



Mate competition, testosterone and intersexual relationships in bonobos, *Pan paniscus*

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Variation in male testosterone levels across and within species is known to be related to mating systems, male dominance rank and aggression rates. When aggression enhances access to mates, dominance status and androgen levels correlate positively. Deviation from this pattern is expected when access to females is determined by factors other than male dominance or when high androgen levels interfere with nonaggressive forms of male reproductive strategies such as paternal care and pair bonding. Bonobos offer an interesting study species to test the relationship between male dominance, aggression and intersexual relationships. On the one hand, males form dominance hierarchies and compete for access to females and mating success varies with rank. On the other hand, males and females are equally dominant, male rank is not only the result of aggression, and strong intersexual relationships might be crucial to male reproductive success. We used behavioural and physiological data from wild bonobos to test relationships between behavioural correlates of mate competition and androgen levels. Aggression and rank were positively correlated, as were aggression and mating success. In the presence of potentially fertile females, male aggression increased but only low-ranking, less aggressive males showed increases in testosterone levels, which consequently tended to be negatively related to rank. High-ranking males who had lower testosterone levels and were less responsive in their testosterone increase were more often involved in friendly relationships with unrelated females. These results suggest that, in bonobos, amicable relationships between the sexes rather than aggressive interactions mediate males' physiological reactivity during periods of mate competition.

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Investigating the interaction between hormone excretion, aggressive and affiliative behaviours, and social relationships is crucial to our understanding of the physiological mechanisms underlying male reproductive strategies and individual variation in reproductive success. Across a wide range of species, individual testosterone levels vary in relation to mating systems and male aggression (Hirschenhauser & Oliveira 2006; Goymann et al. 2007; Hau 2007). Species' differences in terms of the interaction between testosterone and behaviour have been attributed to the different costs and benefits that males derive from aggressive competition for mates (Wingfield et al. 1990). During periods of mate competition and rank instability, males are more aggressive and have elevated androgen levels if the detrimental effects of testosterone, such as immunosuppression (Grossman 1985) or higher risk of injury (Dufty 1989), are offset by increased mating success (Wingfield et al. 1990). When male dominance rank is the result of the outcome of aggressive interactions, androgen levels tend to

correlate positively with dominance status during times of contest, and high-ranking males exhibit the strongest increases in their testosterone levels during these times (Sapolsky 1993).

Deviation from the linear relationship of dominance, aggression and androgen levels is expected when access to females is determined by factors other than male dominance and/or when high androgen levels interfere with nonaggressive forms of male reproductive strategies (Strier et al. 1999; Goymann 2009). For instance, it has been found that paternal care is incompatible with high testosterone levels (Hau 2007; McGlothlin et al. 2007), and strong social bonds between males and females may also mediate absolute testosterone levels (Mooring et al. 2006; Van Anders 2009). Furthermore, dominant territorial breeders in striped mice, *Rhabdomys pumilio*, are found to have lower testosterone levels than subordinate roamers; this suggests a trade-off between amicable behaviours and elevated androgen levels (Mooring et al. 2006; Schradin et al. 2009).

In this context, the two *Pan* species, bonobos, *Pan paniscus*, and chimpanzees, *Pan troglodytes*, are particularly interesting as both share traits typical of species in which male mate competition involves elements of contest competition, such as male-biased sexual dimorphism in body mass and canine size (Plavcan 1990;

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Plavcan & van Schaik 1997), visual signs of oestrus (Furuichi & Hashimoto 2002) and linear male dominance hierarchies, that translate into male mating success (Boesch et al. 2006; Surbeck et al. 2011). However, critical components of mate competition seem to differ. In chimpanzees, males are dominant over females and adopt coercive mating strategies (Muller et al. 2009). Furthermore, mate competition is associated with an increase in aggression and testosterone levels and reports from two groups indicate positive correlations between male rank, aggression and testosterone levels (Muller & Wrangham 2004; Muehlenbein et al. 2004). In bonobos, males are equally dominant to females and therefore cannot force them to copulate (Kano 1992; Paoli 2009). As a consequence, males who succeed in male–male competition do not necessarily gain access to females and female choice is likely to influence male mating success. Under such conditions, the costs deriving from high testosterone levels may not be compensated by the benefits of enhanced reproductive success. Accordingly, it has been proposed that males may adopt alternative strategies such as establishing close associations with unrelated females that are characterized by reduced aggression and elevated mating activity and that these relationships might be important in the context of mating (Hohmann et al. 1999; Hohmann & Fruth 2003a). While results from one study indicate that mating activity is associated with increased rates of male aggression (Hohmann & Fruth 2003a), the correlations between male dominance rank and aggression on the one hand, and aggression and testosterone levels on the other, remain inconclusive (Muller & Wrangham 2001). Results of one preliminary field study with a small sample size suggest a positive correlation between rank and testosterone values (Marshall & Hohmann 2005), while data from captive bonobos indicate that aggression and testosterone values are independent of rank (Sannen et al. 2004a, b). Given these differences between the two *Pan* species in the relationship between behavioural patterns and physiology, one would also expect differences in the physiological response to mate competition. By exploring the hormonal response to a contestable food source, it has been found that, in contrast to chimpanzees, salivary testosterone levels did not increase in bonobos (Wobber et al. 2010), a result that may indicate species-specificity in terms of the association between behavioural interactions and physiological response. However, the question of how testosterone levels in male bonobos are related to rank, aggression, mate competition and social relationships with females remains open and is intriguing, especially because of the shifted dominance relations between the sexes.

Here we tested the following hypotheses to evaluate the importance of male rank, male aggression and male–female relationships, as expressed by grooming interactions between males and unrelated females, in shaping the androgen response in the context of mate competition. The first two hypotheses relate to ways of male competition while the third hypothesis includes male–female relationships as an additional factor.

(1) Male mate contest hypothesis. Given that aggression in bonobos was found to increase on mating days (Hohmann & Fruth 2003a) and that male rank translates into copulation with oestrous females (Surbeck et al. 2011), it is reasonable to assume that male mating success reflects the outcome of contest competition. Consequently, we predicted that high-ranking males are more aggressive, that high-ranking, more aggressive males have higher testosterone levels, and that increased aggression during mate competition is linked to an increase in testosterone levels.

