological measure), duration of sample storage in the freezer, height climbed (to account for variation in energy expenditure) and feeding time (to account for variation in energy intake) as control variables. All continuous fixed factors in the models were standardised to a standard deviation of 1 and mean of 0. All models were fitted in R 2.15.0 (R Development Core Team 2010) using the function lmer of the R-package lme4 (Bates & Maechler 2010).

The response variable in the different models was likely to show temporal autocorrelation unexplained by the fixed effects included, potentially leading to violation of the assumption of independent residuals. Therefore, we included a temporal autocorrelation term into the model using an approach developed by Roger Mundry (see Fürtbauer et al. 2011b).

In each model, we checked that the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting a qqplot and the residuals plotted against fitted values. We checked for model stability by excluding data points one by one from the data and comparing the estimates derived with those obtained for the full model. We checked that the range of the estimates derived were symmetric around the estimate value of the full model, which indicates the absence of influential cases. Variance inflation factors (VIF, Field 2005) were derived using the function vif of the R-package car (Fox & Weisberg 2010) applied to a standard linear model excluding the random effect. VIF's which are less than 5 indicate that covariation between the predictors is not a problem (Bowerman & O'Connell 1990; Myers 1990). In all our models VIF's were less than 1.4. The other diagnostics also did not indicate obvious violation of the assumption.

For each model, we first determined the significance of the full model (including all fixed effects, the interaction, the autocorrelation term and the random effects) as compared to the corresponding null model (including all the factors except "the percentage of time spent mate-guarding") using a likelihood ratio test (R function anova with argument test set to "Chisq"). To achieve a more reliable p-value, we fitted the models using Maximum Likelihood (rather than Restricted Maximum Likelihood, Bolker et al. 2009). Only if this likelihood ratio test revealed significance we considered the significance of the individual predictors. P-values for the individual effects were based on Markov Chain Monte Carlo sampling (Baayen 2008 and derived using the functions pvals.fnc and aovlmer.fnc of the R package languageR (Baayen 2010).

Influence of rank on male energetic status

To test whether males who mate-guarded females extensively during the mating period (alpha and beta males) had a lower energetic status than other males, we ran a GLMM including UCP levels as a response, male rank (two categories: high-ranking for alpha and beta males, and low-ranking for two other males in the same group) as a fixed factor, time of sample collection and sample storage length as control fixed effects and animal ID and group as random factors. In this model, rank was used as a proxy of mate-guarding intensity since we did not record behavioural data on low-ranking males. However, previous studies showed that alpha and beta males are the only ones mate-guarding females intensively (de Ruiter et al. 1994; Engelhardt et al. 2006). The GLMM was calculated and the assumptions checked as described above.

Results

Mate-guarding activity

In each of the three groups, the alpha male mate-guarded a higher number of females than the beta male (*Table 3.3*). Males mate-guarded each female on average 4 consecutive days (range 1 - 33, *Table 3.3*) and on average 29.8% (range 8.4 - 53.9%, *Table 3.3*) of their time was devoted to this behaviour.

Mate-guarding and feeding behaviour

The amount of time spent mate-guarding had a strong negative effect on the amount of time males spent feeding (N = 584 days, *estimate ± SE* = -2.16 ± 0.56, $P_{MCMC} < 0.001$, *Table 3.4* and *3.5*, *Figure 3.1a*). Mate-guarding activity also modified the diet of the males (*Figure 3.2*). In particular, the males fed less on fruits and more on leaves and flowers while mate-guarding females extensively as compared to other days (*Figure 3.2*). Overall the amount of time spend mate-guarding had a strong negative effect on the percentage of fruit in the diet (N = 583 days, *estimate ± SE* = -2.97 ± 0.96, $P_{MCMC} = 0.002$, *Table 3.4* and *3.5*, *Figure 3.1b*).

Table 3.4: Results of the Likelihood-ratio-tests (LRT) run to compare full versus null models of the different GLMMs to test the influence of mate-guarding activity on different behavioural and physiological parameters.

Model response	***N* males**	***N* observation days**	**Null vs. full model**		
			df	χ^2	*P*
% time feeding	6	584	1	14.58	<0.001
% fruit in the diet	6	583	1	8.99	0.003
Height climbed	6	584	1	4.39	0.036
Distance travelled	6	413	1	1.48	0.224
Restlessness	6	580	1	0.89	0.343
UCP levels	6	170	1	0.66	0.416

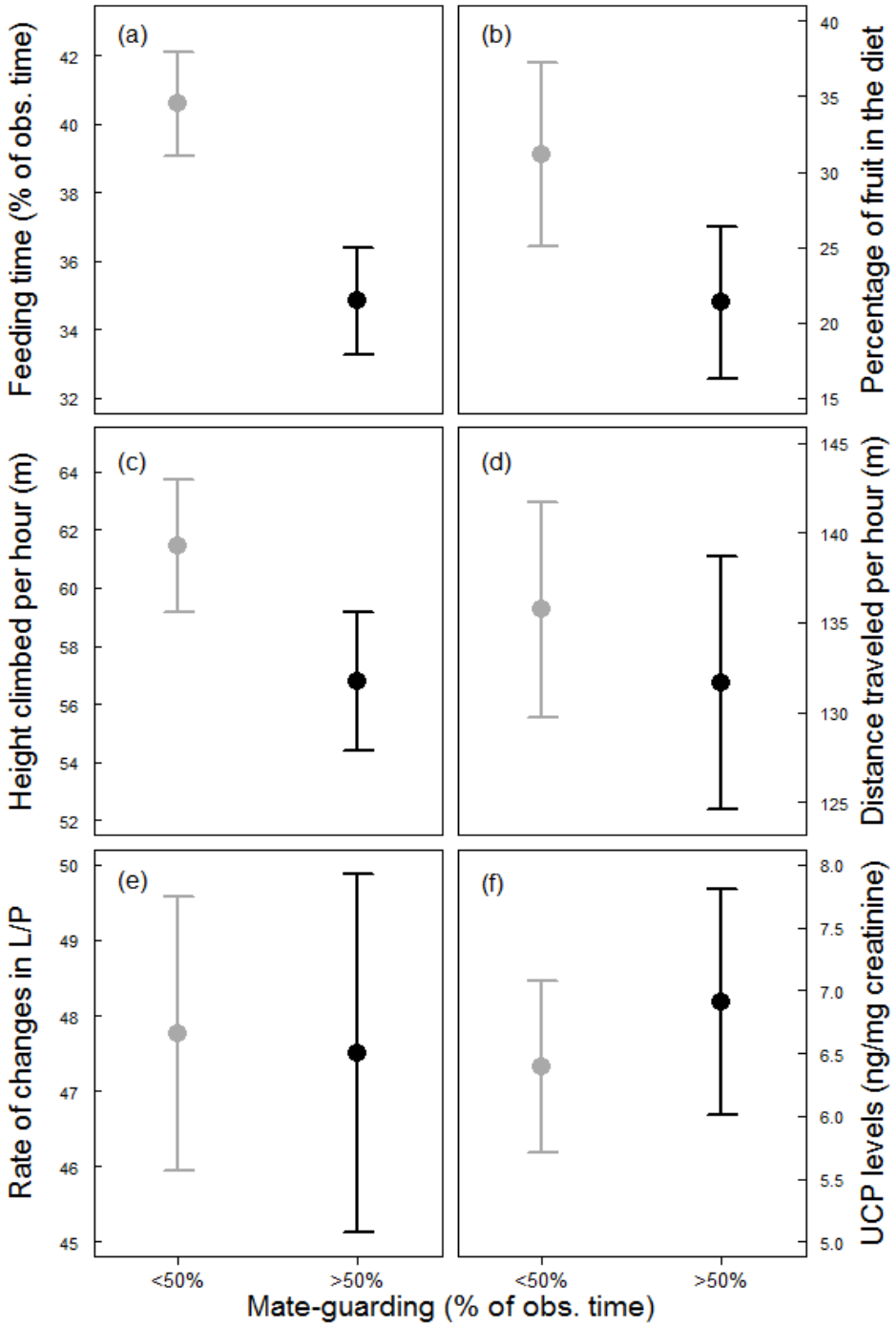

Figure 3.1: Influence of mate-guarding intensity (<50% of observation time on the left in grey and >50% of observation time on the right in black) on males' a) feeding time, b) percentage of fruits in the diet, c) height climbed per hour, d) distance travelled per hour, e) rate of change in locomotion/position (restlessness) and f) UCP levels (energetic status).

The mean ± SE over all males is depicted for each of the parameters. Please note that these graphs are no substitute for the statistical models presented in *Table 3.4* and *3.5*.

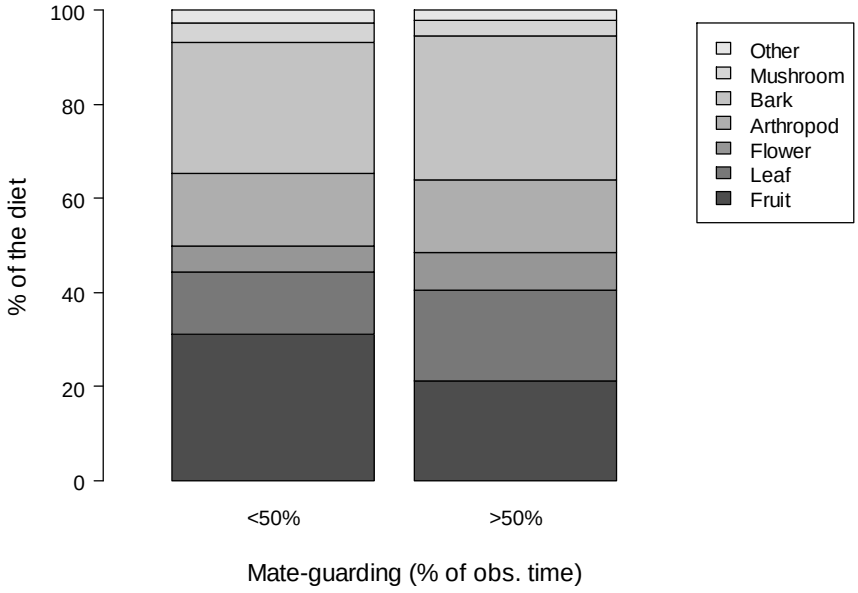

Figure 3.2: Male diet on the day during which they mate-guarded females less than 50% (left bar) and more than 50% (right bar) of the observation time.

Mate-guarding and energy expenditure

Males travelled on average 1.40 - 1.96 km per day (i.e. 117 - 163 m per hour over 12h). The amount of time spent mate-guarding had a statistically significant negative effect on the distance climbed per hour (N = 584 days, *estimate ± SE* = -1.76 ± 0.86, P_{MCMC} = 0.042, *Table 3.4* and *3.5*, *Figure 3.1 c*) but did not significantly affect the distance travelled per hour (N = 413 days, full versus null model, X^2= 1.48, *P* = 0.224, *Table 3.4*, *Figure 3.1 d*) or male's restlessness (N = 580 days, full versus null model, X^2 = 0.89, *P* = 0.343, *Table 3.4*, *Figure 3.1 e*).

Male energetic status

Neither the amount of time spent mate-guarding (N = 170 samples, full versus null model, df = 1, X^2_1 = 0.66, *P* = 0.416, *Table 3.6*, *Figure 3.1f*) nor male dominance status (N = 331 samples, full versus null model, X^2 = 0.354, *P* = 0.552, *Table 3.6*) had a statistically significant influence on UCP levels. In both models, none of the control variables significantly affected the UCP levels. However, there was a trend regarding the effect of height climbed on UCP levels in the first model (mate-guarding - UCP model, *estimate ± SE* = -0.21 ± 0.11, P_{MCMC} = 0.068, *Table 3.6*).

Table 3.5: Estimates ± SE, t-value and MCMC p-values of the different GLMMs to test the influence of mate-guarding activity on different behavioural and physiological parameters.

Model Response		Predictor variables			
		Time spent mate-guarding	*Fruit availability*	*Rainfall*	*Autocorrelation term*
% time feeding	Estimate ± SE	-2.16±0.56	-1.21±0.56	0.22±0.55	3.33±0.55
	t value	-3.84	-2.15	0.42	6.08
	P_{MCMC}	**<0.001**	**0.023**	0.711	**<0.001**
% fruit in the diet	Estimate ± SE	-2.97±0.96	7.08±1.07	-0.95±0.90	5.57±0.91
	t value	-3.11	6.63	-1.05	6.15
	P_{MCMC}	**0.002**	**<0.001**	0.298	**<0.001**
height climbed	Estimate ± SE	-1.76±0.86	-1.87±0.95	-1.18±0.82	3.38±0.83
	t value	-2.05	-1.97	-1.43	4.08
	P_{MCMC}	**0.042**	**0.049**	0.153	**<0.001**
Distance travelled[§]	Estimate ± SE	-2.51±2.19	-2.72±2.40	-2.79±2.02	-3.82±2.12
	t value	-1.15	-1.13	-1.38	-1.80
Restlessness[§]	Estimate ± SE	-0.37±0.39	0.12±0.43	-0.07±0.38	0.29±0.37
	t value	-0.95	0.28	-0.18	0.79

[§] *The MCMC p-values are not shown for the models in which the full model were not significantly different from the null model (LRT, P > 0.05).*

Discussion

Our results indicate that mate-guarding carries no energetic costs in male long-tailed macaques because males seem to compensate any reduction in energy intake by lowering energy expenditure.

In our study, males spent less time feeding and fed less on fruits when mate-guarding and at the same time climbed less distance. UCP levels showed that male energetic status was not affected by mate-guarding, suggesting that the reduction in vertical locomotion counter-balanced reduced food consumption.

Table 3.6: Results of the Likelihood-ratio-tests (LRT) run to compare full versus null models, estimates ± SE, t-value and p-values for the two GLMMs run to test 1) the influence of mate-guarding activity UCP levels and 2) the influence of male rank on UCP levels.

	1/ UCP - Mate-Guarding Model			**2/ UCP – Rank Model**		
N. Samples	170			331		
Null vs. full model	df	χ^2	*P*	df	χ^2	*P*
	1	0.14	0.706	1	0.35	0.552
	Estimate±SE	*t*	P_{MCMC}	*Estimate±SE*	*t*	P_{MCMC}
MG time	0.04±0.10	0.38	0.703			
Height climbed	-0.21±0.11	-1.84	0.068			
Feeding time	0.03±0.12	0.25	0.767			
Rank				0.14±0.15	0.93	0.355
% tree fruiting	-0.20±0.19	-1.05	0.293	-0.15±0.10	-1.47	0.142
Time of sample collection	0.06±0.11	0.55	0.582	0.07±0.07	0.92	0.357
Storage length	0.34±0.32	1.07	0.287	-0.04±0.09	-0.45	0.653

Our study confirms previous findings that mate-guarding leads to reduced feeding time in primate males (baboons: *P. cynocephalus*, Rasmussen 1985, Alberts et al. 1996 and *P. anubis*, Packer 1979; and Japanese macaques, *M. fuscata*, Matsubara 2003) and males of other taxa (e.g. reptiles, Censky 1995, Ancona et al. 2010 and birds, Komdeur 2001). Most likely, mate-guarding males have to adjust their activity to that of the guarded female. In primate species with pronounced sexual size dimorphism, females typically devote less time to feeding activity than males, due to their lower energy demands (e.g. chacma baboons, *P. hamadryas ursinus,* Weingrill et al. 2003). Similarly, in a cetacean (the Dall's porpoise, Ph*ocoenoides dalli*) males undertake shorter dives when paired with females than when paired with males (Willis & Dill 2007) thus potentially lowering their foraging efficiency. Males in general thus seem to trade-off time and energy devoted to feeding with that needed for mate-guarding females and, accordingly, to face costs in terms of reduced feeding time and thus potentially reduced energy intake.

In our case, male long-tailed macaques did not only devote less time to feeding during mate-guarding, they also fed less on fruits, independent of fruit availability. Given that female long-tailed

macaques time reproduction with fruit abundance (van Schaik & van Noordwijk 1985), fruits seem to be an important energy supply in this species. Even though we did not measure energy intake directly, our results suggest that energy intake of male long-tailed macaques during mate-guarding is not only reduced through decreased feeding time, but also through lowered diet quality. Similarly in whiptail lizards (*Aspidoscelis costata*) males feed less during mate-guarding but also capture smaller prey thus not only reducing food intake, but also the quality of their diet (Ancona et al. 2010). Future studies therefore should not only focus on changes in an individual's activity budget, but also on the animal's diet when investigating the costs of behavioural strategies.

Although in our study, male long-tailed macaques faced a clear reduction in time spent feeding and diet quality during mate-guarding, their overall energetic status (as indicated by our measure of UCP levels) was not affected by this behaviour. The most likely explanation is that, during mate-guarding, the reduced feeding time and diet quality is counter-balanced by a reduction in energy expenditure. In fact, in our study, males travelled less distance during mate-guarding, though not horizontally as observed in terrestrial baboons (Altmann et al. 1996), but vertically. Whereas, in terrestrial mammals, locomotion represents only a negligible portion of the total energy expenditure (Altmann 1987), in arboreal species, such as the long-tailed macaque, climbing appears to be an important parameter affecting energetic status (our results *Table 3.6*, see also Hanna et al. 2008). A reduction in vertical locomotion may thus be sufficient to counterbalance any lack in food intake. Whether this reduction occurs as a male strategy to compensate energetic deficiencies or whether it is a side effect of the male adjusting his locomotion to the female cannot be clarified with our data and needs further investigation.

Interestingly, even though-high ranking male long-tailed macaques mate-guard females more extensively than low-ranking ones (Engelhardt et al. 2006), rank did not affect male energetic status, further indicating that mate-guarding is not energetically costly in this species. In contrast, in rhesus macaques, in which mate-guarding intensity is also rank-dependant, high-ranking males bear a clear cost to this behaviour leading to significantly lower UCP levels at the end of the reproductive season (Higham et al. 2011a). The reason why long-tailed macaques differ from rhesus macaques in this respect may lie in the difference of reproductive seasonality between the two species and in the way male hierarchies are formed. First, whereas rhesus macaques are strictly seasonal breeders (Hoffman et al. 2008), long-tailed macaques are not (van Schaik & van Noordwijk 1985). In strictly seasonally breeding species, males typically store fat prior to the mating season and subsequently face a dramatic degradation of their body condition towards the end of this season (Bernstein et al. 1989; Cooper et al. 2004). Males of non-strictly seasonal primates in which females cycle at any time of the

year (though with certain peaks) may adopt a different energy management strategy since they need to maintain a sufficient energy level to respond to the challenges of reproductive competition for much longer (i.e. endurance rivalry, Andersson 1994). Second, whereas male rhesus macaques attain high dominance status through succession or queuing (Berard 1999), high-ranking male long-tailed macaques maintain their rank through contest competition (van Noordwijk & van Schaik 1985). This means that high-ranking male long-tailed macaques, in order to maintain their status, need to be prepared for rank challenges year-round whereas male rhesus macaques don't necessarily have to be prepared for those at all. The combination of endurance rivalry (thought to promote energetic efficiency) and rank challenges, may have, in non-strictly seasonal species, selected for males who are able to limit their locomotion to compensate for the reduced feeding time and diet quality during mate-guarding so as to never reach a critical point at which their energetic status would be substantially negatively affected.

In long-tailed macaques, mate-guarding is one of the main factors determining male reproductive success (Engelhardt et al. 2006). The question therefore remains why alpha males of this species do not always mate-guard females even when there is only a single one being in her fertile phase (Engelhardt et al. 2006) although mate-guarding seems to not bring any energetic costs to them.

One possible explanation may be that alpha males provide reproductive concession to other group males (concession model, Clutton-Brock 1998). The main underlying assumption of the concession model is that the highest-ranking individual has complete control over all the reproductive opportunities in his group (Clutton-Brock 1998). This criteria may be met in long-tailed macaques since alpha males are able to discern the females' fertile phase (Engelhardt et al. 2004), are not energetically limited in their ability to mate-guard females (this study) and female fertile phases do not overlap (Engelhardt et al. 2006). Furthermore, male long-tailed macaques have been observed cooperating with each other to prevent extra group males from accessing females (Girard-Buttoz, unpubl. data). Alpha males may thus reproductively benefit from giving concessions to other group males.