(2) Male mate scramble hypothesis. The findings of two captive studies, which show that rank, aggression and testosterone are independent (Sannen et al. 2004a, b) and that testosterone levels do not increase in a competitive situation (Wobber et al. 2010), imply a minor role of contest competition over females. Accordingly, we

predicted that neither male aggression rates nor testosterone levels rise during times of mate competition and that both parameters are independent of male rank.

(3) Amicability–testosterone trade-off hypothesis. Alternatively, if social relationships between males and females are critical for male mating success, as has been proposed by Hohmann & Fruth (2003a), we would expect a trade-off between amicable relations or social ties between the sexes and elevated androgen levels. Therefore, we predicted that in the context of mate competition, males engaging in amicable relationships with unrelated females would have lower testosterone levels and attenuated testosterone responses compared to males without close relationships.

METHODS

Study Site and Subjects

Data were collected at the long-term LuiKotale field site in Salonga National Park, Democratic Republic of Congo (Hohmann & Fruth 2003b). All members of the bonobo community were habituated to human presence from the start of the study in May 2007. During the data collection period, the community consisted of 33–35 individuals, including nine mature males, 11 parous females and up to five nulliparous immigrant females. Age estimates were based on physical features such as body size, dentition and (in females) genital swellings (Furuichi et al. 1998). All males included in this study were older than 10 years of age (Table 1). All were seen to copulate and groom with oestrous females and engage in aggressive interactions with other males over access to these females. Using behavioural standards from related studies on chimpanzees (Muller & Wrangham 2004; Stumpf & Boesch 2005), we considered the nine males to be socially mature. Sexual maturity in captivity is reached at younger ages (Pereboom & Stevens 2008). Previous genetic analysis of noninvasively collected faecal samples revealed that six of the nine males had their mothers in the group (Surbeck et al. 2011).

Behavioural Observation

Data were collected between May and August 2007 and between December 2007 and July 2009. Parties of males were followed from the time subjects left the nest in the morning until the time they constructed night nests in the evening. Party composition was recorded every full hour (total = 2112 h). All occurrences of aggressive interactions, mating behaviours and grooming events were scored during follows of both the party and focal males (total 470 h of focal time; Altmann 1974). Focal follows of the same individual lasted for 10 min and were separated by at least 1 h. Focal individuals were randomly chosen from the males travelling in the same party.

Table 1
Overview of male subjects included in this study

Individual	Age class	Dominance rank	Mother presence
Ca	B	1	Yes
Ti	B	2	Yes
Ja	C	3	No
Da	A	4	No
Be	C	5	Yes
Ap	D	6	Yes
Pn	D	7	Yes
Em	D	8	Yes
Mx	D	9	No

Age classification is based on estimates: A: >35 years, B: 25–35 years, C: 15–25 years, D: 10–15 years.

Aggression refers to agonistic behaviours directed against individuals of both sexes, including contact aggression (hold-back, hit, pull, bite, kick, jump-on), and interactions without physical contact (charge, chase). Displays were not included as it was often difficult to determine whether they were directed at another individual. Submission refers to various forms of retreat such as flee and jump aside. Multiple aggressive acts within 10 min were scored as a single event if they involved the same individuals and if the direction remained the same. Mating refers to sexual interactions between males and females when intromission was achieved. Individual mating frequencies were calculated for each day from focal follows and all-occurrence observations and were corrected for the time each male was present in the focal party.

Quality of social relations

We used rates of grooming events between males and unrelated females as a measure for the strength of social bonds as this is generally considered to be a meaningful measure of social relationships in nonhuman primates (Cords 1997; Silk et al. 2006). One grooming event consisted of a grooming interaction between two individuals. If the same individuals resumed grooming after less than 10 min after terminating it was counted as one event.

Dominance interactions

Assessments of dominance relationships among male community members were based on dyadic interactions (e.g. Vervaecke et al. 2000). Individuals showing submission in response to aggression or to nonaggressive approaches by another male were classified as inferior. Multiple unidirectional dominance interactions occurring within 10 min were counted as a single event.

Oestrous cycle and fertility

The term 'oestrus' refers to the period when female genital swellings are maximally tumescent (Dixon 1998). We scored genital swellings daily and distinguished between four swelling stages ranging from minimal (stage 1) to maximal (stage 4). Scores were based on firmness of the swelling (tumescent) and skin surface structure (Hohmann & Fruth 2000). Female bonobos may exhibit nonconceptive oestrous cycles for several years (Kano 1992). To distinguish these cycles from cycles at times when the probability for conception was high, oestrous periods 6 months prior to conception were defined as fertile cycles and individuals showing maximally tumescent swellings during this period were classified as potentially fertile females. This time span is comparable to that of oestrous female chimpanzees at Kanyawara, where parous females exhibit a mean of five swelling cycles per conception (approximately 6 months; Muller & Wrangham 2004). To determine months of conception in this study, we counted back 8 months from the birth of the next offspring. During the study period three females conceived and therefore were classified as being potentially fertile for a period of 6 months prior to conception. A total of 16 females were observed at least once during oestrus more than 6 months prior to conception.

Urine Collection

Urine samples from males ($N = 1200$) were collected opportunistically during party follows over the whole study period. Whenever possible, urine was captured on leaves, using the underside of the leaves to avoid contamination. When leaves were not available urine was pipetted from ground vegetation. To avoid cross-contamination, we only collected urine from vegetation if a male was seen urinating and if the collection area did not have any urine from another animal. Urine samples contaminated with faeces were discarded.

Within 12 h from collection, urine samples were transferred into liquid nitrogen. Samples were shipped in nitrogen containers to the Max Planck Institute in Leipzig, Germany, where all hormone analyses were performed. Permits to export urine samples and import them into Germany were issued by the Congolese Wildlife Authority (ICCN) and the head veterinarian of Saxonia/Germany, respectively.

Hormone Measurements

Before measuring testosterone levels, we selected a balanced data set of 510 samples more or less evenly distributed over the entire study period and including representative numbers of samples from all nine males. In addition, the subset accounted for male identity and date and time the sample was collected as well as presence/absence of oestrous and receptive females.

We followed the methods described by Hauser et al. (2008a) to extract testosterone from urine samples. We used 100 μ l of urine for testosterone extraction, adding 50 μ l of internal standard mixture containing d3-testosterone. Steroid glucuronides were hydrolysed using β -glucuronidase from *E. coli* (activity: 200 U/40 μ l); we also hydrolysed the steroid sulphates with ethyl acetate/sulphuric acid to avoid enzymatic conversion of androgens as described for β -glucuronidase/sulphates of *Helix pomatia* juice (Messeri et al. 1984; Massé et al. 1989; Venturelli et al. 1995; Hauser et al. 2008b). If extraction efficiency, considered in the calculation of testosterone concentrations, was lower than 50%, we re-extracted from the sample. If extraction efficiency was again lower than 50%, we used the mean value of both measurements if the two testosterone values deviated from each other by <30%. A deviation of more than this amount resulted in the removal of the sample from our analysis.

Urinary testosterone levels were measured using liquid chromatography–tandem mass spectrometry following the protocol described by Hauser et al. (2008a). Quality control was ensured by adding the deuterated internal standard 16,16,17-d3-testosterone to all urine samples before extraction. Samples that lost more than 50% of the internal standard were remeasured. We measured creatinine (Cr) in each sample to correct for water content in the urine (Erb et al. 1970). All hormone levels are given in ng/mg Cr and we excluded all urine samples with a creatinine value lower than 0.02 mg creatinine/ml from our analysis.

If younger males had lower muscle mass, which would result in lower creatinine values in urine, correction of urinary water content to calculate testosterone levels by creatinine could lead to 'falsely' high testosterone levels in these males. Comparison of mean creatinine levels in samples from younger males with those from older ones did not reveal significant differences (exact Mann–Whitney U test: $U = 4$, $N_1 = N_2 = 9$, $P = 0.19$). Accordingly it was assumed that individual differences in testosterone levels were unlikely to be caused by differences in creatinine values.

Data Analysis

Dominance rank

We determined male rank by carrying out hierarchical rank order analysis with MatMan (version MfW 1.1; earlier version described de Vries et al. 1993) and found a highly linear dominance hierarchy among the males (Surbeck et al. 2011). During a 4-month study period, the dominance relationship between two males of adjacent ranks without mothers was subject to multiple reversals before stabilizing in the same rank order as before the reversals. This period of social instability was included as a control variable in the analysis of determinants of testosterone (see below).

Party rank

For the analysis of determinants of aggression, we ranked individual males in relation to all other males present in a given party, disregarding their dominance status compared to males that were not present. This is important because, owing to the fission–fusion nature of the bonobo social system, the party represents the social environment in which males compete and the dominance status of males that are absent from a given party is unlikely to affect behavioural interactions of male party members. We assigned the highest-ranking individual within a given party (not necessarily the highest-ranking male in the community) a value of 1 and the lowest-ranking individual a value of 0. Other males were ranked equidistantly between these two males. If there was only one male in a given party, it was assigned a value of 1.

Determinants of Aggression

To test whether aggression plays a role in a reproductive context, we evaluated how it was influenced by the presence of oestrous or potentially fertile females. Owing to the fission–fusion grouping pattern of bonobos, party composition and relative male dominance status (party rank) may change frequently. For example, the third-ranking male in the community occupies the highest party rank whenever the highest and the second-highest ranking males of the community are absent from the party.

This enabled us to address the influence of social parameters such as the presence of oestrous females, and a male's party rank on the occurrence of aggression while controlling for different numbers of males or females. We incorporated the variables likely to affect male aggression into a generalized linear mixed model (GLMM; Baayen 2008). The number of aggressive behaviours of a given male while it was in a given party with constant composition was used as a response variable (number of data points: $N = 5805$). We chose this response because changes in party composition were usually accompanied by changes in one or several of the predictor variables. The party rank of males, party composition (number of females as well as number of males) and presence/absence of oestrous or potentially fertile females were included as predictor variables with fixed effects. Male identity and party identity were included as random effects. Since the duration that a specific party with a specific composition lasted varied, we included the duration of a specific party as an offset variable. To test whether the presence or absence of potentially fertile females affected male aggressiveness, we incorporated it as a predictor variable instead of oestrous female presence/absence in an additional model (for more details on the GLMM, see the Appendix). To test the influence of overall dominance rank on individual aggressiveness, we incorporated overall dominance rank instead of party rank in an additional model.

Determinants of Testosterone

To evaluate our predictions concerning the correlates of testosterone levels such as the presence of oestrous or receptive females, male rank and grooming frequency, we incorporated them into a GLMM with testosterone level as the response variable (number of testosterone samples: $N = 505$). In addition to dominance rank of males and the presence/absence of oestrous females in the focal party of a given male during the day of urine collection, we included as predictor variables the period of rank instability, mother's presence in the community (as they intervene on behalf of their sons in mate competition; Surbeck et al. 2011), daily mating frequency (as increased sexual activity has been hypothesized to lead to lower testosterone levels; Gray & Campbell 2009), urine collection time (to control for diurnal patterns of urinary excretion of testosterone), and season (see the Appendix) as fixed effects. We

included day and male identity as random effects. To investigate the influence of presence/absence of potentially fertile females on male testosterone levels, we incorporated it as a predictor variable instead of oestrous female presence/absence in an additional model. To analyse the relationship between testosterone and aggression rates instead of rank, we incorporated mean aggression rates of the males (as this correlated with rank) into the model in a way that allowed us to distinguish within- versus between-subject effects (van de Pol & Wright 2009). To control for individual differences in terms of overall physiological responsiveness, we standardized individual testosterone values to a scale ranging from 0 (mean of the five lowest testosterone values of a given individual) to 1 (mean of the five highest testosterone values of the same individual) and used this value as the response variable instead of the absolute testosterone values in this second model.

As indicated above, the unit of analysis in this model was the day of urine collection, whereas in the model for aggression it was constant party composition. We based our inference on full models (and present their results) to avoid false positives as well as biased effect size estimates and P values (Forstmeier & Schielzeth 2011). We explicitly decided against using a stepwise approach because of the many shortcomings this procedure has (Whittingham et al. 2006; Mundry & Nunn 2009). For more details about the GLMM, see the Appendix.

RESULTS

Male Aggression

We observed 577 aggressive encounters that involved males as aggressors (75% of all aggressive encounters) during 470 h of focal and 2112 h of all-occurrence sampling. The majority (83%) consisted of noncontact aggression while the rest consisted of contact aggression. Male–male aggression accounted for 78% and male–female aggression for 22% of the 535 cases in which the sex of the victim of aggression was known. The proportion of contact aggression was larger when males directed aggression towards females than males (32% versus 14%).