Whether the concession model applies to primates is highly debated (Port & Kappeler 2010) and only one study so far claims to provide empirical evidence for its existence. In male chacma baboons , alpha males seem to reduce potential risk of infanticide and enhance their tenure length through reproductive concession (Henzi et al. 2010). A detailed study on male cooperation and the associated fitness benefits for alpha males will be needed to determine whether reproductive concessions take place in long-tailed macaques or not.

An alternative explanation for why alpha male long-tailed macaques do not mate-guard females as extensively as they theoretically could is that they are limited by other costs than energetic ones. For instance mate-guarding has been shown to be associated with increased aggression rate and/or stress levels in a variety of taxa (baboons Bercovitch 1983; Bergman et al. 2005, lizards Ancona et al. 2010, octopus Huffard et al. 2010).

Overall, our results contradict the thought that mate-guarding is generally energetically costly across all primate species (Alberts et al. 1996; Port & Kappeler 2010) and suggest that under certain circumstances, males may be able to keep their energy budget balanced even when extensively following a female. Whether our finding can be extrapolated to long-tailed macaques in general or not will need further investigation. We can imagine, however, that there are species-dependent differences in the degree to which males are able to balance energetic needs. In particular, males of non-strictly seasonally reproducing species such as long tailed-macaques may be better able than those of strictly seasonal ones to balance the lack of energy intake during reproductive effort by reducing their energy expenditure.

Our study is the first to show that mate-guarding may not necessarily be energetically costly in primates, and possibly also other animals. Our results illustrate the importance of measuring parameters of both energy intake and expenditure and of controlling for environmental factors such as fruit availability when investigating energetic costs of behavioural strategies. Even more importantly, they show that a combination of behavioural observation with more objective measures of physiological status is necessary for a comprehensive picture of this issue.

More studies using physiological measures are now required to further demonstrate and explain the presence or absence of energetic limitations of mate-guarding. Furthermore, assessing the energetic costs associated with this behaviour is just a first step in understanding the factors constraining male reproductive decisions in multi-male primate groups. An evaluation of other costs (e.g. stress and aggression) is also needed for our understanding of the deviance from the Priority of Access model observed in certain species.

Chapter 4

Costs of mate-guarding in wild male long-tailed macaques: physiological stress and aggression

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Abstract

Mate-guarding is an important determinant of male reproductive success in a number of species. Little is known, however, about the physiological and social factors constraining this behaviour. The aim of the study was to assess the effect of mate-guarding on male physiological stress and aggression in long-tailed macaques, a species in which males mate-guard to a lesser extent than predicted by the Priority of Access model (PoA). The study was carried out during two mating periods on three groups of wild long-tailed macaques in Indonesia. We combined behavioural observations and non-invasive measurements of faecal glucocorticoid levels. Mate-guarding was associated with a general rise in male stress levels but from a certain threshold of mate-guarding onwards; vigilance reduced the effect of mate-guarding on these levels. Mate-guarding also increased male-male aggression rate and male vigilance time. Overall, alpha males were more stressed than other males independently of mating competition. Our results suggest that increased glucocorticoid levels during mate-guarding are most likely adaptive since it may help males to maintain a balanced energetic status. However, repeated exposure to high stress levels over an extended period is deleterious to the immune system and thus carries costs. This physiological cost together with the cost of increased aggression may limit the male's ability to mate-guard females, explaining the deviance from the PoA model observed in long-tailed macaques. By comparing our results to previous findings we discuss how ecological factors, reproductive seasonality and rank achievement may modulate the extent to which costs of mate-guarding limit male monopolisation abilities.

Introduction

Mate-guarding of females by males is a common strategy in a broad range of animal taxa (e.g. insects, Alcock 1994; reptiles, Censky 1995, Ancona et al. 2010; crustaceans, Sparkes et al. 1996; birds, Komdeur 2001, Low 2006 and mammals, Alberts et al. 1996, Matsubara 2003, Willis & Dill 2007). The main function of this behaviour is to prevent competitor males from gaining access to females (Andersson 1994), thereby limiting the extent of sperm competition (Birkhead & Moller 1998). As such, mate-guarding has been proven to significantly increase mating and/or reproductive success of males, in particular high-ranking individuals (Censky 1995; Setchell & Kappeler 2003; del Castillo 2003; Engelhardt et al. 2006). Whereas the fitness benefit of mate-guarding is well established, there is a paucity of empirical data on the costs and limitations of this behaviour. Such information is crucial to fully understand the variation in male reproductive skew observed within and across many species (Clutton-Brock 1988; Hager & Jones 2009). It is also needed to improve existing reproductive skew models which, in their current format, have been criticised for their poor applicability to primates (Port & Kappeler 2010). In fact, one of the fundamental parameters in these models is the degree of control top-ranking males have over reproductive output within the group and thus on male reproductive skew (Clutton-Brock 1998; Johnstone 2000; Port & Kappeler 2010).

In primates, the degree of male reproductive skew varies greatly across species living in multi-male multi-female groups (Kutsukake & Nunn 2009). Recent studies have combined modelling and meta-analysis to better comprehend the factors driving this striking variation (Ostner et al. 2008b; Kutsukake & Nunn 2009; Port & Kappeler 2010; Gogarten & Koenig 2013). Given that mate-guarding has been proven to significantly increase mating and/or reproductive success in male primates (rhesus macaques, *Macaca mulatta*, Bercovitch 1997; Berard 1999, long-tailed macaques, *M. fascicularis*, de Ruiter et al. 1994; Engelhardt et al. 2006; Japanese macaques, *M. fuscata*, Matsubara 2003; and mandrills*, Mandrillus sphinx*, Setchell et al. 2005), this behaviour is also likely to be one of the determinants of male reproductive skew. Altmann (1962) developed a verbal model to explain the link between male reproductive skew and mate-guarding in primate species, the Priority of Access model (hereafter the PoA model). This model posits that female cycle synchrony and male rank position are the only limiting factors to female monopolisation and hence fully determine male reproductive output. Yet in several primate species, reproductive output and/or mating frequencies are lower than predicted by the PoA model (savannah baboons, *Papio cynocephalus*, Alberts et al. 2003, rhesus macaques, Dubuc et al. 2011, long-tailed macaques, Engelhardt et al. 2006 and Barbary macaques, *M. sylvanus* Young et al. 2013b). Additional factors other than female monopolisability, such as males' ability to assess the timing of female fertile phases and hence to adjust their mate-

guarding activity accordingly (Dubuc et al. in prep; Engelhardt et al. 2006; Fürtbauer et al. 2011a; Young et al. 2013a) and energetic and physiological costs (Alberts et al. 1996; Bergman et al. 2005) may further limit male mate-guarding activity and success. The ability of males to discern the female fertile phase has been tested in a number of primate species (chimpanzees, *Pan troglodytes*, Deschner et al. 2004; rhesus macaques, Dubuc et al. 2012; long-tailed macaques, Engelhardt et al. 2004; and Hanuman langurs, *Semnopithecus entellus*, Heistermann et al. 2001). In contrast, the costs of mate-guarding still remain largely unclear for primates and this parameter is still missing in primate reproductive skew models (Port & Kappeler 2010).

In a previous study (**chapter 3**), we investigated the energetic costs of mate-guarding in male long-tailed macaques (*Macaca fascicularis*). In this species, reproductive success is highly skewed towards the alpha male (de Ruiter et al. 1994; Engelhardt et al. 2006). Yet high ranked males mate-guard females to a lower extent than predicted by the PoA model (Engelhardt et al. 2006). Since males are able to discern a female's fertile phase (Engelhardt et al. 2004), this lower than expected degree of alpha male mate-guarding could derive from energetic or physiological constraints of the behaviour. However, we did not find any evidence of direct energetic costs (i.e. reduction of energetic status) of mate-guarding in our study population (**chapter 3**). Yet energetic costs may not be the only factor limiting male mate-guarding ability. Physiological stress potentially associated with mate-guarding activity (e.g. Bergman et al. 2005), could also be a cost of this behaviour. In fact, maintaining high levels of stress hormones (e.g. cortisol) for prolonged periods can carry high fitness costs in terms of suppression of the immune system (Grossman 1985; Setchell et al. 2010), sperm production (Sapolsky 1985; Hardy et al. 2005) and general detrimental effects on an animal's health (Sapolsky 2002).

In vertebrates, including primates, male-male competition for accessing fertile females is usually associated with a rise in cortisol/cortisol metabolite levels during the reproductive period (Tokarz et al. 1998; Barrett et al. 2002; Moore & Jessop 2003; Mooring et al. 2006; Fichtel et al. 2007, for a review see Romero 2002). In this highly energetically demanding context, cortisol plays a crucial role by stimulating gluconeogenesis and the mobilisation of fatty acids from body stores (Sapolsky 2002). Whereas males in general exhibit a seasonal and/or short term rise in cortisol levels associated to mating competition, important inter-individual differences in hormone levels can be found between males within the same group (Creel 2001). In primates, these variations are often related to dominance rank, but the direction of the relationship between glucocorticoid (GC) levels and rank may be mediated by several factors, e.g. hierarchy stability (Sapolsky 1983; Bergman et al. 2005; Higham et al. 2013) or opportunities for social support (reviewed in Abbott et al. 2003). Differences in GC levels between high- and low-ranking individuals may also derive from differential rank-related

reproductive strategies. In fact, in many species, only high-ranking males mate-guard females intensively since they are the only ones able to efficiently exclude rival males from accessing the guarded females (Weingrill et al. 2000; Engelhardt et al. 2006; Setchell et al. 2010; Higham et al. 2011a). In baboons, the variation in GC concentrations matches differences in mate-guarding duration at the inter- (alpha vs. beta males, Gesquiere et al. 2011) and intra-individual levels (Bergman et al. 2005). In addition to the potential physiological costs of mate-guarding in terms of increased stress hormone production, males can face increased risk of injuries when monopolising females. In fact, in vertebrates, mate-guarding behaviour is often associated with an increase in aggression rate and/or in time devoted to agonistic interactions (e.g. lemurs, Mass et al. 2009; lizards, Ancona et al. 2010; and birds, Steele et al. 2007) and such interactions involve, by nature, a risk of physical injuries (Clutton-Brock et al. 1979; Blanchard et al. 1988; Drews 1996). Nevertheless, the direct link between stress hormone concentrations, aggression rates and mate-guarding behaviours at a proximate level, and the underlying behavioural factors potentially generating physiological stress during mate-guarding remain to be investigated in primates.

The aim of the current study was to assess the potential aggression and stress-related costs of mate-guarding in wild male long-tailed macaques. This species lives in multi-male multi-female groups and is a non-strictly seasonal breeder (van Schaik & van Noordwijk 1985). Although female long-tailed macaques can conceive year round, birth peaks frequently occur, the timing of which seems to depend on fruit availability (van Schaik & van Noordwijk 1985). The species is therefore classified as capital breeder on the three grade scale of reproductive seasonality in primates by Brockman and van Schaik (2005). In long-tailed macaques, males exhibit a clear seasonal rise in faecal GC (fGC) levels associated with reproductive effort (Girard-Buttoz et al. 2009) but little is known about the proximate factors driving intra- and inter-individual differences in physiological stress levels. Therefore, in the present study, first we used fGC measurements to assess whether mate-guarding effort is associated with an intra-individual rise in physiological stress. In this analysis we controlled for behavioural parameters known to increase cortisol or fGC levels in human and/or non-human primates, i.e. vigilance, aggression, grooming and copulation rates (Ray & Sapolsky 1992; Lynch et al. 2002; Warm et al. 2008; Ostner et al. 2008a; Cheney & Seyfarth 2009; Arlet et al. 2009; Girard-Buttoz et al. 2009; Surbeck et al. 2012). We also controlled for the number of males in proximity as an approximation of the degree of male-male competition. Secondly, we assessed the effect of mate-guarding on those behavioural parameters that are known to have an effect on fGC output as well as on the likelihood of male-male aggression as a possible indicator of the risk of injury for the mate-guarding male. Thirdly, we investigated whether different reproductive tactics, i.e. high versus low investment into mate-guarding, result in inter-individual differences in male physiology by comparing

fGC levels of males mate-guarding females extensively (i.e. high-ranking males) with fGC levels of non-mate-guarding males (i.e. low-ranking males). In order to ensure that a potentially detected effect of dominance rank on fGC derives from reproductive competition during mate-guarding and not from competition between males per se, we tested potential rank effects separately during and outside the mating period.

Methods

Animals and study site

The study was carried out on three groups of wild long-tailed macaques living in the primary lowland rainforest surrounding the Ketambe research Station (3º41'N, 97º39'E), Gunung Leuser National Park, North-Sumatra, Indonesia. The forest structure and phenological composition has been described in detail by Rijksen (1978) and van Schaik and Mirmanto (1985). The long-tailed macaques in the area have been studied since 1979 (van Schaik & van Noordwijk 1985; de Ruiter et al. 1994; Engelhardt et al. 2004). For our study we collected data between March 2010 and April 2011 focusing on three groups: Camp (C), Ketambe Bawa (KB) and Ketambe Atas (KA). Faecal samples were collected regularly during the entire study period and behavioural data during the two consecutive mating periods (see below). All adult individuals were individually known and well habituated to human observers. The total size of a social group varied from 22 to 58 individuals (for details, see **chapter 3**). Between January and April 2011, four males migrated back and forth between the groups KA and KB and associated with one of the groups for periods between a few hours up to 3 weeks before migrating back to the other group. The study was conducted completely non-invasively and under the permission of the authorities of Indonesia. We adhered to the Guidelines of the Use of Animals in Research, the legal requirements of Indonesia and the guidelines of the involved institutes.

Behavioural data collection

Behavioural data were collected by C.G-B and six experienced Indonesian and international field assistants. All assistants were trained by C.G-B and inter-observer reliability was assessed repeatedly (measurement of agreement kappa > 0.8 for each assistant). The observations covered two mating periods for two groups (C and KB) and one mating period for the third group (KA). A mating period was defined as the period between the first mate-guarding day and the last mate-guarding day ever observed in any of the three groups, by any male. From March to July 2010, four observers followed groups C and KB every day and from December 2010 until April 2011 all three groups were generally followed every other day and frequency of observations increased to every day when alpha and/or

beta males were observed mate-guarding. Which male was followed on a given day and whether he was followed for half or entire day was decided the day before depending on the mate-guarding behaviour of the male and on the number of observers available. The focal protocol was never modified in the course of a given day. Accordingly, full day or half day focal protocols (see below) were completed every day regardless of whether the focal male was mate-guarding females or not. Each day, groups were followed from dawn to dusk. We focused our behavioural observations on alpha and beta males because they are known to mate-guard females most extensively (Engelhardt et al. 2006). The activity of the focal animal was recorded every minute using instantaneous sampling (Altmann 1974) and comprised the following categories: resting, being vigilant (monitoring the surrounding environment by looking in different directions, being either still or moving, and while not involved in feeding or social activity except mate-guarding), feeding (handling and consuming food), drinking, travelling (continuous locomotion during at least one minute with no foraging activity and no social interactions), aggressing, affiliating (including copulation), grooming, self-grooming. The mate-guarding behaviour of the focal male and the distance between him and the mate-guarded female was also recorded every minute. Whether a male was mate-guarding or not a female on a given minute was coded *a posteriori*. A male was considered as "mate-guarding" when he followed a sexually active female for more than 5 consecutive minutes and maintained a distance of less than or equal to 10m between him and the female. A female was considered sexually active if she was observed copulating at least once on a given day. If the female moved away from the male and the male did not follow her for more than 2 minutes the mate-guarding activity was considered to have ended. "Extensive mate-guarding days" refers to the days during which the male mate-guarded females more than 50% of the observation time. A mate-guarding period was defined as a period of one to several consecutive extensive mate-guarding days. In addition, all copulations and aggressions (including submissive expressions) between any adult individuals were recorded (all occurrence sampling for the focal male and *ad libitum* for all the other individuals). Aggressions comprised threatening, chasing, hitting and biting. Finally, the identities of all males within 10 m of the focal individual were recorded every 5 minutes.

Determination of fruit availability

Fruit availability is known to significantly affect cortisol levels in primates (e.g. Muller & Wrangham 2004a). It was therefore important to control for this parameter in our analysis and we monitored fruit trees to assess fruit availability. In each of the three studied groups, 40 locations, covering the entire home ranges, were randomly selected (120 locations in total over the three territories). At each location, three trees were randomly selected from three different species among the tree

species producing fruit eaten by *M. fascicularis* (Ungar 1995). In total 360 trees, from 87 different species were selected (120 trees for each group's home range). Each tree was surveyed monthly, within the last 3 days of every month, by a field assistant experienced in phenology and fruit abundance was recorded using a logarithmic scale (**0**: absence, **1**: 1-10 items, **2**: 11-100, **3**: 101-1000, **4**: 1001-10000 and **5**: > 10000). The average monthly score of fruit abundance in each territory was highly correlated with the percentage of trees fruiting. For the analyses, we therefore used percentage of trees fruiting as an index of fruit availability.

Faecal sample collection and hormone analysis

Faecal samples were generally collected once a week from four males in each group: alpha and beta males and two low-ranking males (rank 3 and bellow) as "controls" (males which usually do not mate-guard females extensively, Engelhardt et al. 2006). In addition, during mate-guarding periods, we collected faecal samples every third day from the mate-guarding male. Right after defecation, samples were homogenised and 2-3 g of faces were collected and stored in a polypropylene vial and placed on ice in a thermos bottle. At the end of each fieldwork day, the samples were frozen at -20°C in a freezer. In July 2011, all samples were transported, on ice, to the hormone laboratory of the Bogor Agricultural University (IPB) and then freeze-dried and pulverised before transportation to the Reproductive Biology Unit of the German Primate Centre for glucocorticoid analysis.

For hormone analysis, an aliquot (50-70 mg) of the faecal powder was extracted within 3 ml of 80% methanol by vortexing for 10 min (Heistermann et al. 1995). For monitoring changes in fGC levels, faecal extracts were analysed for immunoreactive 3α,11ß-hydroxyetiocholanolone (3α,11ß-dihydroxy-CM), a group-specific measurement of 5-reduced 3α,11ß-dihydroxylated cortisol metabolites (Möstl & Palme 2002; Ganswindt et al. 2003). The assay has been previously validated for assessing adrenocortical activity from faeces in long-tailed macaques (Heistermann et al. 2006). Hormone measurements were carried out by microtiter plate enzymeimmunoassay according to methods previously described (Möhle et al. 2002; Ganswindt et al. 2003). Intra- and inter-assay coefficients of variation of high- and low- value quality controls were 8.9% and 9.9% (high) and 6.3% and 14.3% (low), respectively.

Statistical analyses

For all analyses, we considered only those days of observation for which at least 1 hour of focal data was recorded. The final data set thus comprised 2.088 hours of focal observations over 600 days (see **chapter 3** for details about observation and mate-guarding time).

Influence of mate-guarding and other behaviours on male fGC levels

For each day, we calculated for the focal male the percentage of time spent mate-guarding, grooming and being vigilant as percentage of the observation time. We also calculated the copulation rate (i.e. number of copulation between the focal male and any female per hour), the rate of male-male aggression (i.e. the number of aggression between the focal male and any other adult male per hour) and the number of males in proximity (defined as the average number of males within 10 m per 5 minute scan). We also calculated the number of sexually active females in each group on each observation day.

We tested whether the percentage of time spent mate-guarding on a given day affected males' stress hormone levels (as assessed by fGC measures, model 1). Since the time-lag for excretion of glucocorticoid metabolites into the faeces is on average 36 h in long-tailed macaques (Heistermann et al. 2006), we matched behavioural observations with fGCs levels measured in samples collected at either day +1 or day +2 after the observations. When samples were available at both days, we used the mean fGCs levels of the two samples. We used a generalised linear mixed model (GLMM, Baayen 2008) to test whether the percentage of time spent mate-guarding influenced fGC levels. The fGC level values were log-transformed to achieve a symmetric distribution and we used a Gaussian error structure in the model. In model 1 we also wanted to determine if, similar to other studies (Ray & Sapolsky 1992; Warm et al. 2008; Cheney & Seyfarth 2009; Girard-Buttoz et al. 2009), vigilance time, copulation rate, aggression rate and grooming rate had a significant effect on fGC levels in our study males. We therefore added these four parameters as independent variables in model 1. To account for the degree of male-male competition for access to receptive females and the potential for male-male interaction we also included as control factors the number of sexually active females and the number of males in proximity in this model. Finally, since ecological conditions are likely to affect fGC levels in primates (Muller & Wrangham 2004a), we included fruit availability as a control factor in model 1. Fruit availability on a given day was approximated using the fruit availability measured on the closest monthly record. For example, the percentage of tree fruiting recorded on the 31st of January was used as the fruit availability score for all the days between the 16th of January and the 15th of February.