Presence of Oestrous and Potentially Fertile Females

While the presence of oestrous females (58% of observation days) had no significant effect on the occurrence of male aggression (Appendix Table A1), male aggression increased significantly in the presence of potentially fertile females (7% of observation days), ranging from a mean of 0.17 acts/h to a mean of 0.26 acts/h (Appendix Table A2). Overall, the absence/presence of potentially fertile females did not affect the proportion of male aggression against females (exact Wilcoxon test: $T^+ = 15$, $N = 6$ males, $P = 0.44$). However, the frequency of contact aggression against females increased from 0.008 to 0.040 acts/h when potentially fertile females were present but it should be noted that in these cases, the target of male aggression was never a potentially fertile female.

Aggression, Rank and Mating Success

High-ranking males had higher aggression rates than low-ranking males (Fig. 1), independent of the presence/absence of potentially fertile females (GLMM with male overall dominance rank: two-way interaction between rank and presence of potentially fertile female: $P = 0.74$; dominance rank: $P < 0.001$; and see Appendix Table A2).

There was no correlation between male overall rank and aggression frequency against females (Spearman rank correlation: $r_s = 0.5$, $N = 9$, $P = 0.18$); instead, the rank-related bias in male

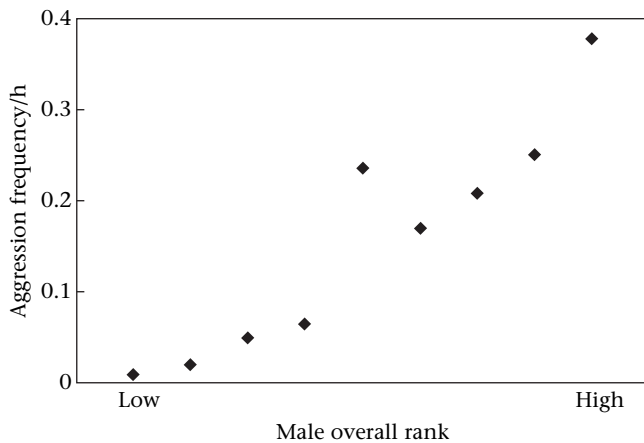


Figure 1. Correlation between male overall rank and aggression frequency. Note that the presented means are calculated from the raw data, but the significance was calculated using a GLMM (dominance rank: GLMM: $P < 0.001$).

aggression was the outcome of male–male interactions (Spearman rank correlation: $r_s = 0.98$, $N = 9$, $P < 0.001$). Males with higher aggression rates also had higher overall mating rates with oestrous females (Spearman rank correlation: $r_s = 0.68$, $N = 9$, $P = 0.050$).

Testosterone, Rank and Presence of Potentially Fertile Females

Mean individual male testosterone levels ranged between 47 and 95 ng/mg creatinine (range of SD 24–43 ng/mg creatinine). Testosterone levels showed a diurnal variation with higher levels early in the morning (Appendix Tables A3–A6). Neither copulation frequency nor the presence of the mother in the community influenced male testosterone levels (Appendix Tables A3, A4). While the presence of oestrous females did not significantly affect male testosterone levels (Appendix Tables A3, A4), the presence of potentially fertile females did. However, these changes in testosterone levels in the presence of potentially fertile females showed a clear rank effect such that the increases in testosterone levels of low-ranking males were significantly larger than those of dominant males (Fig. 2, Appendix Table A5). After we split the data into the two conditions (presence versus absence of potentially fertile females), male rank did not have a significant effect on absolute testosterone levels in either condition. In the presence of potentially fertile females though, there was a nonsignificant tendency for male testosterone levels to correlate negatively with rank (Fig. 2, Appendix Tables A7, A8). A similar result was obtained when standardized individual testosterone values were used as a response variable instead of absolute values to control for differences in mean ranges of testosterone levels that characterized individual males. To test whether the observed effect of male rank reflected rank-related differences in overall testosterone reactivity, we correlated the standard deviation of testosterone values with rank but found no obvious relationship (Spearman rank correlation: $r_s = -0.12$, $N = 9$, $P = 0.78$).

Aggression Rates and Testosterone Levels

Males that were more aggressive showed a lower increase in testosterone in the presence of potentially fertile females than less aggressive males (two-way interaction between mean aggression and potentially fertile females: estimate + SE = $-0.19 + 0.07$, $Z = 2.54$, $P = 0.01$). But testosterone levels did not correlate with mean aggression in either the presence or absence of these females (GLMM: in presence: -0.15 ± 0.10 , $Z = -1.61$, $P = 0.23$; in absence:

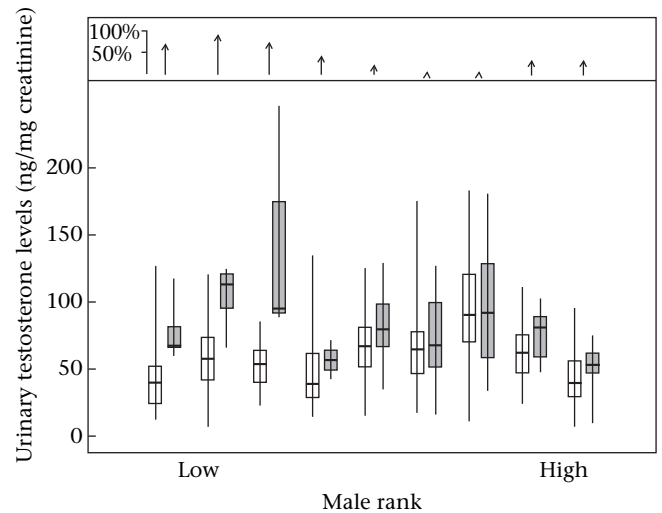


Figure 2. Urinary testosterone levels of bonobo males are shown in relation to rank at times when potentially fertile females were absent (white bars) and present (grey bars). Horizontal lines indicate median, boxes indicate 50% quantiles and vertical lines indicate 2.5% and 97.5% quantiles. Arrows indicate the relative testosterone increase in the presence of potentially fertile females in relation to testosterone values in the absence of potentially fertile females [(median present – median absent)/median absent]. Note that values shown in the lower part of the figure are calculated from the raw data while the test statistics derive from a GLMM: two-way interaction between rank and presence of potentially fertile females in the GLMM: $P < 0.001$.

0.06 ± 0.07 , $Z = 0.95$, $P = 0.44$). Furthermore, there was no correlation between daily aggression rates and testosterone levels within an individual (GLMM: 0.02 ± 0.03 , $Z = 0.68$, $P = 0.53$).

Testosterone, Relationship Quality and Presence of Potentially Fertile Females

Of the 498 observed grooming events between males and females, 61% involved mature males and unrelated females (mature females other than their mother). Males with higher ranks groomed unrelated females more frequently than lower-ranking males (Spearman rank correlation: $r_s = 0.883$, $N = 9$, $P = 0.003$). Furthermore, males who had higher rates of grooming with unrelated females had significantly lower increases in testosterone levels in the presence of potentially fertile females (Appendix Table A6). After splitting the data into presence/absence of potentially fertile females, we found that when potentially fertile females were present, males that groomed unrelated females at the highest rates had the lowest testosterone levels (Fig. 3, Appendix Tables A9, A10).

DISCUSSION

In this study on wild-living bonobos, we found limited support for the male mate contest hypothesis predicting behavioural and hormonal patterns that are indicative for contest for mates among male bonobos. In support of this hypothesis, rates of male aggression and testosterone levels increased in the presence of potentially fertile females and aggression was positively correlated with male overall rank. Contrary to the predictions of this hypothesis, the rise in testosterone levels during competition for potentially fertile females was inversely related to both rank and aggression rates. Only low-ranking, less aggressive individuals showed the predicted increase in testosterone levels. During times of mate competition, there was actually a nonsignificant trend towards a negative correlation between dominance rank and testosterone levels, with high-ranking males exhibiting lower testosterone levels than

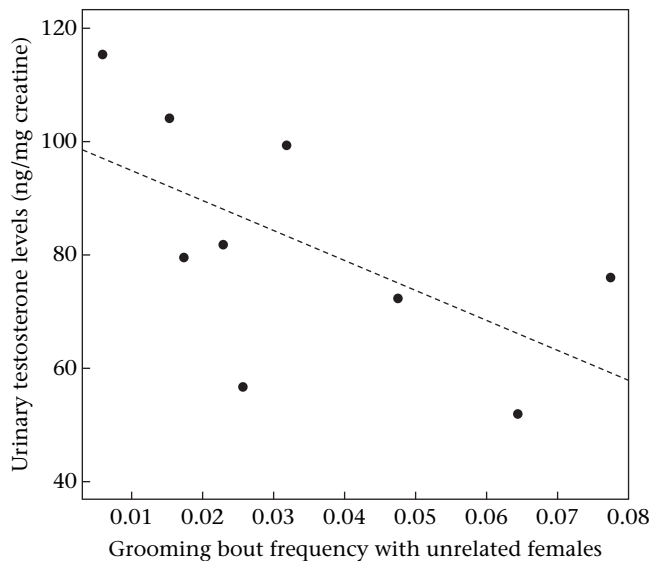


Figure 3. Rates of male grooming events (bouts/h) with unrelated females and urinary testosterone levels at times when potentially fertile females were present. Note that presented means are calculated from the raw data, but the test statistics stem from a GLMM controlling for urine collection time and season ($P = 0.037$).

low-ranking ones. The results did not support the male mate scramble hypothesis predicting no signs of contest competition in bonobos, as we found rank-related aggression rates and competition-dependent variation in male aggression and testosterone levels. Instead, our results are in accordance with the amicability–testosterone trade-off hypothesis predicting an inverse relationship between the strength of male–female social relationships and testosterone levels. As predicted, we found that the frequency of grooming between males and unrelated adult females was negatively correlated with absolute testosterone levels during competition for potentially fertile females as well as with the increase in testosterone levels during these times. Thus, males engaging in amicable relationships with unrelated females had lower testosterone levels and an attenuated testosterone response to the presence of potentially fertile females in comparison to males with weaker relationships to females. This indicates that intersexual relationships are associated with the physiological response of males at times when mating effort intensifies and when the chances of conception are high. A similar association between amicable intersexual relationships and testosterone levels in males has already been reported in other species such as striped mice and humans (Schradin et al. 2009; Van Anders 2009).

In most mammal species investigated thus far, male contest competition for females is associated with an increase in testosterone levels and aggression rates and with high-ranking males having the highest testosterone levels (Hirschenhauser & Oliveira 2006; Fairbanks 2009). Likewise, a number of primate species, such as red-fronted lemurs, *Eulemur fulvus rufus* (Ostner et al. 2008), long-tailed macaques, *Macaca fascicularis*, (Girard-Buttoz et al. 2009) and Assamese macaques, *Macaca assamensis* (Ostner et al. 2011) exhibit increased testosterone levels and aggression during reproductive competition. Evidence from muriquis, *Brachyteles arachnoides*, supports this relationship between aggression and testosterone as males do not compete aggressively over mating and lack the predicted change in testosterone levels (Strier et al. 1999). While many primate species, including Verreaux's sifakas, *Propithecus verreauxi* (Brockman et al. 1998), ring-tailed lemurs, *Lemur catta* (Gould & Ziegler 2007) and chimpanzees (Muller & Wrangham 2004), follow the pattern typical of social mammals

in that dominant males exhibit higher testosterone levels than subordinates during times of mate competition, no such pattern was found in Japanese macaques, *Macaca fuscata* (Barrett et al. 2002) or tufted capuchins, *Cebus apella nigratus* (Lynch et al. 2002). The observed species' differences have been explained as resulting from the increased reproductive success that high-ranking males gain through aggressive competition; if male–male aggression is the primary form of male mate competition, testosterone levels are related to dominance rank and rates of aggression (Anestis 2010).

Similar to species with male contest competition, aggression and testosterone levels of male bonobos increased in the presence of potentially fertile females and high-ranking, more aggressive males had higher mating success than low-ranking, less aggressive males. However, while the increase in aggression in the presence of potentially fertile females was independent of rank, the testosterone response was inversely related to rank. High-ranking, more aggressive males had a lower increase than low-ranking, less aggressive males. Within individuals, there was no correlation between testosterone levels and aggression rates and in the presence of potentially fertile females, high-ranking males tended to have lower absolute testosterone values than low-ranking males. These patterns differed from those expected for species with male contest competition for access to fertile females. While rank-related aggression and mating skew indicated contest competition over access to oestrous females, the testosterone patterns suggested that this was not the primary form of male reproductive competition. In support of this assumption, we never observed potentially fertile females to be the targets of male aggression.