In model 1, we tested for the significance of the interactions between mate-guarding time and 1) aggression rate, 2) number of males in proximity and 3) time being vigilant. Only the latter was significant (likelihood ratio test, $P < 0.05$) and kept in the final model. Male ID and group were included in model 1 as nested random effects.

Influence of mate-guarding on vigilance, proximity of other males and likelihood of male-male aggression

In order to understand better the potential behavioural sources of physiological stress during mate-guarding we analysed the effect of mate-guarding on the parameters which had a significant effect on fGC levels - i.e. vigilance time (although through an interaction with mate-guarding time) and number of males in proximity (see *Table 4.1* and result section). In addition we also tested the effect of mate-guarding on the likelihood of male-male aggression as a possible indicator of the risk of injury. We used a generalised linear mixed model to test whether the percentage of time spent mate-guarding on a given day affected the following parameters in the males: 1) vigilance time (model 2), 2) number of males in proximity (model 3), and 3) likelihood of aggression with other males (model 4). Similar to model 1 we included as control factors fruit availability and the number of sexually active females in all models and the number of males in proximity in models 2 and 4. Male ID and group were included in each model as nested random effects.

The rate of male-male aggressions in our study subjects was very low (0.22 ± 0.04 h^{-1}) and no aggression was recorded on most of the observation days (366 out of 600). Because the resulting distribution of daily aggression rate was thus highly zero inflated, we could not run a model with a Gaussian error structure. We thus coded each day with at least one aggression as an aggression day and other days as non-aggression day and used a model with a binomial error structure to test the influence of mate-guarding on the likelihood of aggression with other males on a given day (model 4). We considered both aggressions given and received by the focal animal. For the other models we used a Gaussian error structure since the response variable was symmetrically distributed. The likelihood of recording any male-male aggression on a given day being dependent on the observation time that day, we included observation time (in minutes) as a control factor in model 4.

Influence of dominance rank on male fGC levels

To test whether males who mate-guarded females extensively during the reproductive periods (alpha and beta males) had higher fGC levels than other males, we ran a GLMM (model 5) with Gaussian error structure including fGC levels as response, male dominance rank (two categories: high-ranking for alpha and beta males, and low-ranking for two other males in the same group), period (mating and non-mating periods) and the interaction between the two as fixed factors, fruit availability as control fixed effects and animal ID and group as nested random factors. Dominance ranks between males were determined using the 'bared-teeth-face' display, a unidirectional submissive display (van Hooff 1967). Bared-teeth-face giver and receiver were entered into a sociometric matrix and dominance ranks were compiled with Matman 1.1.4 using the I&SI method

(de Vries 1998). In addition, to test whether being an alpha male is particularly stressful (Gesquiere et al. 2011), we ran another GLMM (model 6) using the same factors and error structure but the dominance rank categorisation was modified as either alpha or other males. Since the interaction between dominance rank and period was not significant in both models (LRT, $P > 0.4$), we reran the models without the interaction.

Autocorrelation term and assumptions' checking

Each model was fitted in R 2.15.0 (R Development Core Team 2010) using the function lmer of the R-package lme4 (Bates & Maechler 2010). The response variable in the different models with Gaussian error structure (models 1, 2, 3, 5 and 6) was likely to show temporal autocorrelation unexplained by the fixed effects included, potentially leading to violation of the assumption of independent residuals. Therefore, we included a temporal autocorrelation term into these models using an approach developed by Roger Mundry (see Fürtbauer et al. 2011b).

In each model, we checked that the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting a qqplot and the residuals plotted against fitted values. We checked for model stability by excluding data points one by one from the data and comparing the estimates derived with those obtained for the full model. Variance inflation factors (VIF, Field 2005) were derived using the function vif of the R-package car (Fox & Weisberg 2010) applied to a standard linear model excluding the random effects. VIF's which are less than 5 indicate that covariation between the predictors is not a problem (Bowerman & O'Connell 1990; Myers 1990). In all our models VIF's were less than 1.7. The other diagnostics also did not indicate obvious violation of the assumption.

For each model, we first determined the significance of the full model as compared to the corresponding null model (including all the factors except "mate-guarding time", and in addition in model 1, vigilance time and the interaction vigilance*mate-guarding time) using a likelihood ratio test (R function anova with argument test set to "Chisq"). To achieve a more reliable p-value, we fitted the models using Maximum Likelihood rather than Restricted Maximum Likelihood (Bolker et al. 2009). Only if this likelihood ratio test revealed significance we considered the significance of the individual predictors. P-values for the individual effects were based on Markov Chain Monte Carlo sampling (Baayen 2008) and derived using the functions pvals.fnc and aovlmer.fnc of the R package languageR (Baayen 2010).

Results

Mate-guarding activity

In each of the three groups, the alpha male mate-guarded a higher number of females than the beta male (*Table 4.1*). Males mate-guarded each female on average 4 consecutive days (range 1 - 33, *Table 4.1*) and on average 29.8% (range 8.4 - 53.9%, *Table 4.1*) of their time was devoted to this behaviour in general over the entire mating periods.

***Table 4.1*:** Observation time, mate-guarding period length and number of females mate-guarded by the study males.

Group	**Camp**		**Ketambe Atas**		**Ketambe Bawa**	
Male rank	α	β	α	β	α	B
Number of mating periods	2	2	1	1	2	2
Focal observation time (hours)	668	455	185	111	388	323
Number of days of observation	147	114	68	48	122	85
Number of faecal samples	81	47	34	24	52	35
Number of females mate-guarded	5	3	3	2	8	5
Number of MG days	41	4	30	27	49	10
Mean MG period length (days)	3.9	1	4.9	9	3.7	1.5
Range of MG period length (days)	1-18	1-1	1-13	1-33	1-10	1-4
Overall MG time	27.4%	8.4%	40.2%	53.9%	36.9%	12.0%

"MG" refers to mate-guarding. "MG days" refers to days during which the males were mate-guarding female for more than 50% of observation time. The overall MG time is the percentage of observation time during which the male was mate-guarding females.

Mate-guarding, male behaviour and fGC levels

Overall, males had higher fGC levels when mate-guarding females than when not (*Figure 4.1*). However, the model indicates that the effect of mate-guarding on fGC levels was significantly affected by the amount of time a male was vigilant during these days (N = 273 days, interaction between vigilance and mate-guarding time: *P* = 0.028, *Table 4.2*, *Figure 4.1*). On days on which males did not mate-guard females, male fGC levels increased with the amount of time a male was vigilant. On days on which males mate-guarded a female, i.e. on days on which male fGC levels were increased anyway, vigilance however reduced the physiological stress response from a certain degree of mate-guarding onwards (*Figure 4.1*). The more time males invested into mate-guarding the stronger vigilance reduced fGC levels. Yet these levels were always above non-mate-guarding values.

Of the other variables tested, the number of sexually active females, the number of males in proximity and fruit availability (all P ≤ 0.05, *Table 4.2*) also had a significant effect on fGC levels whereas copulation rate, male-male aggression rate and grooming rate did not (all *P* > 0.37, *Table 4.2*). Male fGC levels increased with increasing numbers of sexually active females, with declining numbers of males in proximity and with diminishing fruit availability.

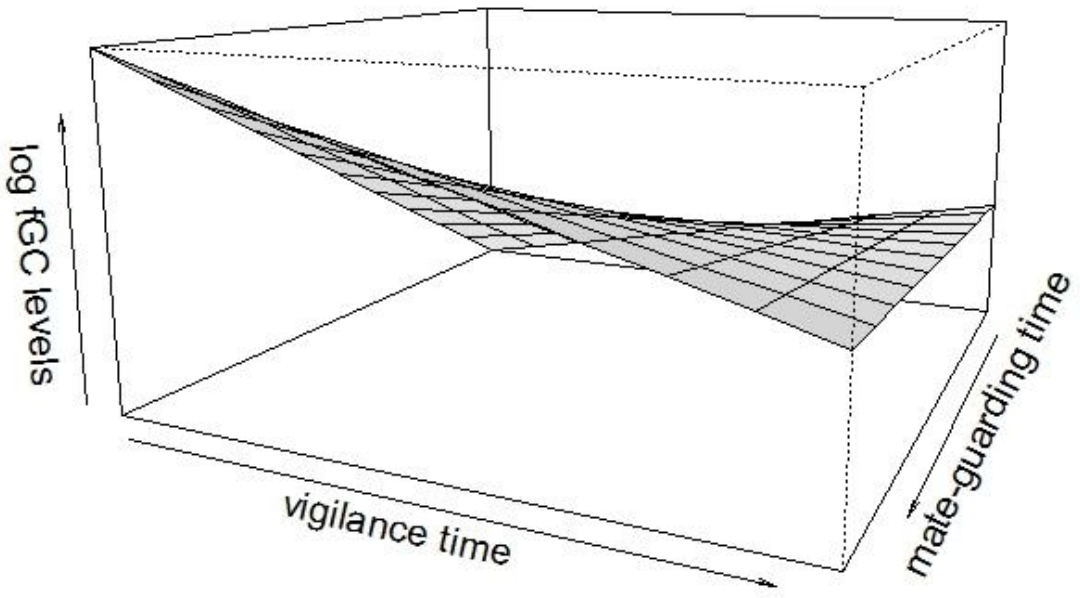

Figure 4.1: Effect of mate-guarding time and vigilance time on fGC levels.

The plane depicts values predicted by model 1.

Table 4.2: Results of the Likelihood-ratio-tests (LRT) run to compare full versus null models, estimates ± SE, t-value/Z-value and p-values for the different GLMMs run to test the influence of mate-guarding activity on fGC levels and behavioral parameters.

	Model 1			**Model 2**			**Model 3**			**Model 4**		
	fGC levels			Vigilance time			N. males			Aggression (Y/N)		
N. obs. days	273			600			600			600		
Null vs.	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
full model	1	38.87	<0.001	1	15.17	<0.001	1	6.97	0.008	1	11.23	<0.001
	Estimate±SE	t	P_{MCMC}	Estimate±SE	t	P_{MCMC}	Estimate±SE	t	P_{MCMC}	Estimate±SE	Z	P
Intercept	6.11±0.08	76.30	<0.001	42.72±0.83	51.46	0.003	0.50±0.05	10.51	0.004	-0.54±0.15	-3.56	<0.001
MG time	0.18±0.03	6.03	<0.001	1.87±0.46	4.01	<0.001	0.02±0.01	2.65	0.009	0.35±0.10	3.45	<0.001
N. males	-0.06±0.03	-2.08	0.039	-1.23±0.45	-2.71	0.016				0.52±0.10	5.05	<0.001
% tree fruiting	-0.29±0.03	-9.03	<0.001	0.24±0.52	0.45	0.521	-0.04±0.01	-3.71	<0.001	-0.11±0.12	-0.92	0.358
N. sex. act. fem.	0.06±0.03	1.99	0.050	1.07±0.48	2.23	0.025	0.04±0.01	3.93	<0.001	0.15±0.11	1.31	0.191
AC term	0.08±0.03	3.09	0.002	2.90±0.43	6.75	<0.001	0.03±0.01	3.13	0.003			
Observation time										0.85±0.12	6.87	<0.001
Copulation rate	-0.02±0.03	-0.66	0.554									
Aggression rate	0.02±0.03	0.9	0.371									
Grooming time	0.02±0.03	0.6	0.578									
Vigilance time	0.02±0.03	0.84	0.442									
MG * Vigilance	-0.06±0.03	-2.28	0.028									

"MG" refers to mate-guarding, "N. males" to the number of males in proximity, "N. sex. act. fem." to the number of sexually active females and "AC term" to the autocorrelation term.

Mate-guarding, male behaviour and proximity of other males

The amount of time a male spent mate-guarding had a significant positive effect on vigilance time (N = 600 observation days, $P < 0.01$, *Table 4.2*). In other words, the more time a male spent mate-guarding the more vigilant he was (*Figure 4.2a*). Independent of whether and how much a male spent time on mate-guarding, increasing numbers of sexually active females also increased vigilance behaviour in males ($P = 0.025$, *Table 4.2*). Vigilance however decreased with increasing numbers of males in proximity ($P = 0.016$, *Table 4.2*).

Mate-guarding also significantly increased the number of males in proximity and the likelihood of male-male aggression (both $P < 0.01$, *Table 4.2*, *Figure 4.2b* and *4.2c*). The latter may have been interdependent, because increasing numbers of males in proximity increased the likelihood of male-male aggression even independent of mate-guarding ($P < 0.01$, *Table 4.2*).

Finally, the number of males in proximity was also significantly affected by the number of sexually active females ($P < 0.01$, *Table 4.2*). Males were more cohesive the more sexually active females were present in the group.

Dominance rank, period, fruit availability and fGC levels

Males had significantly higher fGC levels during the mating than during the non-mating periods (N = 771 samples, $P < 0.001$, mean±SE mating periods: 573.9±44.4 ng/g faeces, mean±SE non-mating period: 412.5±45.3 ng/g faeces, *Table 4.3*, *Figure 4.3a*). There was no significant difference between high-ranking (alpha and beta) and low-ranking (all others) males in fGC levels (Model 5, $P = 0.45$, *Table 4.3*, *Figure 4.3b*). However, alpha males alone had significantly higher fGC levels than other males, independent of period and fruit availability (Model 6, $P = 0.03$, *Table 4.3*, *Figure 4.3c*). Fruit availability in turn had a highly significant negative effect on fGC levels independent of period and male rank ($P < 0.001$, *Table 4.3*).

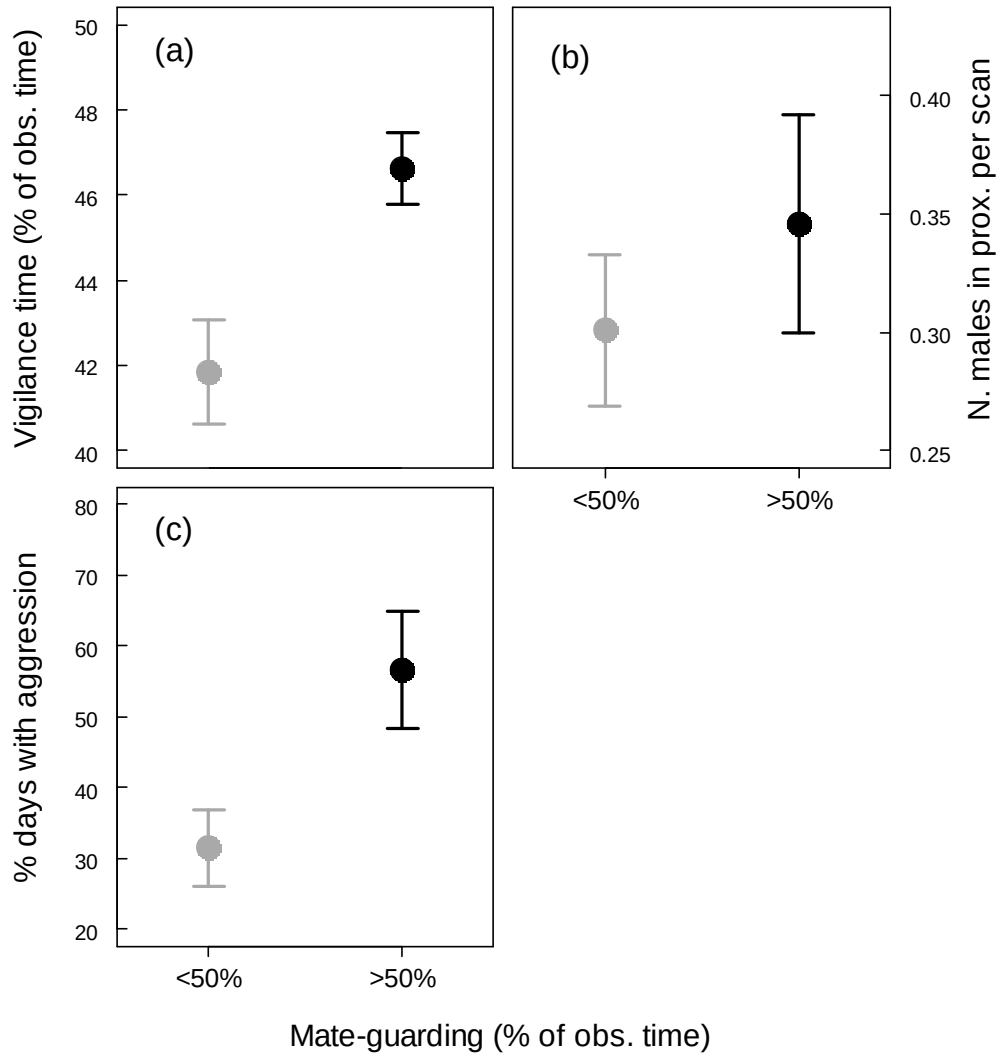

Figure 4.2: Influence of mate-guarding intensity on a) males' vigilance time, b) number of males in proximity, and c) percentage of day with male-male aggression. Grey dots depict results from days in which males spent <50% of observation time mate-guarding and black dots those in which they did this >50% of observation time. The mean ± SE over all males is depicted for each of the parameters. Please note that these graphs are no substitute for the statistical models presented in *Table 4.1*.

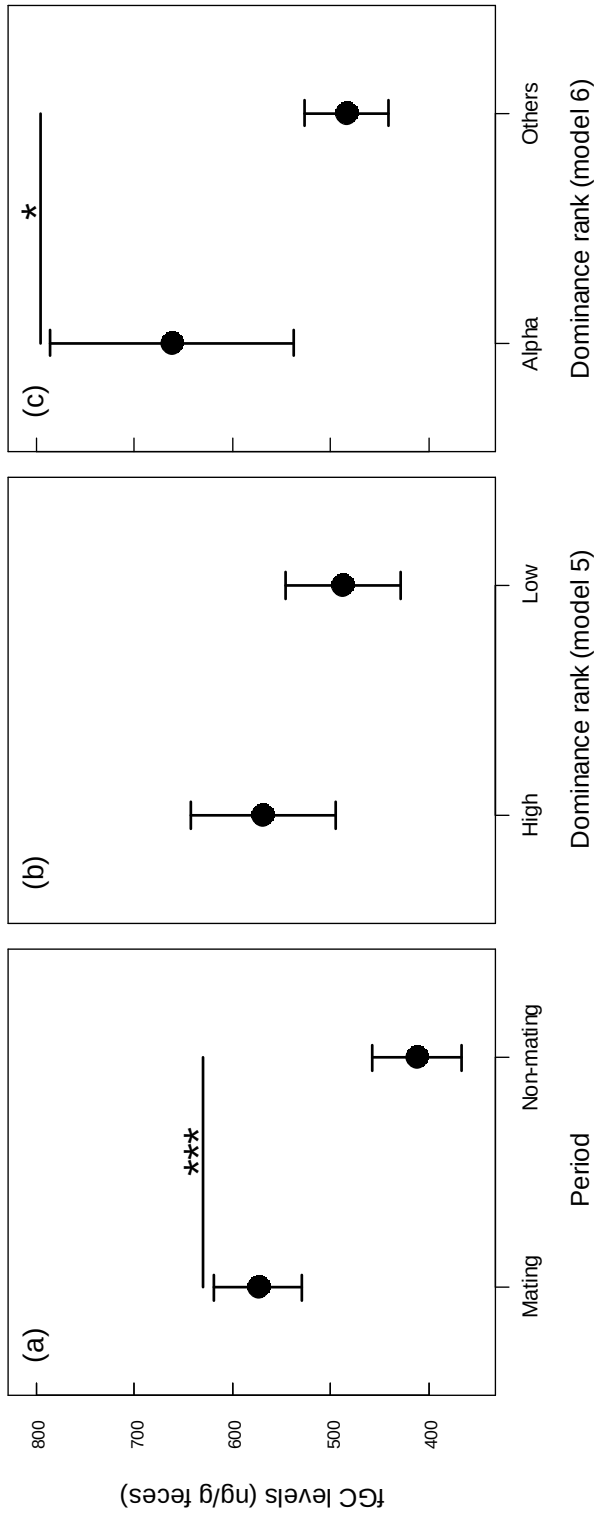

Figure 4.3: Influence of period (a) and rank (b and c) on fGC levels.