It has been hypothesized that the permanently elevated testosterone patterns of high-ranking male chimpanzees reflect the need for constant aggression to maintain high social status in the unpredictable social environment of a fission–fusion society (Muller & Wrangham 2004). Although bonobos also exhibit a fission–fusion grouping pattern, high-ranking males did not have the elevated testosterone levels found in male chimpanzees. This indicates that fission–fusion does not lead to a constant challenge of ranks in bonobos necessitating permanently elevated testosterone levels. One stabilizing factor might be that maternal support is an important factor influencing male rank in bonobos (Furuichi 1997).

Comparing the response of male bonobos and chimpanzees in the context of food competition, Wobber et al. (2010) found that chimpanzees (but not bonobos) responded to competition with increased testosterone levels while bonobos responded with a rise in cortisol levels. Our results on male bonobos show that competition for potentially fertile females is associated with an increase in testosterone levels, but unlike results from field studies of chimpanzees, in bonobos the increase in testosterone levels differed between individuals according to dominance rank. Because the increase in testosterone was negatively correlated with the overall aggression of the males and testosterone levels did not correlate with aggression rates within an individual, we can exclude the possibility that differences in aggressive behaviour are related to different testosterone reactivity in high- and low-ranking males. A similar result was obtained in a study of captive bonobos by Sannen et al. (2004b) but the use of an inadequate enzyme for testosterone extraction restricts the generalization of that study (Messeri et al. 1984; Massé et al. 1989; Venturelli et al. 1995; Hauser et al. 2008b). While all bonobo males showed an increase in aggression during mate competition, indicating a contesting species, the hormonal response, particularly in high-ranking males, was different from that in species that engage in contest competition over access to females.

Furthermore, the high testosterone levels during mate competition in low-ranking males in combination with low aggression

rates and low mating success are still puzzling. To investigate whether the differences in testosterone reactivity of high- and low-ranking bonobo males reflect alternative reproductive strategies (Moore 1991), data on reproductive success are needed in combination with longitudinal data on shifts in individual male behaviour, rank and testosterone levels.

Rank-related differences in testosterone responsiveness may be a spurious outcome if high-ranking males have permanently elevated testosterone levels (Goymann 2009). However, in our study this was clearly not the case since the highest-ranking male had the lowest testosterone levels, and there was no rank-related difference in testosterone level ranges covered by the individual males. Low levels of testosterone can also be associated with progressing senescence (Harman et al. 2001) and overall testosterone levels and reactivity to competition may be triggered by differences in social and physical development (Martin et al. 1977). While we do not have precise information on age (all males were born before the start of the LuiKotal field project), none of the focal males showed physical signs of ageing, making such an explanation unlikely. Additionally, the younger age of the four lowest-ranking males could have influenced their testosterone reactivity. We cannot exclude this possibility, but a captive study indicates that this age class did not differ in their testosterone levels from older individuals (Sannen et al. 2004c). Given that one of the four younger males in our study was similar to older males in terms of both overall testosterone levels and reactivity, it seems that explanations for the observed differences in behaviour and physiology require a more complex approach. Further studies will address how testosterone reactivity in bonobos changes over the course of a lifetime.

The finding that differences in testosterone levels and response during mate competition for potentially fertile females were associated with interindividual differences in grooming relationships with unrelated females are in line with the amicability–testosterone trade-off hypothesis, which has been used to explain variation in male–female relations in species with paternal care. In a number of mammalian species, including humans, the strength of male social relationships with females has been found to be associated with male testosterone levels. For example, dominant male striped mice living in close association with females have lower testosterone levels than lower-ranking males who are not associated with females (Schradin et al. 2009). Common marmoset, *Callithrix jacchus*, males that are isolated from other group members show a stronger increase in testosterone levels in response to female ovulatory cues than males paired with a female (Ziegler et al. 2005). Finally, human men in romantic relationships have lower testosterone levels and lower testosterone responses to the presence of a woman than unpaired men (Burnham et al. 2003; van der Meij et al. 2008). However, in all these species, males also invest in paternal care, a behaviour that appears to be incompatible with elevated testosterone levels (Wingfield et al. 1990). Therefore, it remains unclear whether these changes in testosterone levels are ultimately linked to close relationships with females or to imminent paternal care. However, while male bonobos may maintain friendly relationships with immature individuals, there is no evidence for paternal care that compares to that practised by species with cooperative breeding systems such as marmosets (Tardiff 1997). Therefore, the results of our study suggest that amicable relationships between unrelated males and females may be associated with similar male testosterone patterns as paternal investment. The fitness benefit of maintaining close relationships with unrelated females as a means to maximize reproductive success might be especially relevant in bonobos because females have high social status (Kano 1992), and unrelated females form coalitions in conflicts against males (Parish 1996). What prevents

low-ranking males from establishing grooming relationships with females and whether the reproductive status of females influences grooming interactions are questions that will be addressed in future studies.

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Appendix

Specifications of GLMM for determinants of male aggression

We used the number of initiated aggressive interactions per male during a constant party composition as a response variable. As predictor variables with fixed effects we included the presence of oestrous or potentially fertile females (one at a time), the relative rank of males (party rank), the number of males and the number of females. In addition to these main effects, we also included several two-way interactions: (1) party rank and number of males, (2) presence of oestrous or potentially fertile females and party rank, and (3) presence of an oestrous or potentially fertile female and number of males. The reasons for including these two-way interactions were that it appeared likely that (1) the effect of rank on aggressiveness would increase with the number of males within a party, (2) dominant individuals would be more aggressive only in the presence of oestrous or potentially fertile females, and (3) the number of males in a party would influence male aggressiveness only when males compete for access to oestrous or potentially fertile females. We also included the three-way interactions between party rank, number of males and presence of an oestrous or potentially fertile female because we assumed that the number of males in a group would influence changes in aggressiveness in dominant male individuals, but only in situations in which oestrous or potentially fertile females were present. To test for the influence of male dominance rank on aggressiveness, we ran an additional model that included dominance rank instead of party rank in the same model.

We based our inference on full models (and present their results) to avoid false positives as well as biased effect size estimates and *P* values (Forstmeier & Schielzeth 2011). We did not interpret *P* values of terms involved in a significant interaction because 'it is generally not meaningful to speak of a factor-effect – even if its *p*-value is significant – if there is a significant interaction effect' (Zar 1999, page 242). However, when an interaction appeared to be nonsignificant, we removed it from the model (to be able to interpret the terms it comprises), but only when the full model revealed significance.