The mean ± SE fGC levels over all males in each of the periods (mating and non-mating) (a) and the mean ± SE fGC levels over males in each rank category as defined in model 5 (b) and in model 6 (c) are also depicted. In model 5 dominance rank is categorized as high (alpha+beta males) or low (other males) and in model 6 as alpha or other males. "*" $P = 0.029$, *** $P < 0.001$.

Discussion

Our results suggest that mate-guarding may carry two types of costs in male long-tailed macaques: physiological and physical. In our study, males generally faced increased fGC levels when mate-guarding females. Repeated exposure to high GC levels over an extended period may have deleterious impacts on male immunity and reproduction (Sapolsky 2002, but see Boonstra 2013) and may as such constitute a cost. The effect of mate-guarding on fGC levels however interacted with vigilance: from a certain degree of mate-guarding onwards vigilance reduced the effect of mate-guarding on stress hormone levels. Mate-guarding also increased the rate with which males were involved in aggressive interactions with other males. Although aggression did not significantly affect male fGC levels, increased aggression brings an extra risk of injury. Mate-guarding male long-tailed macaques may thus bear potential physical costs as well.

***Table 4.3**:* Results of the Likelihood-ratio-tests (LRT) run to compare full versus null models, estimates ± SE, t-value and MCMC p-values for the two GLMMs run to test the influence of dominance rank and period (mating or non-mating) on fGC levels.

	Model 5 fGC levels Rank: High (α+β) vs. low (others)			**Model 6** fGC levels Rank: Alpha vs. others		
Null vs. full model	df	χ^2	*P*	df	χ^2	*P*
	1	134.9	**<0.001**	1	139.22	**<0.001**
	Estimate±SE	*t*	P_{MCMC}	*Estimate±SE*	*t*	P_{MCMC}
Intercept	5.82±0.09	62.23	**<0.001**	5.99±0.10	57.49	**<0.001**
Rank (others)	-0.09±0.13	-0.72	0.454	-0.29±0.12	-2.46	**0.029**
Period (mating)	0.40±0.03	12.11	**<0.001**	0.40±0.03	12.12	**<0.001**
% tree fruiting	-0.24±0.02	-14.64	**<0.001**	-0.24±0.02	-14.64	**<0.001**
AC term	0.18±0.01	12.12	**<0.001**	0.18±0.01	12.21	**<0.001**

In model 5 dominance rank is categorised as high (alpha and beta males) or low (other males) and in model 6 as alpha or other males. "AC term" refers to the autocorrelation term.

Altogether, male long-tailed macaques appear to be physiologically stressed during mate-guarding (as indicated by our measure of fGC levels), which confirms similar findings in chacma baboons (Bergman et al. 2005). Whereas in primates a rise in fGC levels often results from increased aggression and copulation rates (Ray & Sapolsky 1992; Lynch et al. 2002; Ostner et al. 2008a; Arlet et al. 2009; Girard-Buttoz et al. 2009; Surbeck et al. 2012), two behaviours that generally accompany male mate-guarding activities, this was not the case in our study males. The question thus remains: what increased fGC levels during-mate-guarding? Males may increase glucocorticoid production in order to maintain a balanced energetic status (**chapter 3**) in a context in which they trade-off feeding time (**chapter 3**) against vigilance time (this study) and face a potential decrease in energy intake. Similar to long-tailed macaques during mate-guarding, mammals commonly trade-off feeding time/efficiency against vigilance (Illius & Fitzgibbon 1994; Fortin et al. 2004) and males of diverse taxa are, generally, more vigilant during the reproductive season (birds, Reboreda & Fernandez 1997; mammals, Li et al. 2012) and particularly when paired to females (Guillemain et al. 2003). Overall, increased vigilance derives from the need to monitor conspecifics in a highly competitive context. The increase in fGC levels during mate-guarding in our study males may provide the male with more readily available energy (Sapolsky 2002). This energy may compensate for the reduced food intake and may also be allocated towards vigilance, which is by nature energetically demanding and stressful (Warm et al. 2008). Interestingly, the importance/quality of the female may be a source of additional psychological stress. From a certain degree of investment into mate-guarding (and thus most likely also interest in the female), our study males had higher fGC levels when they were less vigilant. High-ranking male long-tailed macaques can assess female fertile phase and increase their mate-guarding effort around this period (Engelhardt et al. 2006). When males mate-guard a female intensively (i.e. when females are of high reproductive value) psychological stress may stem from the fear of failing to monopolise the female efficiently. A way for the male to reduce this stress is to be more vigilant and as such better prevent other males from accessing the female.

In addition to its effect on male physiological stress response, similar to other vertebrates (e.g. mammals, Mass et al. 2009; reptiles, Ancona et al. 2010; and birds Steele et al. 2007), mate-guarding also increased male-male aggressions. We did not measure injuries systematically in our study animals, but aggression is known to inherently increase the risk of injury in vertebrates (Clutton-Brock et al. 1979; Blanchard et al. 1988; Drews 1996). During mate-guarding, males of capital breeder species with a low degree of female cycle synchrony, such as long-tailed macaques (Engelhardt et al. 2006), might be more likely to engage in male-male aggressions than males from income breeders. In fact, in capital breeders, the guarded female is often the only fertile female in the group (e.g. Engelhardt et al. 2006), thus further concentrating male-male competition. In income breeders, in

contrast, several females can be sexually receptive at the same time so that several high-ranking males may concurrently access different females, leading to a reduction in intensity of male-male competition. Surprisingly, in our study, male-male aggression rate did not significantly influence male fGC levels, although increased aggression rate during male-male competition for access to mates often leads to a concurrent increase in fGC levels in primates (Ostner et al. 2008a; Arlet et al. 2009; Surbeck et al. 2012). This might be explained by the low rate of aggression in our study males (0.2 h^{-1}). Increased aggression rate during mate-guarding may thus not in itself dramatically impact male stress physiology. It may constitute, however, a physical cost since, in long-tailed macaques, male-male aggressions sometimes result in severe injuries directly impairing male ability to mate-guard females. For example, during our study period, two high-ranking males (one alpha and one beta) from two different groups got severely injured and had to isolate themselves socially from the group for over a week to recover. During this period, they did not access/mate-guard females despite the presence of sexually active females in the group.

In our study, males had more males in proximity on mate-guarding days than on other days most likely resulting from males' interest in the guarded female. Interestingly, although mate-guarding increased aggression between males, having more males around during mate-guarding reduced male stress hormone levels. It may be that the presence of other males in proximity provides a mate-guarding male with the benefit of collective vigilance against predators (reviewed in Elgar 1989 and Quenette 1990) or extra-group males attempting to enter the group and access females (e.g. Engelhardt et al. 2006). Our finding that our study males were less vigilant when they had more males in proximity supports this idea. The presence of other male group members may thus alleviate the need to monitor the surrounding and better focus on monitoring the guarded female hence reducing his physiological stress levels. This might be particularly the case in a species like long-tailed macaques where alpha male tenure and male residence duration are relatively long (on average 25 and 45 months respectively, van Noordwijk & van Schaik 2001), which provides the opportunity for stable long-term alliances and coalitionary support against extra-group males. Under such conditions and in periods of hierarchy stability (as in our study), group males can thus be allies rather than challengers so that their presence will be beneficial to high-ranking males. Strong male-male mutual support may explain why "bluff immigrants" trying to take over alpha position from outside the groups are rarely successful (van Noordwijk & van Schaik 2001).

Beyond the direct effect of mate-guarding on male fGC levels, in our study, all males (i.e. mate-guarding and non-mate-guarding males) were in general more physiologically stressed during the mating periods than during the non-mating ones confirming previous finding in the same population (Girard-Buttoz et al. 2009). This pattern is in line with many studies in vertebrates that found a clear

rise in glucocorticoid levels during the reproductive period (Tokarz et al. 1998; Barrett et al. 2002; Moore & Jessop 2003; Mooring et al. 2006; Fichtel et al. 2007; for a review see Romero 2002). Interestingly, being at the top of the dominance hierarchy appears to be physiologically stressful for male long-tailed macaques independently of competition for access to females. In our study, alpha males had on average higher fGC levels than other males in the group during but also outside of the mating season. A similar finding has been recently shown for savannah baboons (Gesquiere et al. 2011). Alpha male long-tailed macaques maintain their rank through contest competition (van Noordwijk & van Schaik 1985) and face the risk of rank challenges year-round. In contrast, in species in which males attain high dominance status through succession, such as rhesus macaques (Berard 1999), dominance rank influences fGC levels only during a period of the reproductive season with an unstable dominance hierarchy (Higham et al. 2013). These differences illustrate how the process of rank achievement can modulate the relationship between dominance rank and stress hormone levels in primates and potentially in other group living mammals as well.

The potential physiological and physical costs of mate-guarding and the cost of being alpha-male per se may altogether explain, at least partially, the deviation from the PoA model observed in long-tailed macaques (Engelhardt et al. 2006). Whereas short term increases in glucocorticoid levels during mate-guarding is most likely a proximate adaptive mechanism favouring the maintenance of a balanced energetic status (**chapter 3**, see also discussion above), long-term exposure to high cortisol levels can be highly deleterious for the males. Chronic stress may suppress the immune system (Grossman 1985; Setchell et al. 2010) and testicular function (Sapolsky 1985; Hardy et al. 2005) and hence affect males' health and ability to reproduce. In long-tailed macaques, the need for the males to prevent the detrimental effects of aggression and exposure to chronic stress may prevent them from mate-guarding all the females in a group even when their fertile phases do not overlap (Engelhardt et al. 2006).

The possibility for the alpha male to monopolise as many females as expected by the PoA model and/or the need to limit his monopolisation potential to certain females may depend on the degree of reproductive seasonality in primates. In long-tailed macaques the timing of female fertility is unpredictable and females can potentially cycle year round (van Schaik & van Noordwijk 1985). Males thus face a high risk of exposure to chronic stress since, in order to monopolise access to all females, they would have to mate-guard females over extended periods of time. Mountain chacma baboons, in contrast, live in a seasonal and predictable environment. Conceptions are clustered during the first half of the year and males mate-guard females to the extent predicted by the PoA model (Weingrill et al. 2000). In this species, males do not seem to be limited in their monopolisation

potential even though they bear the cost of elevated fGC levels during mate-guarding (Bergman et al. 2005).

Given the strong effect of fruit availability on fGC levels in our study males, ecological factors may in addition play an important role in male mate-guarding decisions and may further explain the deviation from the PoA model. In order to prevent the exposure to chronic stress, the males may need to stop mate-guarding females in periods of food shortage (i.e. when their fGC levels are naturally high). Such a phenomenon has been described in other taxa: food availability influenced the decision to engage or not in costly courtship and/or mate-guarding for example in crabs (Kim et al. 2008) and fish (Kolluru et al. 2009). The influence of food availability on mate-guarding decisions in male long-tailed macaques remains to be investigated but may be challenging to assess under natural conditions.

Our study shows that male long-tailed macaques may endure physiological (in the form of exposure to chronic stress) and potentially also physical (in the form of increased aggression and associated injuries) costs of mate-guarding. Even though the rise in cortisol most likely serves an adaptive proximate function – i.e. reallocating resources during mate-guarding - it may, ultimately limit male mate-guarding abilities. We suggest that the degree to which these costs of mate-guarding act to limit male monopolisation potential in different species depends on the species' reproductive seasonality. Males of seasonally reproducing species can most likely afford to engage fully in stressful, aggressive male-male competition and female guarding over a short period of time without facing the high risk of exposure to chronic stress. In contrast, males of species with highly unpredictable timing of reproduction are more likely to face long-term exposure to physiological stress and may thus have evolved an "incomplete female monopolisation strategy" in order to avoid this cost. We therefore encourage future model developers to incorporate physiological costs of mate-guarding into reproductive skew models and to take into account the extent to which reproductive seasonality and rank achievement modes influence the interplay between costs of mate-guarding, dominance and male monopolisation potential.

Chapter 5

Costs of and investment into mate-guarding in wild long-tailed macaques: the impact of female characteristics and male-female social bonds

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Abstract

Male primates living in multi-male groups tend to exert mate and mate-guarding choices towards females of high reproductive value (i.e. high ranking parous females) or females with whom they share strong bonds with. Little is known however about the constraints which may limit male mate-guarding choices, (e.g. the costs of this behaviour) and the influence of the females' quality on the males' investment into mate-guarding. The aim of our study was to investigate the effect of female rank, parity status and male-female social bond strength on a male's costs of and investment into mate-guarding. The study was carried out during two reproductive seasons on three groups of wild long-tailed macaques in Indonesia. We combined behavioural observations on males' locomotion and activity, and non-invasive measurements of faecal glucocorticoids (fGC). Males fed less time when guarding nulliparous females and tended to have higher fGC levels when guarding low-ranking nulliparous ones. Evolution should thus favour male choice towards monopolising high-ranking parous females since such a decision brings benefits at proximate (reduced costs of mate-guarding) and ultimate (higher reproductive value) levels. Furthermore male's investment into mate-guarding was flexible and contingent on female reproductive and social value. Males were more vigilant and more aggressive towards other males when guarding females with whom they were strongly bonded and/or high ranking ones. Our findings bring a new dimension to the study of mate-choice by showing that males not only mate preferentially with high quality females but may also aim at better securing paternity with these females through optimised monopolisation.

Introduction

The study of mate-choice traditionally focused on females since they are usually the sex investing the most into reproduction and hence should be more choosy in regard to their mating partners (Trivers 1972; Andersson 1994). Variability in the quality of available females and the costs of mating per-se may, however, favour the evolution of mate-choice in males as well, even in species with no sex-role reversal (Kokko & Monaghan 2001; Edward & Chapman 2011). In particular, male mate-choice is particularly likely to evolve when multiple females are available at the same time and when the rate of encounters with females is high (Kokko & Monaghan 2001) such as in many group living primates.

Several primate species have evolved a certain degree of male mate-choice to limit the costs of reproducing and allocate limited sperm resources towards the most valuable females (reviewed in Keddy-Hector 1992; Setchell & Kappeler 2003; Kappeler 2012). Male-male competition for access to mates including monopolisation of females is often costly for male primates since it may increase the risk of injury (e.g. Drews 1996) and affect a male's feeding time, energy balance or physiological stress levels (**chapter 4**; Alberts et al. 1996; Bergman et al. 2005; Georgiev 2012). In addition, successive ejaculations may impair sperm performance (e.g. Marson et al. 1989). Sperm is therefore a limited resource (Wallen 2001) and when several females are receptive simultaneously males may face a trade-off between current and future mating opportunities (Kappeler 2012). Males should therefore choose to compete for and mate with the females most likely to be fertile and to produce offspring surviving until the next generation (Setchell & Kappeler 2003). Indeed, males of several species have been observed to concentrate their mating effort on females during their conceptive cycles (e.g. chimpanzees, *Pan troglodytes schweinfurthii*, Emery Thompson & Wrangham 2008; and chacma baboons, *Papio hamadryas ursinus,* Weingrill et al. 2003) and to preferentially mate with high-ranking females (e.g. Barbary macaques, *Macaca sylvanus*, Kuester & Paul 1996; and long-tailed macaques, *M. fascicularis*, de Ruiter et al. 1994, reviewed in Berenstain & Wade 1983) more likely to produce offspring of better quality, surviving until adulthood and achieving a high rank position in the future (van Noordwijk & van Schaik 1999, 2001; Setchell et al. 2002; Robbins et al. 2011; Majolo et al. 2012). For similar reasons, males may mate more frequently with "experienced" parous females who already produced and successfully raised an infant than with nulliparous ones (e.g. mandrills, *Mandrillus sphinx,* Setchell 1999; and savannah baboons, *Papio cynocephalus*, Smuts 1985). Finally, in some species, male exhibit mating preferences towards females with whom they have strong social bonds, independently of female rank, parity or fertility status (e.g. rhesus

macaques, *M. mulatta*, Chapais 1983, Japanese macaques, *M. fuscata,* Takahata 1982; and savannah baboons, Smuts 1985).

Being a choosy male may particularly make sense in species in which males need to engage into costly female monopolisation (i.e. mate-guarding) over an extended period of time in order to secure paternity (reviewed in Manson 1997). While mate-guarding significantly increases mating/reproductive success of male primates (rhesus macaques, Berard et al. 1994; Bercovitch 1997, long-tailed macaques, de Ruiter et al. 1994; Engelhardt et al. 2006; Japanese macaques, Matsubara 2003; mandrills*,* Setchell et al. 2005) this behaviour also entails certain costs in at least some species. In a number of baboon and macaque species, mate-guarding led to a reduction in male-feeding time (**chapter 3**;Packer 1979; Rasmussen 1985; Alberts et al. 1996; Matsubara 2003). Males may also face physiological constrains during mate-guarding. Male long-tailed macaques and chacma baboons have higher faecal glucocorticoid levels (a marker of physiological stress) when mate-guarding than when not mate-guarding females (**chapter 4**; Bergman et al. 2005). These costs may, however, vary depending on which female is mate-guarded. It is known for example that high-ranking females travel less distance than low-ranking females, have priority of access to high quality food and face less risk of predation since they spend usually more time in the core of the group (van Noordwijk & van Schaik 1987; Ron et al. 1996; Saito 1996; Vogel 2005). These benefits of being a high-ranking female are likely to translate into a reduction of the costs of mate-guarding for the males since during mate-guarding, males adjust their activity, locomotion and spatial positioning to the guarded female. At the same time males may face additional costs stemming from increased male-male competition associated with the reproductive value of high-ranking females.

In addition to female reproductive value, male-female social bonds are also likely to impact the costs of mate-guarding since they can affect females' cooperation during mate-guarding (e.g. yellow baboons, Rasmussen 1980) which may in turn reduce the costs of monopolisation. Concurrently, males may invest more energy and mate-guard more thoroughly females with whom they are strongly bonded in order to maintain the direct fitness benefit of long-term male-female social bond (Kulik et al. 2012; Massen et al. 2012). However, to our knowledge, no study ever empirically tested if and how female identity impact the costs of and the investment of males into mate-guarding.

In our study we therefore aimed at quantifying the influence of female characteristics (rank and parity status) and male-female social bonds on the costs of mate-guarding in male long-tailed macaques. In this species, male reproductive success is highly skewed towards the alpha male (de Ruiter et al. 1994; Engelhardt et al. 2006). Yet high ranked males mate-guard females to a lower extent than predicted by the Priority of Access model (Altmann 1962; Engelhardt et al. 2006). Since

males are able to discern a female's fertile phase (Engelhardt et al. 2004), we suggest that this lower than expected degree of alpha male monopoly derive from behavioural or social constraints associated with the costs of mate-guarding (**chapter 4**). In previous studies, we found that mate-guarding impacts males' behaviour and physiology in long-tailed macaques. Males fed less time, climbed less distance, received more aggression and were more vigilant and exhibited higher levels of stress hormones while mate-guarding females than when not (**chapter 3** and **4**). Male energetic status (assessed through urinary C-peptide measures, **chapter 2**) was, however, not affected (**chapter 3**). Even though we documented some costs of mate-guarding in long-tailed macaques, it remains unclear in how far the magnitude of these costs varied across the guarded females. In this species top-ranking males concentrate their mate-guarding effort on high-ranking and parous females (de Ruiter et al. 1994; Engelhardt et al. 2006) since they produce better quality offspring (van Noordwijk & van Schaik 1987). In how far this choice is based on differences in costs during mate-guarding remains, however, so far unclear.

In the current study we thus first investigated the effect of female characteristic and male-female social bonds on behavioural and physiological parameters which have been shown to be affected by mate-guarding – i.e. feeding time, climbing distance and physiological stress levels (**chapter 3** and **4**). Secondly, we examined whether males were more thorough and more physically engaged when mate-guarding female of high reproductive value or with whom they were closely bonded. We quantified, during mate-guarding, the investment of males into 1) aggressions towards other males, 2) distance maintained with the guarded female and 3) time being vigilant.

Methods

Animals and study site

The study was carried out on three groups of wild long-tailed macaques (*Macaca fascicularis*) living in the primary lowland rainforest surrounding the Ketambe research Station (3º41'N, 97º39'E), Gunung Leuser National Park, North-Sumatra, Indonesia. The forest structure and phenological composition has been described in detail by Rijksen (1978) and van Schaik and Mirmanto (1985). The long-tailed macaques in the area have been studied since 1979 (van Schaik & van Noordwijk 1985; de Ruiter et al. 1994; Engelhardt et al. 2004). For our study we collected data between March 2010 and April 2011 focusing on three groups: Camp (C), Ketambe Bawa (KB) and Ketambe Atas (KA). Faecal samples and behavioural data were collected during two consecutive mating periods. A mating period was defined as the period between the first mate-guarding day and the last mate-guarding day observed in any of the three groups, by any male (see below for definition of mate-guarding). All adult

individuals were individually known and well habituated to human observers. The total size of the social groups varied from 22 to 58 individuals (for details, see **chapter 3**). Between January and April 2011, four males travelled back and forth between the groups KA and KB and associated with one of the groups for periods between a few hours up to 3 weeks before travelling back to the other group.

The study was conducted completely non-invasively and under the permission of the authorities of Indonesia. We adhered to the Guidelines of the Use of Animals in Research, the legal requirements of Indonesia and the guidelines of the involved institutes.

Behavioural data collection

Behavioural data were collected by C.G-B and six experienced Indonesian and international field assistants. All assistants were trained by C.G-B and inter-observer reliability was assessed repeatedly (measurement of agreement kappa > 0.8 for each assistant and for all behaviours). The observations covered two mating periods for two groups (C and KB) and one mating period for the third group (KA). From March to July 2010, four observers followed groups C and KB every day and from December 2010 until April 2011 all three groups were generally followed every other day and frequency of observations increased to every day when alpha and/or beta males were observed mate-guarding. Each day, groups were followed from dawn to dusk. We focused our behavioural observations on alpha and beta males because they are known to mate-guard females most extensively (Engelhardt et al. 2006). Alpha and beta males of each group were the focal animals for half or entire days depending on the number of observers available. The activity of the focal animal was recorded every minute using instantaneous sampling (Altmann 1974) and comprised the following categories: resting, being vigilant (monitoring the surrounding environment by looking in different directions, being either still or moving, and while not involved in feeding or social activity), feeding (handling and consuming food), drinking, travelling (continuous locomotion during at least one minute with no foraging activity and no social interactions), aggressing, affiliating (including copulation), grooming, self-grooming. The canopy height (six categories: **0**: focal animal on the ground, **1**: 1-5 m; **2**: 5-10 m; **3**: 10-15 m; **4**: 15-20 m; **5:** 20-25 m; **6**: > 25 m), the mate-guarding behaviour of the focal male and the distance between him and the mate-guarded female was also recorded every minute. Whether a male was mate-guarding or not a female on a given minute was coded *a posteriori*. A male was considered as "mate-guarding" when he followed a sexually active female for more than 5 consecutive minutes and maintained a distance of less than or equal to 10m between him and the female. A female was considered sexually active if she was observed copulating at least once on a given day. If the female moved away from the male and the male did not follow her for more than 2 minutes the mate-guarding activity was considered to have ended. "Mate-

guarding days" refers to the days during which the male mate-guarded females for at least 25% of the observation time. In addition, all copulations and aggressions (including submissive expressions) between any adult individuals were recorded (all occurrence sampling for the focal male and *ad libitum* for all the other individuals). Aggressions comprised threatening, chasing, hitting and biting. We also recorded approaches (defined as one individual entering within 1m radius of another individual) between the focal male and any other adult individual in the group. Finally, the identities of all adult males within 10 m of the focal individual were recorded every 5 minutes.

Vertical travelling distance

In order to calculate the vertical distance travelled, we first used the centre of each height category as an estimate for the height at which the male was at each minute-scan-point (e.g. 7.5m for category 2 or 12.5m for category 3). Subsequently we calculated the height difference between each minute-scan-height estimate.

Determination of female dominance hierarchy and parity status

During the focal protocols any agonistic interaction and the occurrence of 'bared-teeth-face', a unidirectional submissive display (van Hooff 1967) between any adult member of the groups were recorded *ad libitum* (Altmann 1974). The dominance hierarchy for females was built based on 488 dyadic aggressive or submissive interactions (312, 132 and 64 in groups C, KA and KB respectively) where a clear winner and loser could be identified. The agonistic interactions used in this analysis were chase and displacement. Winner and loser were then entered into a sociometric matrix and dominance ranks were compiled with Matman 1.1.4 using the I&SI method (de Vries 1998). Landau's corrected linearity indexes were 0.54, 0.89 and 0.74 and the percentages of unknown relationships were 32.4%, 19.1% and 33.3% in group C, KA and KB. Prior to statistical analysis (see below) female ordinal rank was standardised to a mean of 0 and a standard deviation of 1 in each group to obtain a range of values comparable between the three groups containing different number of females (*Table 5.1*).

The parity status of the female was assessed visually based on the size of the nipples. In long-tailed macaques nulliparous female nipples are similar to male's nipples and distinctively shorter than parous female nipples.

Table 5.1: Observation time on mate-guarding days, number of faecal samples measured and characteristics of the guarded females for each of the study males.

Group	**Camp**		**Ketambe Atas**		**Ketambe Bawa**	
Male rank	α	β	α	β	α	B
Number of mating periods	2	2	1	1	2	2
Focal observation time on MG days (h)	253.6	73.6	118.4	75.3	160.7	51.6
Number of MG days of observation	47	15	39	31	45	15
Number of faecal samples	32	6	24	16	26	9
Number of adult males in the group	6-9	6-9	4-7	4-7	4-8	4-8
Number of adult females in the group	14-15	14-15	7	7	9-10	9-10
Number of females mate-guarded	6	8	4	3	7	6
Number of nulliparous females guarded	1	1	1	0	5	4
Range of guarded female ranks	1-15	4-15	1-7	1-4	1-9	1-9

"MG" refers to mate-guarding.

Determination of male-female social bond

Dyadic male-female social bond strength was measured using an approach inspired from the calculation of the "composite index of sociality" (Silk et al. 2006). However, since we did not collect focal behavioural observations on all the males present in each of the groups we could not compute a "composite index of sociality". Instead, we used the number of approaches and grooming time to calculate a "male-centred" association index (hereafter AI) between males and females. The AI was computed for each male-female dyad as follow: $AI = [(G_{ij}/G_{ix})+(A_{ij}/A_{ix})]/2$ where G_{ij}/G_{ix} is the grooming time of male i with female j (G_{ij}) relative to the total grooming time of male i with all females in the group (G_{ix}). Similarly A_{ij}/A_{ix} is the number of time male i approached or was approached by female j (A_{ij}) relative to the total number of time male i was approached or approached all females in the group (A_{ij}). In order to obtain a measure independent of male mate-guarding activity, we used only data collected on days during which the male did not mate-guard females at all.

Determination of fruit availability

Fruit availability is known to significantly affect feeding time, distance climbed and glucocorticoid levels in male long-tailed macaques (**chapter 3** and **4**). It was therefore important to control for this

parameter in our analysis and we monitored fruit trees to assess fruit availability. In each of the three studied groups, 40 locations, covering the entire home ranges, were randomly selected (120 locations in total over the three home ranges). At each location, three trees were randomly selected from three different species among the tree species producing fruit eaten by *M. fascicularis* (Ungar 1995). In total 360 trees, from 87 different species were selected (120 trees for each group's home range). Each tree was surveyed monthly, within the last 3 days of every month, by a field assistant experienced in phenology and fruit abundance was recorded using a logarithmic scale (**0**: absence, **1**: 1-10 items, **2**: 11-100, **3**: 101-1000, **4**: 1001-10000 and **5**: > 10000). The average monthly score of fruit abundance in each territory was highly correlated with the percentage of trees fruiting. For the analyses, we therefore used percentage of trees fruiting as an index of fruit availability. This index varied between 6.8 and 30.9%. During the mating periods the fruit availability was 71.4% of the time below the mean of the range of the index - i.e. 15 out of the 21 group-months had an index below 18.9%.

Faecal sample collection and hormone analysis

During mate-guarding periods, we collected faecal samples every third day from the mate-guarding male. Right after defecation, samples were homogenised and 2-3 g of faeces were collected and stored in a polypropylene vial and placed on ice in a thermos bottle. At the end of each fieldwork day, the samples were frozen at -20°C in a freezer. In July 2011, all samples were transported, on ice, to the hormone laboratory of the Bogor Agricultural University (IPB) and then freeze-dried and pulverised before transportation to the Endocrinology Laboratory of the German Primate Centre for glucocorticoid (fGC) analysis.

For hormone analysis, an aliquot (50-70 mg) of the faecal powder was extracted within 3 ml of 80% methanol by vortexing for 10 min (Heistermann et al. 1995). For monitoring changes in fGC levels, faecal extracts were analysed for immunoreactive 11ß-hydroxyetiocholanolone (3α,11ß-dihydroxy-CM), a group-specific measurement of 5-reduced 3α,11ß-dihydroxylated cortisol metabolites (Möstl & Palme 2002; Ganswindt et al. 2003). The assay has been previously validated for assessing adrenocortical activity from faeces in long-tailed macaques (Heistermann et al. 2006). Hormone measurements were carried out by microtiter plate enzymeimmunoassay according to methods previously described (Ganswindt et al. 2003; Girard-Buttoz et al. 2009). Intra- and inter-assay coefficients of variation of high- and low- value quality controls were 8.9% and 9.9% (high) and 6.3% and 14.3% (low), respectively.

Statistical analyses

For all analyses, we considered only those days of observation for which at least 1 hour of focal data was recorded and the male mate-guarded a female for at least 25% of observation time. The day during which the male did not mate-guard the same female for at least 70% of its mate-guarding time as well as days during which females where mate-guarded after conception (see Engelhardt et al. 2007) were discarded from the analyses. We determined the likely date of conception for each new born in each group each year by counting back 163 days (average gestation length in this study population, Engelhardt et al. 2006) from the date of birth of each new born. Each mate-guarding day occurring more than 2 weeks after the likely date of conception was considered to be a post-conception day. For the females who did not conceive in a given year, every day was kept since they might have been potentially cycling at the time even if they did not conceive. The final data set comprised 733 hours of focal observations over 192 days (see details in *Table 5.1*).

Influence of female rank, parity status and male-female AI on the costs of mate-guarding

For each day, we calculated for the focal male the percentage of time spent feeding, grooming and being vigilant as percentage of the observation time. In addition, we determined the average canopy height difference (i.e. vertical locomotion) per minute (in meters). We also calculated the copulation rate (i.e. number of copulations between the focal male and any female per hour), the rate of male-male aggression (i.e. the number of aggressions between the focal male and any other adult male per hour), the number of males in proximity (defined as the average number of males within 10 m per 5 minute scan) and the number of sexually active females in each group on each observation day. We assessed male's stress hormone levels (fGC measures) on days for which we had matching faecal samples. Since the time-lag for excretion of glucocorticoid metabolites into the faeces is on average 36 h in long-tailed macaques (Heistermann et al. 2006), we matched behavioural observations with fGCs levels measured in samples collected at either day +1 or day +2 after the observations. When samples were available at both days, we used the mean fGCs levels of the two samples.

In previous studies we found that mate-guarding affect the males feeding time, climbing distances and fGC levels (**chapter 3** and **4**). In the present study we wanted to assess in how far female characteristics and male-female social bonds affected these parameters. We used generalised linear mixed models (GLMM, Baayen 2008) to test whether the rank of the guarded female, her parity status and male-female AI had an effect on a male's 1) feeding time (Model 1), 2) climbing distance (Model 2), and 3) fGC levels (Model 3). The structure of each model is summarised in *Table 5.2*. The fGC level values were log-transformed and climbing distances were power transformed (*^0.7) to achieve a symmetric distribution. We used a Gaussian error structure in the models. Since fruit

availability significantly affects feeding time, distance climbed and fGC levels in our study males (**chapter 3** and **4**) we included fruit availability as control factor in each model. Fruit availability on a given day was approximated using the fruit availability measured on the closest monthly record (details in **chapter 3**). We also included percentage daily mate-guarding time (as percentage of observation time) and number of females in the group as control factors. In addition, in Model 3, we also included as control factors variables which are known to affect fGC excretion in primates in general and/or in our population in particular (chapter 4;Ray & Sapolsky 1992; Warm et al. 2008; Cheney & Seyfarth 2009; Girard-Buttoz et al. 2009), i.e. male-male aggression rate, copulation rate, grooming time, number of male in proximity, the number of sexually active females and the interaction between vigilance time and mate-guarding time.

Since female reproductive quality is a combination of female parity status and rank we tested the significance of the interaction between parity status and rank in all of the three models. This interaction was not significant in Model 1 and 2 (likelihood ratio test (LRT), $P > 0.1$) but there was a trend towards significance in Model 3 (LRT, $P = 0.055$). To assess whether ecological factors modulate the relationship between costs of mate-guarding and female rank, parity status and male-female AI, we also tested for the significance of the interaction between these three parameters and fruit availability in each model. Only the interaction between male-female AI and fruit availability was significant in Model 1 and 2 (LRT, $P < 0.05$).

Influence of female rank, parity status, and male-female AI on the investment of males into mate-guarding.

In a second set of models we aimed at assessing whether males are more motivated and engage in more costly behaviours when they mate-guard females of high reproductive values (i.e. high-ranking parous females) or with whom they are closely bonded (high AI). We used GLMMs to analyse the effect of female rank, parity status and male-female AI on 1) likelihood of severely aggressing other males (Model 4), 2) distance to the mate-guarded female (Model 5), and 3) vigilance time (Model 6). The structure of each model is summarised in *Table 5.2*. Severe aggressions comprised chase, hit and bite. The focal males did not severely aggress any male during over half of the observation days (101/192) and hence the resulting distribution of daily aggression given was highly zero inflated. Accordingly we could not run a model with a Gaussian error structure. We thus coded each day with at least one severe aggression given by the focal male towards any other male in the group as an "aggressing" day and other days as non-aggressing days. We used a model with a binomial error structure to test the influence of mate-guarding on the likelihood of aggression with other males on a given day (model 4). For the other models we used a Gaussian error structure since the response

variable was symmetrically distributed. The likelihood of recording severe aggression given on a given day being dependent on the observation time that day, we included observation time (in minutes) as a control factor in model 4. In long-tailed macaques, fruit availability affects male locomotion (and hence also potentially the distance a male maintains with the female during mate-guarding), the trade-off between vigilance and feeding (**chapter 4**) and potentially also the degree of male-male competition for access to food. Similar to Models 1-3 we thus included this parameter as a control factor in Models 4-6. Finally, since the investment of males into mate-guarding can be modulated by socio-sexual context and degree of male-male competition we also included number of sexually active females on a given day and number of males in proximity (see definition above) as control factors into these models.

Table 5.2: Structure of models 1-6.

	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**
	Feeding time	Climbing distance (^0.7)	Log (fGC levels)	Aggression given (Y/N)	Distance to the female	Vigilance time
Fixed factors	-female rank	-female rank	-female rank	-female rank	-female rank	-female rank
	-female parity status	-female parity status	-female parity status	-female parity status	-female parity status	-female parity status
	-male-female AI	-male-female AI	-male-female AI	-male-female AI	-male-female AI	-male-female AI
	-male-female AI *fruit availability	-male-female AI *fruit availability	-female rank *female parity status		-male-female AI *fruit availability	-male-female AI *fruit availability
	-fruit availability	*-fruit availability*	*-fruit availability*	*-fruit availability*	*-fruit availability*	*-fruit availability*
	-N. females in the group	*-N. females in the group*	*-N. females in the group*	*-N. females in the group*	*-N. females in the group.*	*-N. females in the group*
	-mate-guarding time	*-mate-guarding time*	*-mate-guarding time*	*-mate-guarding time*	*-mate-guarding time*	*-mate-guarding time*
			-N. males in proximity	*-N. males in proximity*	*-N. males in proximity*	*-N. males in proximity*
			-N. sexually active females	*-N. sexually active females*	*-N. sexually active females*	*-N. sexually active females*
			-Copulation rate			
			-Grooming time			
			-vigilance time			
			*-vigilance*mate-guarding time*			
				-Observation time		
Random factors	-male ID	-male ID	-male ID	-male ID	-male ID	-male ID
	-guarded female ID	-guarded female ID	-guarded female ID	-guarded female ID	-guarded female ID	-guarded female ID
	-group	-group	-group	-group	-group	-group

The control fixed factors are indicated in italic

In this set of models we also tested for the significance of several interactions: 1) the interactions between fruit availability and female rank, parity status and male-female AI to assess whether ecological factors modulate the relationship between these three parameters and male investment during mate-guarding and 2) the interaction between female rank and parity status for the same rational basis as in Models 1-3. The interaction between male-female AI and fruit availability was significant in Model 5 (LRT, $P = 0.024$) and revealed a trend towards significance (LRT, $P = 0.072$) in Model 6. All other LRT tests revealed $P > 0.1$.

For all models (Models 1 to 6) all interactions with $P < 0.1$ for the LRT were kept in the final models. Finally, in addition of all the fixed factors, male ID and group were included in all models as nested random effects.

Models fitting and assumptions' checking

Each model was fitted in R 2.15.0 (R Development Core Team 2012) using the function lmer of the R-package lme4 (Bates & Maechler 2010).

In each model, we checked that the assumptions of normally distributed and homogeneous residuals were fulfilled by visually inspecting a qqplot and the residuals plotted against fitted values. We checked for model stability by excluding data points one by one from the data and comparing the estimates derived with those obtained for the full model. In some models we identified influential cases which rendered the model unstable. We then reran the models without these particular data points. If the results were similar with or without influential cases we present the outcome of the model run on the full data set. If the results were different, we present the outcome of the models run with a reduced data set (i.e. not comprising influential cases). Variance inflation factors (VIF, Field 2005) were derived using the function vif of the R-package car (Fox & Weisberg 2010) applied to a standard linear model excluding the random effects. VIF's which are less than 5 indicate that covariation between the predictors is not a problem (Bowerman & O'Connell 1990; Myers 1990). In all our models VIF's were less than 2.2. The other diagnostics did not indicate obvious violation of the assumption.

For each model, we first determined the significance of the full model as compared to the corresponding null model (including all the factors except "female rank", "female parity status", "male-female AI" and the interactions) using a likelihood ratio test (R function anova with argument test set to "Chisq"). To achieve a more reliable p-value, we fitted the models using Maximum Likelihood rather than Restricted Maximum Likelihood (Bolker et al. 2009). Only if this likelihood ratio

test revealed $P < 0.1$ we considered the significance of the individual predictors. P-values for the individual effects were based on Markov Chain Monte Carlo sampling (Baayen 2008) and derived using the functions pvals.fnc and aovlmer.fnc of the R package languageR (Baayen 2010, number of simulations = 10000).

Results

Mate-guarding duration, female value and male-female AI.

All the males but one (α male of group KA) mate-guarded nulliparous and parous females and all the males but one (β male in group KA) mate-guarded females ranging from high to low-ranking (*Table 5.1*). Our data set is thus not biased towards certain males mate-guarding exclusively females with certain characteristics (i.e. high-ranking males mate-guarding only high-ranking or parous females).

Whereas in two groups (C and KA) males spent more time on mate-guarding parous than nulliparous females, in the third group (KB) it was the opposite (*Table 5.1*). The number of days a given male mate-guarded a given female was unrelated to its AI with the female or to the female's rank or parity status (*Figure 5.1*). Among all the females which were observed being mate-guarded, nulliparous females were guarded by a given male between 1 and 20 days and parous females between 1 and 28 days. Furthermore, several females with an AI with the guarding male above average (mean AI = 0.121, range = 0.015-0.350) were mate-guarded only for one or two days, whereas several females with AI with the guarding male below average were guarded for over 5 days or even for 14 days in the case of one female. Finally, a few low-ranking females were guarded for over 10 days whereas many high-ranking females were mate-guarded for only 1 or 2 days.