We included male identity and the identity of the specific party as random effects. To account for variations in stable party composition, we included the duration of a specific party as an offset variable. Specifically, we included the natural logarithm of (individual party duration × total number of all copulations/total duration of all parties). We accounted for temporal autocorrelation in the response (potentially violating the assumption of independent residuals) by first running a full model that included all fixed and random effects and derived residuals from it. We then calculated for each individual observation the weighted average of all other residuals of the same male, with the weight equalling one divided by the time lag between two observations (measured in days and fractions of days). We then ran the full model again with the derived values included as an additional fixed effect ('autocorrelation term'). Models were fitted in R version 2.9.1 (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>) using the function `lmer` of the R package `lme4` version 0.999375–28 (Bates & Maechler 2009), with the Poisson family and log link function. We used a likelihood ratio test to compare the overall fit of the full model with that of the null model (including only the autocorrelation term, the offset term and the random effects) as a global test of the significance of the set of all fixed effects. We did this using the R function `ANOVA` with the argument 'test' set to 'chisq'. To achieve more reliable likelihood ratio tests, we used maximum likelihood estimation (`lmer` argument `REML = F`). We tested for the effects of individual fixed effects or

interactions using the *z* test provided by the function `lmer`. We *z* transformed all covariates prior to fitting the model (including the autocorrelation term) to a mean of zero and a standard deviation of one. Testing the residuals revealed that overdispersion was not a problem in our model ($\chi^2_{5791} = 3887.987$, $P = 1$, dispersion parameter = 0.6713845).

The results of the GLMM for determinants of male aggression are shown in Tables A1 and A2.

Specifications of GLMM for determinants of testosterone levels

We used the log-transformed testosterone level of a given urine sample as a response variable. As predictor variables with fixed effects we included the presence/absence of oestrous or potentially fertile females (one at a time) in the focal party of a given male during the day of urine collection, the dominance rank of males, daily mating frequency, mother's presence in the community (as they are known to intervene on behalf of their sons in mate competition) and a period of rank instability (the 3-month period in which the third- and fourth-ranked males had no unidirectional outcomes during agonistic interactions). We controlled for diurnal and seasonal patterns of urinary excretion by including collection time and seasonal effects as additional fixed effects. Seasonal effects were accounted for by first transforming the days within a given year to a variable ranging from 0 to $2 \times \pi$. Then we included the sine and the cosine of this variable as additional fixed effects in the model. Because grooming bout frequency and dominance rank were strongly correlated (Spearman rank correlation: $r_s = -0.883$, $N = 9$, $P = 0.003$), we ran all models with grooming bout frequency or with dominance rank as a predictor variable. In addition to these main effects, we also included the two-way interaction between male dominance rank/grooming bout frequency and presence of oestrous or potentially fertile females because it seemed likely that rank or grooming bout frequency would influence testosterone levels only when oestrous or potentially fertile females are present.

We based our inference on the full models (and present their results) to avoid false positives as well as biased effect size estimates and *P* values (Forstmeier & Schielzeth 2011). With regard to the interpretation of main effects involved in an interaction, we followed the same rule as described for the previous model.

We included male identity as a random effect and accounted for temporal autocorrelation as described for the previous model. Models were fitted in R version 2.9.1, using the function `lmer` of the R-package `lme4` version 0.999375–28 with the Gaussian family and identity link function. Estimated *P* values for the effects of individual fixed effects or interactions were based on the Markov chain Monte Carlo simulation provided by the function `pvals.fnc` from the R package `languageR`. A likelihood ratio test was used to compare the overall fit of the full model with that of the null model (including only the autocorrelation term and the random effects) as a global test of the significance of the set of all fixed effects. We did this using the R function `ANOVA` with the argument 'test' set to 'chisq'. To achieve more reliable likelihood ratio tests, we used maximum likelihood estimation (`lmer` argument `REML = F`). We *z* transformed all covariates prior to fitting the model (including the autocorrelation term) to a mean of zero and a standard deviation of one. Visual inspection of plots of residuals against fitted values did not reveal any obvious deviations from the assumptions of normally distributed and homogeneous residuals. We interpreted significant interactions by testing for the effect of one variable (e.g. dominance rank) after splitting the data by the other (e.g. potentially fertile female present/absent) and after excluding nonsignificant predictors (mother present and rank instability).

The results of the GLMM for determinants of testosterone levels are shown in Tables A3–A10.

Table A1

Results from the model including the presence/absence of oestrous females after nonsignificant interactions were removed

	Estimate	SE	z	P
Intercept	-1.10	0.22		
Party rank	0.57	0.07	8.02	
Number of males	0.11	0.06	1.83	
Number of females	-0.01	0.06	-0.20	0.836
Oestrous females present/absent	0.04	0.10	0.46	0.645
Autocorrelation term	0.25	0.02	10.78	<0.001
Party rank: Number of males	0.10	0.04	2.68	0.007

The full model, compared to the null model comprising only the random effects, the offset and the autocorrelation term, was highly significant (likelihood ratio test: $\chi^2_{12} = 68.9$, $P < 0.001$). The removed nonsignificant interactions were those between party rank, number of males and presence/absence of oestrous females ($z = -1.28$, $P = 0.20$), oestrous female present/absent and number of males ($z = 0.08$, $P = 0.94$) and oestrous female present/absent and male party rank ($z = -1.42$, $P = 0.16$; statistics for the two-way interactions were derived from a model not including the three-way interaction). P values for party rank and number of males are not indicated because these predictors were involved in a significant interaction.

Table A2

Results from the model including the presence/absence of potentially fertile females after nonsignificant interactions were removed

	Estimate	SE	z	P
Intercept	-1.09	0.20		
Party rank	0.58	0.07	8.17	
Number of males	0.11	0.06	1.82	
Number of females	-0.01	0.06	-0.18	0.860
Potentially fertile females present/absent	0.40	0.18	2.25	0.024
Autocorrelation term	0.25	0.02	10.77	<0.001
Party rank: Number of males	0.11	0.04	2.79	<0.005

The full model, as compared to the null model comprising only the random effects, the offset and the autocorrelation term, was highly significant (likelihood ratio test: $\chi^2_{12} = 37.9$, $P < 0.001$). The removed nonsignificant interactions were those between party rank, number of males and presence/absence of potentially fertile females ($z = 1.62$, $P = 0.10$), potentially fertile female presence/absence and number of males ($z = 0.66$, $P = 0.50$) and potentially fertile female presence/absence and male party rank ($z = 0.13$, $P = 0.90$; statistics for the two-way interactions were derived from a model not including the three-way interaction). P values for party rank and number of males are not indicated because these predictors were involved in a significant interaction. Results from the model including dominance rank instead of party rank for the males were similar but the two-way interaction between number of males and dominance rank was no longer significant (model with oestrous female present/absent: $z = -0.91$, $P = 0.36$; model with potentially fertile female present/absent: $z = -0.59$, $P = 0.55$). Dominance rank in both models had a significant influence on male aggressiveness (model with oestrous female present/absent: $z = -9.26$, $P < 0.001$; model with potentially fertile female present/absent: $z = -9.26$, $P < 0.001$).

Table A3

Results from the model including rank and the presence/absence of oestrous females after nonsignificant interactions were removed

	Estimate	SE	P
Intercept	4.07	0.12	<0.001
Oestrous female absent/present	-0.03	0.05	0.590
Male dominance rank	0.05	0.07	0.507
Copulation frequency	-0.02	0.03	0.387
Mother absent/present	-0.02	0.14	0.881
Rank instability	-0.01	0.09	0.912
Sine of day of the year	-0.23	0.06	<0.001
Cosine of day of the year	-0.19	0.03	<0.001
Collection time	-0.11	0.03	<0.001
Autocorrelation term	0.09	0.03	<0.001

The full model was highly significant compared to the null model comprising only the random effects and the autocorrelation term (likelihood ratio test: $\chi^2_{14} = 67.27$, $P < 0.001$). The removed nonsignificant two-way interaction was the one between rank and presence/absence of oestrous females = 0.50. Significant results are shown in bold.

Table A4

Results from the model including grooming frequency with unrelated females and the presence/absence of oestrous females after nonsignificant interactions were removed

	Estimate	SE	P
Intercept	4.05	0.12	<0.001
Oestrous female absent/present	-0.03	0.05	0.600
Grooming bout frequency with unrelated females	0.00	0.07	0.999
Copulation frequency	-0.02	0.03	0.421
Mother absent/present	0.00	0.14	0.784
Rank instability	-0.01	0.09	0.895
Sine of day of the year	-0.23	0.06	<0.001
Cosine of day of the year	-0.19	0.03	<0.001
Collection time	-0.11	0.03	<0.001
Autocorrelation term	0.09	0.03	<0.001

The full model was highly significant compared to the null model comprising only the random effects and the autocorrelation term (model including grooming frequency: likelihood ratio test: $\chi^2_{14} = 66.24$, $P < 0.001$). The removed nonsignificant two-way interaction was the one between grooming and presence/absence of oestrous females = 0.99. Significant results are shown in bold.

Table A5

Results from the model including rank and the presence/absence of potentially fertile females

	Estimate	SE	P
(Intercept)	4.06	0.11	<0.001
Potentially fertile female absent/present: Male dominance rank	-0.23	0.07	0.002
Male dominance rank	0.08	0.06	
Potentially fertile female absent/present	0.05	0.10	
Copulation frequency	-0.02	0.03	0.555
Mother absent/present	-0.02	0.13	0.870
Rank instability	-0.01	0.11	0.935
Sine of day of the year	-0.23	0.06	<0.001
Cosine of day of the year	-0.19	0.04	<0.001
Collection time	-0.11	0.03	<0.001
Autocorrelation term	0.09	0.03	<0.001

The full model was highly significant compared to the null model comprising only the random effects and the autocorrelation term (likelihood ratio test: $\chi^2_{14} = 75.08$, $P < 0.001$). Significant results are shown in bold.

Table A6

Results from the model including grooming frequency with unrelated females and the presence/absence of potentially fertile females

	Estimate	SE	P
(Intercept)	4.04	0.12	<0.001
Potentially fertile female absent/present: Grooming bout frequency with unrelated females	-0.18	0.07	0.015
Grooming bout frequency with unrelated females	0.03	0.07	
Potentially fertile female absent/present	0.03	0.10	
Copulation frequency	-0.02	0.03	0.580
Mother absent/present	-0.01	0.14	0.812
Rank instability	0.00	0.11	0.956
Sine of day of the year	-0.23	0.06	<0.001
Cosine of day of the year	-0.19	0.04	<0.001
Collection time	-0.11	0.03	<0.001
Autocorrelation term	0.09	0.03	0.001

The full model was highly significant compared to the null model comprising only the random effects and the autocorrelation term (likelihood ratio test: $\chi^2_{14} = 71.15$, $P < 0.001$). Significant results are shown in bold.

Table A7

Results from the model including rank if potentially receptive females were present

	Estimate	SE	<i>P</i>
Intercept	3.93	0.24	<0.001
Male dominance rank	−0.20	0.08	0.064
Sine of day of the year	−0.37	0.18	0.069
Cosine of day of the year	−0.27	0.26	0.347
Collection time	−0.14	0.07	0.054
Autocorrelation term	0.02	0.07	0.819

Significant result is shown in bold.

Table A8

Results from the model including rank if potentially receptive females were absent

	Estimate	SE	<i>P</i>
Intercept	4.03	0.07	<0.001
Male dominance rank	0.08	0.07	0.329
Sine of day of the year	−0.21	0.04	<0.001
Cosine of day of the year	−0.18	0.04	<0.001
Collection time	−0.11	0.03	<0.001
Autocorrelation term	0.10	0.03	<0.001

Significant results are shown in bold.

Table A9

Results from the model including grooming when potentially fertile females were present

	Estimate	SE	<i>P</i>
Intercept	3.99	0.24	<0.001
Grooming bout frequency with unrelated females	−0.22	0.08	0.037
Sine of day of the year	−0.41	0.18	0.039
Cosine of day of the year	−0.19	0.27	0.520
Collection time	−0.13	0.07	0.068
Autocorrelation term	0.03	0.07	0.829

Significant results are shown in bold.

Table A10

Results from the model including grooming when potentially fertile females were absent

	Estimate	SE	<i>P</i>
Intercept	4.03	0.07	<0.001
Grooming bout frequency with unrelated females	0.02	0.07	0.786
Sine of day of the year	−0.41	0.18	0.04
Cosine of day of the year	−0.21	0.04	<0.001
Collection time	−0.18	0.04	<0.001
Autocorrelation term	0.09	0.03	0.001

Significant results are shown in bold.