Influence of female rank, parity status, and male-female AI on the costs of mate-guarding

Feeding time (Model 1)

The null model was significantly different from the full model in Model 1 (Feeding time, $P = 0.006$, *Table 5.3*). Males spent more time feeding when mate-guarding parous females than when guarding nulliparous ones (N = 192 days, at reference level "parous" *estimate ± SE* = 5.79 ± 2.31, $P_{MCMC} = 0.023$, *Table 5.3*), but female rank did not affect a male's feeding time ($P_{MCMC} = 0.997$). A male's AI with the guarded female influenced his feeding time through an interaction with fruit availability ($P_{MCMC} < 0.001$, *Table 5.3*). When fruit availability was low, males fed less time while guarding females with whom they had a high AI. However, from a certain degree of fruit availability onwards the pattern was reversed and males spent more time feeding while mate-guarding these females when fruit availability was high (*Figure 5.2*).

Table 5.3: Results of the Likelihood-ratio-tests (LRT) run to compare full versus null models, estimates ± SE, t/Z-value and p-values for the GLMMs run to test the influence (during mate-guarding) of male-female AI, female rank and female parity status on male's 1) Feeding time (Model 1), 2) fGC levels (Model 3), 3) likelihood of aggressing other males (Model 4) and 4) vigilance time (Model 6).

	Model 1 Feeding time			**Model 3** fGC levels			**Model 4** Aggression given			**Model 6** Vigilance time		
N. of observation days	192			113			189			191		
Null vs. full model	*df*	χ^2	*P*	*df*	χ^2	*P*	*df*	χ^2	*P*	*df*	χ^2	*P*
	4	14.42	0.006	4	8.23	0.084	3	8.57	0.035	4	9.08	0.059
	Estimate±SE	*t*	P_{MCMC}	*Estimate±SE*	*t*	P_{MCMC}	*Estimate±SE*	*Z*	*P*	*Estimate±SE*	*t*	P_{MCMC}
Intercept	33.11±1.93	17.14	0.003	6.51±0.10	66.05	<0.001	0.47±0.46	1.02	0.308	44.27±1.34	33.01	<0.001
Male-female AI	In an Interaction			-0.02±0.05	-0.38	0.738	0.76±0.29	2.61	0.009	In an interaction		
Female rank	-0.06±1.02	-0.06	0.997	In an Interaction			-0.46±0.26	-1.77	0.077	-2.01±0.78	-2.58	0.048
Female parity status (parous)	5.79±2.31	2.50	0.023	In an Interaction			-0.54±0.58	-0.94	0.348	0.40±1.75	0.23	0.749
Fem. rank * Fem. parity				0.21±0.10	-2.17	0.071						
Assoc. index * Fruit avail.	3.07±0.88	3.50	<0.001							-1.57±0.75	-2.10	0.148
Fruit availability	In an interaction			-0.28±0.05	-5.83	<0.001	-0.38±0.26	-1.47	0.142	In an interaction		
Number of females	-2.80±1.37	-2.04	0.204	0.04±0.08	0.45	0.659	0.09±0.33	0.28	0.779	0.72±0.99	0.73	0.546
MG time	0.70±0.90	0.78	0.484	In an Interaction			0.14±0.21	0.67	0.504	1.13±0.78	1.46	0.335
N. males in proximity				-0.07±0.04	-1.64	0.132	1.23±0.27	4.49	<0.001	-1.60±0.81	-1.97	0.095
N sex. act. fem.				0.05±0.04	1.32	0.204	0.16±0.22	0.75	0.457	-0.98±0.79	-1.23	0.204
Copulation rate				0.01±0.04	0.15	0.889						
Grooming time				-0.02±0.04	-0.37	0.697						
Obervation time							1.60±0.34	4.75	<0.001			
Vigilance time				In an Interaction								
MG time * vigilance				-0.02±0.04	-0.59	0.606						

In this table only the models for which LRT to compare null vs. full models revealed P<0.1 are presented.

Climbing distance (Model 2)

In Model 2, the full model was not significantly different from the null model (N = 191 days, LRT, df = 4, χ^2 = 7.53, *P* = 0.110) indicating that neither female rank nor parity status nor male-female AI significantly affected male climbing distance.

fGC levels (Model 3)

In model 3 the LRT to compare the null and the full model revealed a trend toward significance *P* = 0.084 (*Table 5.3*). The AI between the male and the guarded female did not influence male's fGC levels (N = 113 days, *P* = 0.738). Female rank, however, did so in nulliparous females (interaction "parity status * female rank" close to significance, *P* = 0.071, *Figure 5.3*). Males had higher stress hormone levels when mate-guarding low-ranking nulliparous females than when guarding high-ranking nulliparous females (*Figure 5.3*). Finally fruit availability had a significant and negative effect on a male's fGC levels (*estimate ± SE* = 1.23 ± 0.27, P_{MCMC} < 0.001, *Table 5.3*). The more fruits were available the lower were the males´ fGC levels.

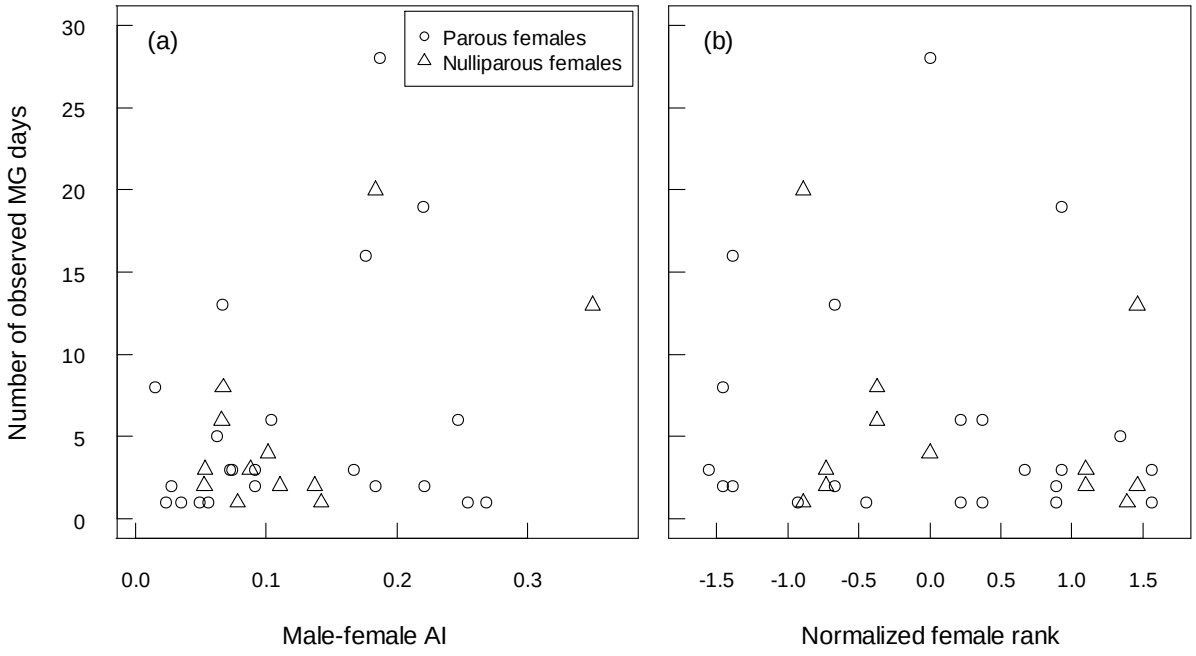

Figure 5.1: Number of days each female was observed being mate-guarded by a given male depending on male-female association index (a) and female rank (b).

Parous females are depicted with circles and nulliparous ones with triangles. Each point represents a given male-female guarding dyad.

Influence of female rank, parity status and male-female AI on male investment during mate-guarding

Aggression given (Model 4)

The null model was significantly different from the full model in Model 4 (P = 0.035, *Table 5.3*). The likelihood of aggressing other males was affected by male-female AI (N = 189 days, *estimate ± SE* = 0.76 ± 0.29, P_{MCMC} = 0.009, *Table 5.3*) and female rank (*estimate ± SE* = -0.46 ± 0.26, P_{MCMC} = 0.077, *Table 5.3*) but not by female parity status (P_{MCMC} = 0.348). Males were more likely to aggress other males when mate guarding high-ranking females and those with whom they had a high AI.

Distance to the female (Model 5)

In Model 5, the full model was not significantly different from the null model (LRT, df = 4, χ^2 = 6.44, P = 0.168) indicating that neither male-female AI nor female rank or parity status significantly affected male climbing distance during mate-guarding.

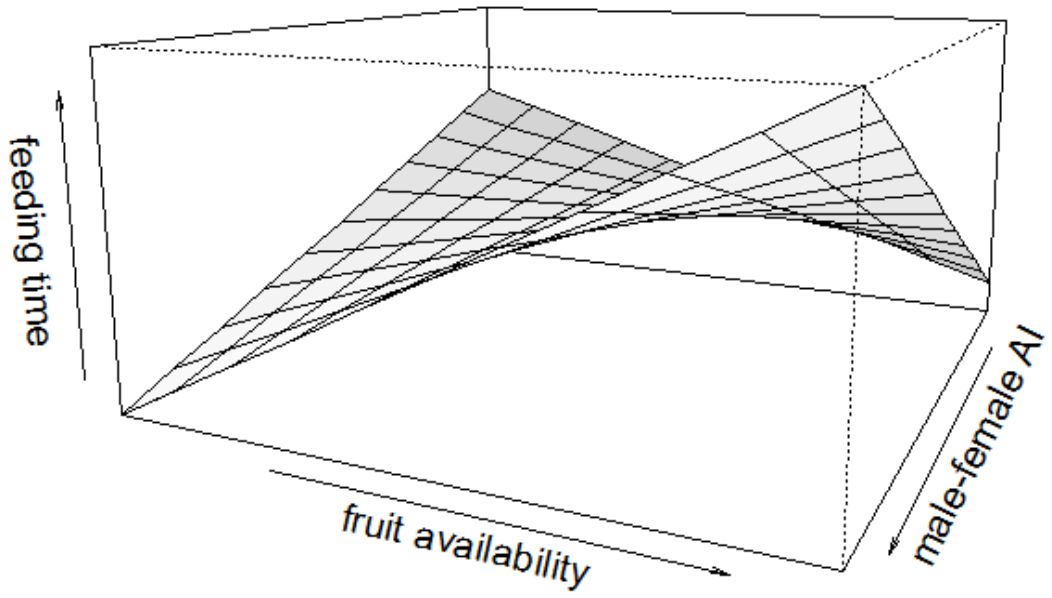

Figure 5.2: Effect of male-female Association Index (AI) and fruit availability on male feeding time.

The plane depicts values predicted by model 1.

Vigilance time (Model 6)

In model 3 the LRT to compare the null and the full model revealed a trend towards significance (P = 0.059, *Table 5.3*). A male's vigilance time during mate-guarding was significantly affected by female rank (N = 191, *estimate ± SE* = -2.01 ± 0.78, P_{MCMC} = 0.048, *Table 5.3*) but not by female parity status or male-female AI (both P > 0.15, *Table 5.3*). Males were more vigilant when mate-guarding high-ranking females than when guarding low-ranking ones.

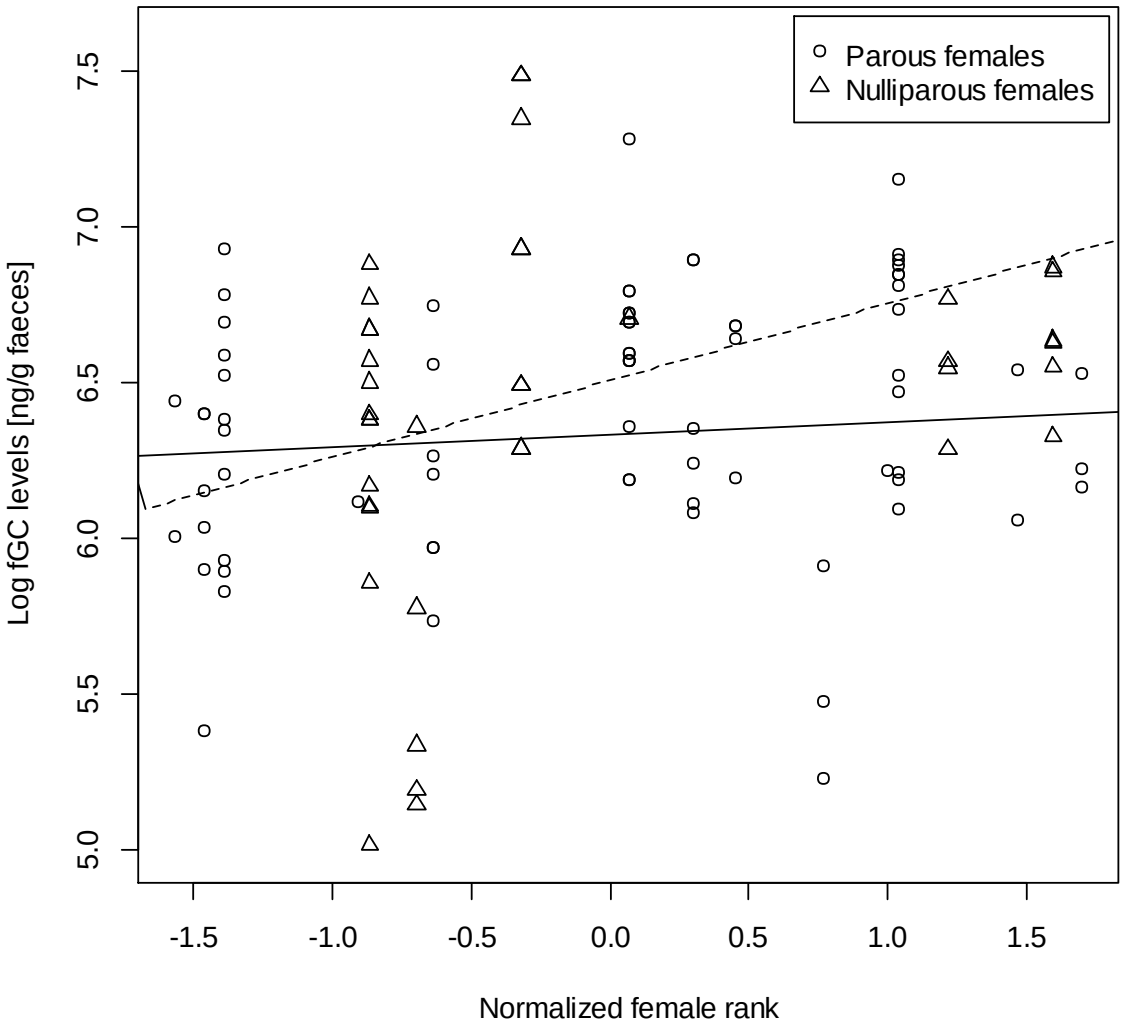

Figure 5.3: Effect of the guarded female's rank on male's fGC levels for nulliparous females (triangles) and parous females (circles).

The lines depict the linear relationship predicted by model 3 for nulliparous (dashed line) and parous females (solid line).

Discussion

Our results indicate that female rank, parity and the strength of male-female social bonds affect behavioural and physiological costs of mate-guarding and male investment into this behaviour in wild long-tailed macaques. Specifically, our results suggest that females of lower reproductive value (i.e. nulliparous and low-ranking females) might be more costly to mate-guard. Males fed less time when mate-guarding nulliparous than parous females and tended to be more physiologically stressed when guarding low-ranking nulliparous females than other females. Further, the male-female social bond strength (AI) influenced a male's feeding time during mate-guarding but this effect was dependent on fruit availability. When fruit availability was high, males fed longer while guarding females with whom they were strongly bonded; this pattern was reversed when fruit availability was low. Finally, males appear to invest more in females of high reproductive value or in females with whom they are strongly bonded. Males were more vigilant and aggressive when mate-guarding high ranking and/or females with whom they were strongly bonded (high AI) than when guarding other females.

While mate-guarding low-ranking nulliparous females, male long-tailed macaques, may face a twofold set of costs: 1) proximate behavioural and physiological costs related to variation in food intake and in physiological stress and 2) ultimate costs linked to the lower reproductive value of these females.

Feeding and stress related costs of mate-guarding have been reported in different primate species, including long-tailed macaques (Packer 1979; Rasmussen 1985; Alberts et al. 1996; Matsubara 2003; Bergman et al. 2005). Our study shows that male long-tailed macaques may have the opportunity to limit these costs by mate-guarding preferentially parous high-ranking females.

First, in our study, males fed more time when guarding parous than nulliparous females. Males may thus benefit in terms of balancing their energetic status from preferentially mate-guarding parous females. Furthermore, despite the absence of an effect of female rank on a male´s feeding time, males may also benefit from mate-guarding high-ranking females. Whereas male long-tailed macaques trade-off vigilance time against feeding time during mate-guarding in general (**chapter 4**), this might less be the case when they mate-guard high-ranking females. In fact, males were more vigilant when guarding high-ranking females than low-ranking ones but achieved this without reducing their feeding time. The absence/reduction of the trade-off between feeding and vigilance when guarding females of high rank might be related to the fact that high-ranking females have priority of access to high quality food patches (van Noordwijk & van Schaik 1987) from which guarding males may also benefit.. Consequently, males may mate-guard females of high reproductive

value more thoroughly by being more vigilant (see also below) without paying extra costs of reduced feeding time.

Second, our data suggest that the males may lower the risk of exposure to repeated elevation of physiological stress by preferentially mate-guarding high-ranking parous females compared to low-ranking nulliparous ones. This might be particularly important in a non-strictly seasonal species, such as long-tailed macaques, in which an increase in stress levels during mate-guarding (**chapter 4**) might have deleterious consequences. In this species, the timing of female fertility periods is unpredictable and females can conceive year round (van Schaik & van Noordwijk 1985). Furthermore, female fertile phases are usually asynchronous (Engelhardt et al. 2006). Therefore, by mate-guarding one female after the other, males may be exposed repeatedly to elevated stress levels and eventually face the risk of becoming chronically stressed (**chapter 4**). This might be highly costly for the males since chronic stress may suppress the immune system (Grossman 1985; Setchell et al. 2010) and testicular function (Hardy et al. 2005; Sapolsky 1985) and hence affect males' health and ability to reproduce (but see Boonstra 2013).

In long-tailed macaques and other primate species, low-ranking and/or nulliparous females produce offspring which are less likely to survive until adulthood and to achieve a high rank position in the future than offspring of high-ranking and/or parous females (Bercovitch et al. 1998; van Noordwijk & van Schaik 1999, 2001; Setchell et al. 2002; Robbins et al. 2006, 2011; Majolo et al. 2012). Evolutionary pressures at both proximate and ultimate levels may therefore enhance male mate-guarding choice towards high-ranking parous females which appear to be less costly to monopolise and are likely to achieve a higher reproductive success. In view of this, it is surprising that in our study males did not obviously choose females of high reproductive value as preferred mate-guarding partner. This pattern contrasts, at least partially, with results from a previous study on the same population (Engelhardt et al. 2006) which found that higher-ranking females were mate-guarded longer by the alpha male during their fertile phase than lower-ranking ones. In this study, female fertile phases did not overlap, however the study group comprised of only 8 females. Our study groups had up to 15 females and thus the likelihood of temporal overlap of fertile phases is much greater. However, we did not have the logistical power to collect regular faecal samples from all the females of all the study groups so as to be able to assess temporal overlap of female fertile phases. We may therefore not draw definite conclusion regarding male mate-guarding choice. For example it may well be that, when two females are fertile at the same time, high-ranking males mate-guard the high-ranking/parous ones preferentially.

Beyond the time spent mate-guarding different females, the choosiness of males towards certain females may also be expressed at the level of the investment and thoroughness with which males mate-guard the females. We found that males were more aggressive and more vigilant when mate-guarding high-ranking females than when guarding low-ranking ones. These two parameters may reflect an active and conscientious decision of the males to enhance the efficiency of monopolising females of high rank and thus higher reproductive value. In this respect, our study males follow patterns described in long-tailed macaques and other species of primates with male mating and/or mate-guarding preference towards higher-ranking females (de Ruiter et al. 1994; Kuester & Paul 1996; Engelhardt et al. 2006; Setchell & Wickings 2006). By aggressing other males, the guarding male may face counter-aggressions and is thus exposed to a higher risk of injuries (Clutton-Brock et al. 1979; Blanchard et al. 1988; Drews 1996). Being injured may in turn prevent high-ranking males from guarding current or subsequent fertile females and/or to maintain their hierarchical status (Drews 1996, personal observations). Given the strong link between rank and reproductive success in male long-tailed macaques (de Ruiter et al. 1994; Engelhardt et al. 2006) males may be willing to face the risk of aggressive retaliation, and the likelihood of associated deleterious consequences, only to monopolise females of high reproductive value.

In addition to enhancing the efficiency of monopolisation, being more vigilant while mate-guarding high-ranking females may also partially modify male physiological stress levels. In a previous study we found that being vigilant during mate-guarding reduces male physiological stress levels (**chapter 4**). This may explain our finding of lower glucocorticoid levels when males were guarding high-ranking compared to low-ranking nulliparous females.

In addition to female rank and parity status, male-female social bonds also affected costs of mate-guarding and male investment into this behaviour. In our study, the strength of male-female social bonds affected the feeding time of the guarding male, although this effect was contingent on fruit availability. In periods of high fruit availability, males benefited from being strongly bonded to the female since they fed more when the association index with the guarded female was higher. In this context, an increased feeding time may stem from a higher cooperation of the female. It has in fact been suggested and reported that male-female friendship influences female cooperation during mate-guarding in primates (Rasmussen 1980; Smuts 1985). In contrast, when fruit availability was low, males fed less time when mate-guarding females with whom they were strongly bonded. This may indicate a higher degree of investment of the males into mate-guarding strongly bonded females. In the context of fruit scarcity, males may face a trade-off between feeding long enough to meet their energetic requirement and being able to thoroughly mate-guard females. Males may

therefore, under such conditions, thoroughly mate-guard only the female with whom they are strongly bonded at the costs of reduced feeding. In contrast, if the female is not strongly bonded to him, the male may favour energetic needs over mate-guarding investment and relax mate-guarding attention to feed longer. The fact that males were also more aggressive when mate-guarding females with whom they are strongly bonded supports the evidence of their increased investment in these females.

Mate-guarding females more thoroughly and preventing other males from accessing them more aggressively might be a mechanism for the guarding male to maintain strong social bonds with certain females. In turn, this bond may have direct fitness benefit for the males, as shown in rhesus macaques (Kulik et al. 2012; Massen et al. 2012). In long-tailed macaques, females are not fully monopolisable since females can gain copulations with non-mate-guarding males (de Ruiter et al. 1994). Accordingly, cryptic post-copulatory female choice has been hypothesized to play a role in offspring paternity (Engelhardt et al. 2006, see also Kappeler 2012 for possible mechanisms). It may therefore be of high importance for males, even high-ranking ones, to maintain strong social bonds with certain females to enhance their probability of post-copulatory sperm selection.

Our study brings a new dimension to the study of mate-choice in primates (Keddy-Hector 1992; Setchell & Kappeler 2003; Kappeler 2012) by showing that males might be constrained in their mate-guarding choices by both social and ecological factors. Further, we showed that beyond differential time allocation of mate-guarding (Engelhardt et al. 2006; Setchell & Wickings 2006), males may express some preference towards highly valuable females (i.e. with high reproductive potential or those to which they are closely bonded) by investing more into aggression and vigilance while mate-guarding these females. A male may thus not only mate preferentially with the most valuable females (reviewed in Setchell & Kappeler 2003; Kappeler 2012) but also aim at better securing paternity through optimised monopolisation (our study). Our findings support mathematical modelling and evolutionary theories (Kokko & Monaghan 2001; Edward & Chapman 2011) predicting that when females vary in quality (van Noordwijk & van Schaik 1999, 2001) and the access to and monopolisation of females is costly (**chapter 4**), male may express choosiness towards certain females. Future studies should investigate how, at a given point in time, males adjust their mating and/or mate-guarding decisions depending on the interplay between their physiological and physical conditions, the food available and the quality and diversity of females in their fertile phase. Such studies will require hormonal assessment of female fertile phase (Heistermann et al. 2001; Engelhardt et al. 2004; Dubuc et al. 2012; Young et al. 2013a) but also the use of non-invasive physiological markers of male body condition and energetic status, such as urinary C-peptides (Sherry

& Ellison 2007; Deschner et al. 2008; Emery Thompson & Knott 2008; Emery Thompson et al. 2009) or stable isotopes (Deschner et al. 2012).

Chapter 6

General discussion

The overall aim of this thesis was to broaden our understanding of the factors constraining male reproductive decisions and female monopolisation potential by quantifying a variety of possible costs of mate-guarding for males. I combined behavioural measurements of aggression rates and energy intake and expenditure with physiological measures of energetic status and stress levels to derive a comprehensive picture of the costs of female monopolisation in a wild mammalian species, the long-tailed macaque.

In the following discussion, I will first summarise the results of this thesis (*section 6.1*). I will then highlight the influence of ecological factors on male reproductive effort (*section 6.2*) and discuss how males may finance the costs of reproduction through different energy management strategies (capital vs. income breeder, *section 6.3*). In the following section I will show that, male reproductive effort is not necessarily isolated to the reproductive period and may be expressed year round through social interactions and competition for dominance rank (*section 6.4*). Finally I will discuss the link between energy management strategies, costs of mate-guarding and male reproductive skew in long-tailed macaques within the framework of reproductive skew theories (*section 6.5*) before providing a brief general conclusion and outline some ideas for future research (*section 6.6*).

6.1 Summary of results

In **chapter 2,** I validated the suitability of urinary C-peptide (UCP) as a reliable non-invasive marker of inter- and intra-individual variation in energetic status and body condition in macaques. This provides the first validation of the usefulness of this marker in a non-hominid primate and was an essential prerequisite to study the energetic costs of mate-guarding in male long-tailed macaques. The main results of **chapter 3** and **4** are summarised in *Figure 6.1*. In these chapters, I found that mate-guarding, but also fruit availability, had significant effects on several behavioural and physiological parameters in high-ranking males (*Figure 6.1*). In **chapter 3,** I focused on the energetic costs of mate-guarding and showed that, in long-tailed macaques, mate-guarding reduced a male's feeding time, percentage of fruit in the diet and height climbed (*Figure 6.1*). However, mate-guarding had no significant effect on a male's horizontal distance travelled or restlessness (*Figure 6.1*). Altogether, I could not detect any significant effect of mate-guarding on a male's UCP levels (*Figure 6.1*) suggesting that energy intake and expenditure were balanced.

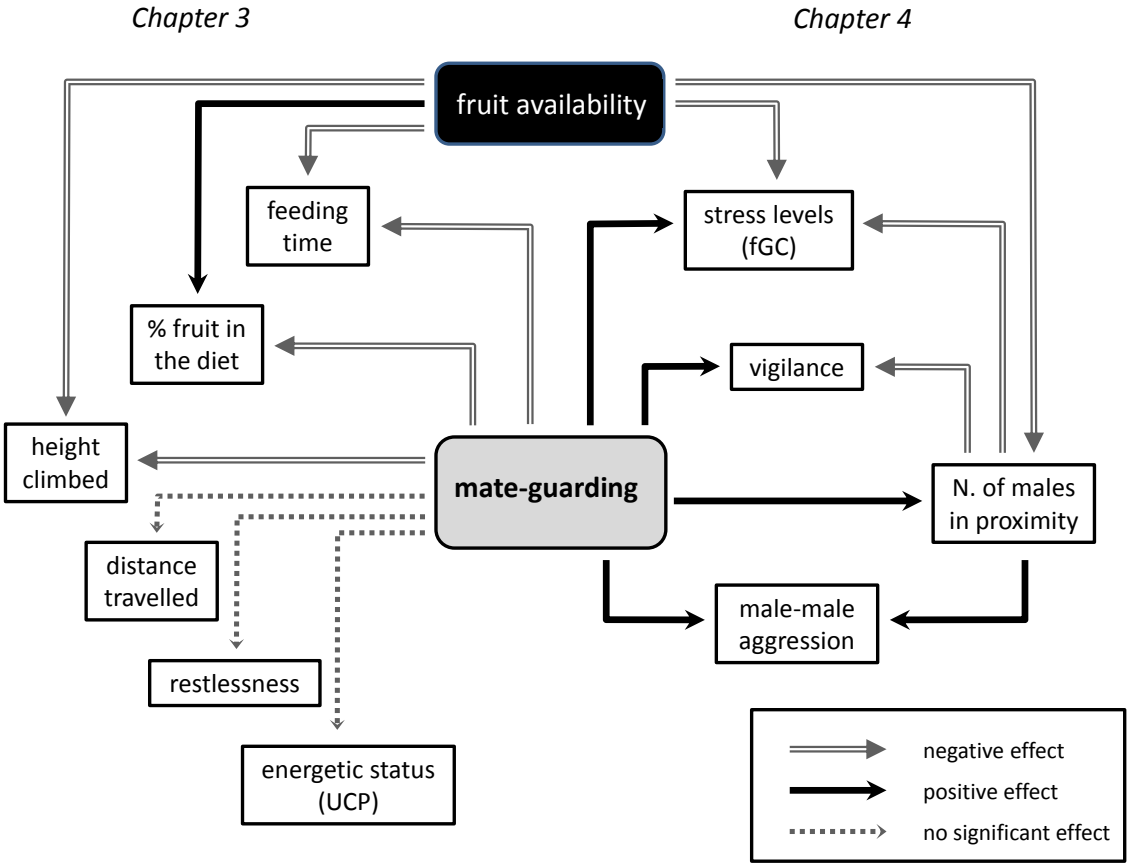

Figure 6.1: The interplay between high-ranking male mate-guarding effort, ecological, energetic, physiological and social factors in long-tailed macaques.

This chart summarizes the main results of chapter 3 and 4.

In **chapter 4,** I extended the investigation of the costs of mate-guarding by assessing aggression and stress related costs. Male stress levels were elevated during mate-guarding in general (*Figure 6.1*), but the magnitude of this relationship was contingent on male vigilance behaviour. Furthermore, males were more vigilant, had more males in proximity and were more likely to be involved in aggressions with other males when mate-guarding females than when not (*Figure 6.1*). The number of males in proximity itself reduced male vigilance time and fGC levels but increased the rate of male-male aggressions (*Figure 6.1*). Independently of the effect of mate-guarding, when fruit availability was high males had more other males in proximity, spent less time feeding, climbed less distance, had lower fGC levels and had a higher percentage of fruit in their diet (*Figure 6.1*).

Beside what is shown in *Figure 6.1*, I also found that dominance rank did not have a significant effect on male UCP levels during the mating period (**chapter 3**). However, alpha males were overall more physiologically stressed than other males year round and the magnitude of the difference between alpha and other males did not vary between mating and non-mating periods. In the last study

(**chapter 5**) I aimed to better comprehend if and to which extent female reproductive value (parity status and dominance rank) and the strength of male-female social bonds modulate the costs of mate-guarding and the investment of males in this behaviour. When mate-guarding females of low reproductive value (low-ranking and/or nulliparous) males fed less time and had higher fGC levels than when mate-guarding other females. Males also invested more into females of high reproductive or social value (high ranking females or females with whom they were strongly socially bonded) by being more vigilant and acting more aggressively towards other males.

6.2 Food availability and reproductive effort

The ability of an organism to meet the energetic needs of reproduction depends on its capacity to acquire, through feeding, sufficient resources in the environment (Boggs 1992; McEwen & Wingfield 2010). The efficiency of resource acquisition (i.e. energy intake, see *Figure 1.1*, **chapter 1**) is affected not only by feeding time and ingestion rate but also by the availability of resources in the environment (Boggs 1992). In turn, animals may adjust their investment into energetically costly mating tactics depending on food availability. For example, food supplementation increased the intensity and duration of male dawn and dusk songs (a form of mate-guarding) in blackbirds (*Tordus merula*, Cuthill & Macdonald 1990) and the intensity of courtship behaviours in fiddler crabs (*Uca lacteal*, Kim & Choe 2003; Kim et al. 2008) and in guppies (*Poecilia reticulate,* Kolluru & Grether 2005*).*

Similarly, ecological factors may play a role in male mate-guarding decisions in long-tailed macaques (**chapter 4**). In my study, I found that fruit availability not only influenced some fundamental components of males' energetics (feeding time, diet and locomotion) but also males' stress levels (**chapter 3** and **4**, *Figure 6.1*). If males monopolise females in periods of low fruit availability, they face concurrently two sources of elevation of their physiological stress: mate-guarding behaviour and low fruit availability. During these periods, males may thus need to stop mate-guarding females to avoid such increases in their stress levels or alternatively endure such a physiological challenge only to mate-guard the most valuable females (i.e. high-ranking or strongly bonded ones, **chapter 5**, Engelhardt et al. 2006). Fruit availability may thus be an important part of a male's energetic management strategy (see below, *section 6.3*).

Considering the broad effect of fruit availability on an animal's energetics but also on male spatial dynamics (number of males in proximity, **chapter 4**, *Figure 6.1*), assessments of ecological conditions should be systematically carried out in any study investigating the energetic, physiological and/or social costs of male reproductive effort. Furthermore, future studies could build upon the costs of mate-guarding identified in my thesis to investigate how ecological factors, availability and quality of

fertile females and male physiological and energetic status interact to determine a male's decision to mate-guard a female or not. Finally, seasonal fluctuations in food availability may play a key role in determining the overall energy management strategies of animals and the timing of their reproductive effort.

6.3 Reproductive effort and energy management strategies

Temporal and quantitative dynamics through which animals acquire resources and allocate energy into survival, growth and reproduction is at the core of life-history evolution theories (Stearns 1989; Boggs 1992). In regard to reproduction, the concept of capital and income breeding offers a comprehensive framework to understand individual energetic management strategies (Stearns 1989; Jonsson 1997; Brockman & van Schaik 2005). Following Stearns (1992, pp. 221-222) I define here a capital breeder as "an organism that uses stored energy for reproduction" and an income breeder as one that "uses energy acquired during the reproductive period rather than stored energy for reproduction". In environments with temporal variation in resource availability, the timing of female conception periods is contingent on female breeding strategies (van Schaik & Brockman 2005). Since the most energetically demanding period differs between females (mainly lactation, i.e. birth season) and males (mate acquisition, i.e. mating season) (Trivers 1972), the overall energy management strategy may differ between the sexes. Income breeder females time their reproduction so that the

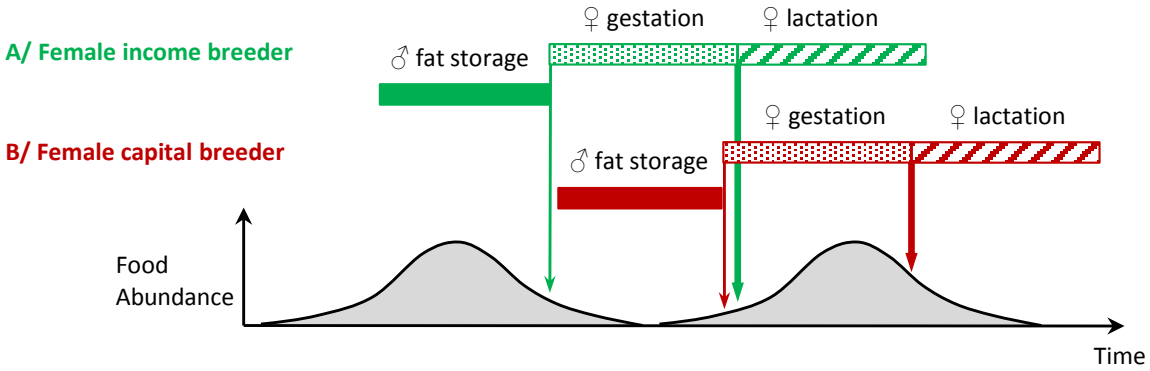

Figure 6.2: Two distinct strategies of energy management in response to seasonality in the abundance of food resource (modified from van Schaik and Brockman 2005).

*In **A** (green) females are selected to give birth before the annual peak in food availability and therefore have evolved sensitivity to exogenous cues that lead to conception one gestation period before this optimal timing (income breeding). Here food availability is high during the pre-mating period and males can therefore store fat during this period. In **B** females respond to increases in food abundance as cues for conception, leading to birth one gestation period following that event, often coinciding with the end of food availability peak (capital breeder). Here food availability is low during the pre-mating period and males have little opportunity to store fat. Thin arrows indicate the approximate timing of conception and fat arrows the approximate timing of birth.*

birth season coincides with the period of maximum food availability (*Figure 6.2*, van Schaik & Brockman 2005). In this context, males have the opportunity to accumulate energy (fat reserves) prior to the mating season and utilise this energy for subsequent male-male competition for access to females and hence use a capital breeder strategy (*Figure 6.2*). In contrast, capital breeder females time their periods of fertility with rise in food availability. This allows females to store energy during the gestation period that they can subsequently utilise for lactation during periods of low food availability (*Figure 6.2*). Here food availability is low prior to the mating season and males have little opportunity to store energy (fat) and need therefore to acquire resources while reproducing (income breeder strategy).

In conclusion, whether an energy management strategy is capital or income breeder should be determined at the sex-specific level within a species rather than at the overall species level, even though the degree to which males are constrained in their strategy may also depend on the species' social dynamic. For example, in grey seals (*Halichoerus grypus*), females adopt a capital breeder strategy but males and females forage independently (Beck et al. 2003b). Males therefore have the opportunity to forage at different times than females, store fat prior to the mating season and hence employ a capital breeder strategy as well (Beck et al. 2003a).

The capacity of males to store fat prior to the mating season determines the energy males can invest into their reproduction, reproductive tenure, and consequently, their reproductive success (e.g. in pinnipedes, Lidgard et al. 2005; Galimberti et al. 2007; Crocker et al. 2012). Quantifying the costs of male reproductive effort may shed light on male energy management strategies (**chapter 3**). If males are able to store fat before the mating periods, they are expected to use these reserves during mating competition and hence pay an energetic cost leading to decreases in their body condition and energetic status (e.g. rhesus macaques, Bernstein et al. 1989; Higham et al. 2011a; elephant seals Galimberti et al. 2007). In long-tailed macaques, in contrast, females are strict capital breeders and males have hence little opportunity of fat storage prior to the mating season (van Schaik & van Noordwijk 1985, see above) and should therefore use an income breeder strategy. In this species, mate-guarding represents an energetic challenge for males since their physiological stress levels increase when they engage in this behaviour (**chapter 4**). I thus suggest that the absence of a detectable effect of mate-guarding on males' energetic status (**chapter 3**) indicates that high-ranking males in this species finance the costs of reproduction (here mate-guarding effort) using resources acquired during the reproductive period and not stored beforehand. Males may have evolved a fine-tuned energy management strategy whereby they maintain their energetic status balanced by using cortisol as a physiological tool (**chapter 4**) and by limiting their vertical locomotion during mate-guarding (**chapter 3**), and circumvent the energetic costs of mate-guarding by not monopolising all

fertile females (Engelhardt et al. 2006) or by mate-guarding females of low reproductive and social value less aggressively (**chapter 5**).

In other vertebrate (Ancona et al. 2010) and invertebrate (Suzuki et al. 2012) taxa, males were also more aggressive when guarding females of high reproductive value or prolonged their mate-guarding effort over longer periods. Theoretical models predict that the costs of reproductive effort drive the evolution of male mate-choice (Kokko & Monaghan 2001). A flexible mate-guarding effort adjusted to female reproductive quality (**chapter 5**) is a form of male mate-choice (Edward & Chapman 2011) which may have evolved under constraints linked to the costs of female monopolisation (**chapter 4**, Ancona et al. 2010). Mate-guarding choices may thus be an integral part of an overall male energy management strategy giving males more magnitude to maximise the trade-offs between the costs and benefits of reproductive effort.

6.4 Reproductive effort, dominance status and sociality

In this thesis I focused on the costs of mate-guarding. However, as argued by Georgiev (2012), the costs of male reproductive effort are not limited to the periods when sexually receptive females are present. In species in which male reproductive success is highly skewed towards the highest-ranking males, such as long-tailed macaques, male lifetime reproductive success depends on his ability to acquire high dominance rank and on the duration of his tenure at high-rank (e.g. de Ruiter et al. 1994). Therefore, the energy allocated to dominance status acquisition and maintenance can be an important, albeit less direct, part of male reproductive effort (Georgiev 2012). The fraction of this overall effort allocated to dominance status may be contingent on the interplay between 1) dominance rank achievement processes, 2) the degree of male reproductive skew and 3) male energy management strategy (capital vs. income breeder). I would suggest that the energy allocated by high-ranking males to maintaining their dominance status is important in species with a steep reproductive skew, a process of rank achievement through contest competition and in which males employ an income breeding strategy. In this case, being an income breeder allows males to distribute their reproductive effort over the whole year and to finance it by continuously acquiring resources. These predictions fit with my findings on male long-tailed macaques in which the alpha male showed higher stress hormone levels during mating-periods but also outside of mating periods (**chapter 4**). This indicates that alpha males allocated a larger portion of their reproductive effort into maintaining their dominance status than other males. During the reproductive period, however, males all experienced a similar rise in stress hormone levels (**chapter 4**, see also Girard-Buttoz et al. 2009) indicating a similar reproductive effort across all males during this period regardless of the rank or mating tactics. Similarly in chimpanzees, dominance rank had no effect on male energetic status

when oestrus females were present in the group. In the absence of oestrus females, however, higher-ranking males had lower UCP levels than low-ranking ones (Georgiev 2012). In chimpanzees, high-ranking males may thus invest more of their reproductive effort into maintaining their rank and less into direct male-male competition for access to mates than other males. In certain primate species where paternity success is highly skewed towards highest-ranking males, investment of high-ranking males into maintaining their dominance status may explain the impact of hierarchy stability on the relationship between dominance rank and males' physiology. In chacma baboons (Bergman et al. 2005), and mandrills (Setchell et al. 2010) low-ranking males had higher stress hormone levels than high-ranking males during stable periods but this relationship was reversed during periods of hierarchy instability. This suggests that, in periods of hierarchy instability, high-ranking males need to invest more energy than other males into maintaining their rank since they are usually the ones challenged.

In contrast, males should allocate little energy to maintaining their dominance status in species with shallow reproductive skew, rank achievement through queuing and in which males employ a capital breeder strategy. Rhesus macaques, for example, show all these characteristics (Bernstein et al. 1989; Berard 1999; Dubuc et al. 2011), and a recent study suggested that high-ranking males invest little into dominance status maintenance but invest more than other males into direct male-male competition for access to mates during the mating season (Higham et al. 2011a). In this study, high-ranking males had better body condition and fatness than lower-ranking ones at the beginning of the reproductive season but, by the end of the reproductive season, high-ranking males were in the worst energetic condition.

Beyond direct male-male competition for dominance status and access to females, males may also invest resources into establishing and maintaining strong social-bonds with targeted conspecifics if sociality enhances male fitness (discussed in Schülke et al. submitted and Georgiev 2012). In rhesus macaques, the strength of male-female social bonds were directly linked to male reproductive success even after controlling for dominance rank (Kulik et al. 2012). In Assamese macaques, strong male-male social bonds were shown to enhance male reproductive success through coalition formation and dominance status (Schülke et al. 2010). Males may balance their investment between sociality and direct contest competition based on their need for support from other males. In chimpanzees, a comparison of grooming behaviour of three consecutive alpha males (within the same group) showed that the smallest male, when he was alpha, groomed other male group members more and more reciprocally than the two other alpha males (Foster et al. 2009). This small alpha male also avoided contact aggression unlike the two other males when they were alpha themselves. Social relationships with females might also be important for male fitness (Kulik et al.

2012; Massen et al. 2012). In this thesis I showed that male long-tailed macaques mate-guard females with whom they are closely bonded more thoroughly (i.e. more vigilantly) and more aggressively (**chapter 5**). This result further highlights the interplay between male reproductive effort into sociality and direct male-male contest competition for access to mates.

Obviously, the fitness benefits of forming strong social bonds and/or maintaining top dominance status over extended periods (e.g. over 5 and 10 years for long-tailed macaques and chimpanzees respectively, de Ruiter et al. 1994; Georgiev 2012) are tightly linked to the species' social organisation. In species in which males and females do not associate year round, such as elephants and seals, male reproductive effort is entirely allocated to direct male-male competition for access to mates (Le Boeuf 1974; Poole 1989; Lidgard et al. 2005). In these species, male reproductive effort is limited to the period when females are sexually receptive. The rest of the year can be utilised by males to store energy in preparation of the upcoming mating periods or to recover afterwards, free of social or dominance hierarchical constraints.

Male reproductive effort hence comprises different non-mutually exclusive components such as sociality, mate-guarding and male-male competition for dominance rank or direct access to mates. In species in which males mate-guard females, the interplay between the timing and degree to which high-ranking males invest into these different components, environmental resource availability and male mate-choices determine when and to what extent high-ranking males mate-guard females (chapter 4, see above). In turn, the investment of top-ranking males into mate-guarding and the degree of control of these males over reproduction may impact the pattern of reproductive skew among males.

6.5 Costs of mate-guarding and reproductive skew in primates

As predicted by the PoA model and confirmed by meta-analyses, the degree of control of the highest-ranking male primates over reproduction (i.e. alpha male paternity) is strongly affected by female reproductive synchrony (Ostner et al. 2008b; Gogarten & Koenig 2013). Interestingly, and independently of the effect of synchrony, this degree of control is also influenced by the number of males in the group (Ostner et al. 2008b; Gogarten & Koenig 2013). Therefore, in primates in general, the more male competitors are present in the group the less the highest-ranking male is able to monopolise females. This supports the limited control model (Reeve et al. 1998) in which reproduction cannot be fully controlled by the top ranking male and is partitioned among male group members based on their competitive abilities (i.e. dominance rank in primates, Majolo et al. 2012).

In long-tailed macaques, alpha males were found to monopolise females less than predicted by the PoA model (Engelhardt et al. 2006). In this species, the percentage of alpha male paternity decreased when the number of males in the group increased, suggesting that the limited control model applies to long-tailed macaques as well (de Ruiter et al. 1994). This conclusion is further reinforced by the results of this thesis identifying physiological and physical costs of mate-guarding (**chapter 4**). These costs may constrain high-ranking male mate-guarding decisions and force them to adopt an "incomplete female monopolisation strategy" whereby males do not mate-guard all the females in the group in order to decrease their chance of exposure to aggressions with other males and chronic stress. However, the number of males in long-tailed macaque groups might not directly impact the ability of the alpha male to monopolise females but rather be an index of the reproductive quality of the females in the group (i.e. of the attractiveness of the group for other males). In fact, I found that the number of males in proximity decreased high-ranking males' hormone stress levels and vigilance time (**chapter 4,** *Figure 6.1*), hence reducing some of the costs of mate-guarding for these males. In long-tailed macaques, alpha males frequently form coalitions with other males to defend their rank against challenges from young extra-group males (van Noordwijk & van Schaik 2001). During my study, coalitions involving high-ranking males were exclusively directed towards extra group males attempting to challenge alpha or beta males and access the guarded females (unpubl. data). The presence of other males in the group may thus be beneficial for high-ranking males and enhance both their female monopolisation potential and rank tenure.

The limited control model posits that the reproductive share of the dominant male (i.e. the highest-ranking male) is the result of within group competition and solely depends on dominant and subordinate competitive ability (Reeve et al. 1998). This model may only partially apply to long-tailed macaques since, as described above, costs of mate-guarding and associated limited monopolisation potential might be driven by contest from outside the group rather than from within group competition. Therefore, reproductive skew models would benefit from incorporating threats from outside the group as a factor affecting how the reproduction is partitioned within the group (see Ostner et al. 2008b).

In long-tailed macaques, the costs of female monopolisation, alpha males' reproductive effort and energy management, male-male support, and overall male reproductive skew, may be linked to each other through a positive feedback loop. Since mate-guarding is physically and physiologically challenging (**chapter 4**) an alpha male may need to adjust his mate-guarding effort to female quality (**chapter 5,** Engelhardt et al. 2006) in order to balance his energetic status (**chapter 3**) and be able to respond at any time to rank challenges from other males and ultimately maintain his alpha position over several years (de Ruiter et al. 1994). As part of their overall energy management strategy, males

may have been selected to maximise the trade-off between current and future reproduction (Stearns 1989) through an incomplete female monopolisation strategy. In turn this strategy of the top-ranking male provides some reproductive share to other males in the group which may generate a staying incentive for these males. This is particularly important for the stability of the group since adult male long-tailed macaques within a group are unlikely to be genetically related (average index of kinship R bellow 0, de Ruiter & Geffen 1998) and hence do not derive inclusive fitness benefits from the alpha male's reproductive success. Finally, the presence of the other males most likely benefits the alpha male since it may enhance his tenure (through coalitionary support during challenge from extra group males, van Noordwijk & van Schaik 2001) and by reducing the physiological costs of mate-guarding (**chapter 4**). Overall the factors driving male reproductive skew in long-tailed macaques might be a modified version of the limited control model (Reeve et al. 1998) whereby limitation arises from outsider males challenges and not from within group male-male competition.

6.6 Conclusions and outlook

By combining behavioural observations and physiological and ecological measurements this thesis provides a detailed picture of the factors constraining mate-guarding decisions in high-ranking male long-tailed macaques. In perspective of the previous knowledge on long-tailed macaques reproductive biology and ecology (van Noordwijk 1985b; van Schaik 1985; de Ruiter et al. 1994; Engelhardt 2004), my results provide a comprehensive picture of the factors driving male mating decisions and reproductive skew in this species. An "incomplete female monopolisation strategy" may have evolved in males constrained by several factors 1) the unpredictable reproductive seasonality of the species (van Schaik & van Noordwijk 1985) 2) the low food availability before the reproductive period preventing the males from storing energy prior to this period (van Schaik & van Noordwijk 1985), 3) the physiological and physical costs of mate-guarding (**chapter 4**) which may impose a trade-off on males between current and future reproduction (Stearns 1989) and 4) the mode of rank achievement (through contest competition, van Noordwijk & van Schaik 2001) which forces alpha males to be energetically ready to respond to rank challenges year round. Within this overall strategy, mate-guarding high quality females preferentially, longer and more thoroughly (**chapter 5**, Engelhardt et al. 2006) might play an important role in maximising a male's fitness.

By comparing my results with those on the costs of male reproductive effort in other vertebrates I show in this thesis that these costs and their effect on male reproductive skew result from a complex interaction between 1) reproductive and resource seasonality, 2) male energy management strategies (capital vs. income breeder), 3) males' dominance rank achievement processes and 4) social structure. Male reproductive effort is a complex phenomenon which is not limited to the

actual mating activity and reproductive period but might be distributed over the whole year and may be expressed in the form of direct competition for dominance status or non-sexual social interactions.

This thesis highlights the need to complement behavioural assessment of male energy intake and expenditure with physiological measures to provide comprehensive pictures on the costs of male reproductive effort. The forthcoming special issue in the International Journal of Primatology on "the costs of success in male primates" combining empirical field studies on monkeys, apes and humans, theoretical models and reviews, (and in which the **chapter 5** of this thesis will be published) highlights the growing interest on this topic in primatology. The next step into our understanding of the evolution and expression of male reproductive tactics is to go beyond the quantification of the costs of reproductive effort *per se* and start to analyse how males adjust their mating decisions depending on their physiological and physical conditions, the quality of available fertile females and ecological factors. These questions have already been partially addressed through several experimental manipulations in birds, crustaceans and fish (see *section 6.3*). However its investigation under natural conditions and non-invasively for large mammals may be more problematic. For mammals it would require the assessment at a given point in time of 1) the number of fertile females available (e.g. through hormonal measurements in faeces) and their quality (rank and social bond with the male), 2) the food available (through ecological measures), 3) the number of males in the group, 4) male dominance rank and 5) male physiological status (e.g. stress hormone levels and energetic status). Only then by answering the question "what reproductive decision will a male take given all these parameters?" will we be able to understand if male reproductive decisions are directly affected by the costs of reproduction.

In addition to urinary C-peptide, which is now validated as a reliable marker of a male's energetic status for some non-hominid primate species as well (**chapter 2**), other non-invasive markers open promising avenues for future research. In particular, measures of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and of urea concentrations were linked to nitrogen balance and dietary composition in mammals and birds (Hobson et al. 1993; Deschner et al. 2012; Vogel et al. 2012) and are likely to allow for determining whether the energy expenditure is derived from dietary intake or from fat and protein storage (Vogel et al. 2012). These markers would be particularly useful to determine whether males use, during the mating period, energy stored beforehand or acquired directly through feeding (Stephens et al. 2009). In turn, it would provide better classification of males on the income-capital breeders continuum (Jonsson 1997; Brockman & van Schaik 2005) and a better understanding of their overall energy management strategy and timing of reproductive effort.

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CURRICULUM VITAE

Cédric GIRARD-BUTTOZ

Born in Grenoble, France | 21 April 1983

Education

2008 - Present	**PhD thesis** on *"Costs of mate-guarding in male long-tailed macaques (Macaca fascicularis)"*. German Primate Centre and Georg August University – Göttingen
2005 – 2006	**Second year of Master's Degree in Biodiversity, Ecology and Evolution** Paul Sabatier University – Toulouse
2004 – 2005	**First year of Master's degree in Ecology** Paul Sabatier University – Toulouse
2003 – 2004	**Bachelor Degree in Organic Biology** University Montpellier II
2001 - 2003	**DEUG (a two year course)** specialising in biology Joseph Fourrier University – Grenoble

Skills

Languages	French | native	English | fluent
	German and Indonesian | basic.	

Computer Excel, Word, Power-Point, Statistical program: R, PRAAT (acoustic analysis), Zotero, End Note, basic ArcView and Q GIS.

Research and field experiences

Nov. 2008 – Jul. 2011 **Costs of mate-guarding in wild male long-tailed macaques**

Field work for my PhD thesis, Aras Napal and Ketambe, Gunung Leuser National Park, Sumatra, Indonesia

Feb. – Dec. 2007 **Vocalisations and scent marking behaviour of Milne-Edwards Sifaka (*Propithecus edwardsi)***

Field research assistant of Erik Patel (Cornell University, USA)
Ranomafana National park, Madagascar.

Nov. 2005 - Jun. 2006 **Functional links between landscape pattern and community structure on insects and birds communities in rural landscapes**

Master's research trainee with INRA (National Institute of Research in Agronomy), Toulouse, France (Supervisors: Prof. Dr. Gérard Balent, Dr. Annie Ouin)

Aug. – Sept. 2005 **Pairbond attachement in titi monkeys (*Callicebus brunneus*)**

Field research assistant for PhD Student (Columbia University,USA)
Rio Los Amigos Reserve, CICRA Research Station, Peru

Feb. - May 2005 **Habituation of roe deer *(Capreolus capreolus)* to human presence**

Trainee with INRA, laboratory CEFS (Behaviour and Ecology of Wild Animals), Toulouse, France

Jul. 2004 **Olfactory communication of dung beetles (*Scarabeus laticollis*)**

Trainee with CEFE (Centre of Evolutionary and Functional Ecology) Montpellier, France

Jul. 2003 **Protection of iguanas**

Voluntary work with « Iguana station », Utila Island, Honduras

Aug. 2002 **Protection of marine turtles**

Voluntary work with "Archelon" Association, Crete, Greece.

Publications

-peer reviewed-

Girard-Buttoz, C., Heistermann, M., Rahmi, E., Marzec, A., Agil, M., Ahmad Fauzan, P., Engelhardt, A. (in press). Mate-guarding constrains feeding activity but not energetic status of wild male long-tailed macaques. *Behavioural Ecology and Sociobiology: DOI 10.1007/s00265-013-1673-8*

Higham, J.P., **Girard-Buttoz, C.**, Engelhardt, A., Heistermann, M. (2011). Urinary c-peptide of insulin as a non-invasive marker of nutritional status: some practicalities. *PLoS One* 6(7): e22398.

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Girard-Buttoz, C., Heistermann, M., Krummel, S., Engelhardt, A. (2009). Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*). *Physiology and Behavior:* 98: 168-175.

-in revision-

Girard-Buttoz, C., Heistermann, M., Rahmi, E., Agil, M., Ahmad Fauzan, P., Engelhardt, A. (in revision). Costs of mate-guarding in wild male long-tailed macaques (*Macaca fascicularis*): physiological stress and aggression. *Hormones and Behavior*

Girard-Buttoz, C., Heistermann, M., Rahmi, E., Agil, M., Ahmad Fauzan, P., Engelhardt, A. (in revision). Costs of and investment into mate-guarding in wild long-tailed macaques: the impact of female characteristics and male-female social bonds. *International Journal of Primatology*

Conference contributions

Girard-Buttoz, C., Heistermann, M., Muhammad, A., Ahmad Fauzan, P., Engelhardt, A. (2012). The energetics of mate-guarding in wild long-tailed macaques (*Macaca fascicularis*). 82nd annual meeting of the American Association of Physical Anthropologists. Knoxville, Tennessee, USA. **Invited Paper**, Symposium on "The high price of success: Costs of reproductive effort in male primates and humans". (**Oral Presentation**)

Girard-Buttoz, C., Heistermann, M., Muhammad, A., Ahmad Fauzan, P., Engelhardt, A. (2012). The influence of mate-guarding on glucocorticoid excretion and vigilance in wild male long-tailed macaques (*Macaca fascicularis*), 13th meeting of the German Primatological Society, Hamburg, Germany. (**Oral Presentation**)

Girard-Buttoz C. (2012). Peerage of science: discussing a new peer review process. 3rd PhD workshop of the Courant Research Centre "Evolution of Social Behaviours", Göttingen, Germany (**Oral presentation**)

Girard-Buttoz, C., Higham, J. P., Engelhardt, A., Heistermann, M. (2012). Practicalities of urinary c-peptide measurements for monitoring the nutritional status of wild animals. 3rd conference of the International Society of Wildlife Endocrinology, Vienna, Austria. (**Oral Presentation**)

Girard-Buttoz, C., Heistermann, M., Muhammad, A., Ahmad Fauzan, P., Engelhardt, A. (2012). The energetics of mate-guarding in wild long-tailed macaques (*Macaca fascicularis*). 1st joint congress on Evolutionary Biology, Ottawa, Canada. (**Oral Presentation**)

Girard-Buttoz C. (2011). Discussing methods to study the energetic status of wild primates: advantages, drawbacks and perspectives. 2nd PhD workshop of the Courant Research Centre "Evolution of Social Behaviours", Göttingen, Germany. (**Oral Presentation**)

Girard-Buttoz, C., Heistermann, M., Higham, J.P., Wedegärtner, S., Maestripieri, D., Engelhardt, A. (2010). Validation of urinary c-peptide measurement as a potential marker of energetic condition in macaques. 23rd congress of the International Primatological Society, Kyoto, Japan. **Invited Paper**, Symposium on "Energetics: Measurement and Interpretation" (**Oral Presentation**)

Patel, E.R. and **Girard-Buttoz, C.** (2008). Non-nutritive tree-gouging in wild Milne-Edwards' sifakas (*Propithecus edwardsi*): Description and potential communicative function. 22nd congress of the International Primatological Society, Edinburgh, Scotland. (**Oral Presentation**)

Ouin A., **Girard-Buttoz, C.,** Burel, F., Gergaud, J., Tessier M., Sarthou J.-P., Balent, G. (2006). Étude de la relation entre structure des communautés et distribution des ressources dans le paysage par l'utilisation des Diagrammes Rang-Fréquence (DRF). Symposium on Landscape Ecology, Rennes, France. (**Poster**)

Grants awarded

2009	13,442 $	Research Grant of the **Leakey Foundation**
2008	145,900 €	Evolutionary Biology Grant for PhD, **VolkswagenStiftung**
2008	24,992 $	Dissertation fieldwork Grant of the **Wenner Gren Foundation**.