

1 **The relationship between testosterone and long-distance calling in wild male chimpanzees**

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21 Abstract

22 Long-distance calling is a common behaviour in animals that has various important
23 social functions. At a physiological level, calling is often mediated by gonadal hormones such
24 as testosterone (T), particularly when its function is linked to intra-sexual competition for
25 mates or territory. T also plays an important role in the development of vocal characteristics
26 associated with dominance in humans. However, the few available studies of T and vocal
27 behaviour in non-human primates suggest that in primates T has less influence on call
28 production than in other animals. We tested this hypothesis by studying the relationship
29 between T concentrations and pant hooting in wild male chimpanzees (*Pan troglodytes*
30 *schweinfurthii*) of the Kanyawara community in the Kibale National Park, Uganda. We found
31 three kinds of correlation. Hourly T averages were positively associated with hourly rates of
32 pant-hooting. Monthly T levels were likewise correlated with monthly rates of pant hooting
33 after controlling for other influences such as fission-fusion rates. Finally, males with high T
34 levels had higher peak frequency at the start of the call climax. These results suggest that T
35 affects the production of pant-hoots in chimpanzees. This implies that the pant-hoot call
36 plays a role in male-male competition. We propose that even in cognitively sophisticated
37 species, endocrine mechanisms can contribute to regulating vocal production.

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40 Key words: chimpanzee, testosterone, vocal behaviour, pant hooting, acoustic structure

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43 Significance Statement

44 Many animals, ranging from amphibians to mammals, produce long-distance calls. The
45 production of these calls is often modulated by gonadal hormones such as testosterone,
46 especially if they are involved in competition between males for mates or territory.
47 However, little is known about the role of testosterone in vocal behaviour of non-human
48 primates, especially among great apes. In this study, we examined the relationship between
49 testosterone and pant hooting in wild male chimpanzees. We found that testosterone levels
50 were associated with pant hoot rates and one acoustic feature of the call. More specifically,
51 males pant hooted more often and produced pant hoots with higher peak frequencies
52 during periods of elevated testosterone levels. These results imply that gonadal hormones
53 are involved in regulating vocal behaviour in chimpanzees, and support the view that pant
54 hoots play a role in male-male competition.

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57 Introduction

58 Long distance calls have been identified in a variety of animal species, ranging from
59 insects to mammals (Bailey 2003; Delgado 2006; Hall 2009). Multiple functions have been
60 attributed to these calls, including attracting mates, repelling sexual rivals, alarm-calling,
61 signalling the identity and location of the caller, advertising social bonds, and defending
62 territorial boundaries (Waser 1977; McComb 1991; Geissmann 1999; Furmankiewicz et al.
63 2001; Zuberbühler 2001). On a physiological level, animal vocal behaviour is often
64 influenced by hormones, and gonadal hormones such as testosterone (T) and its
65 metabolites, are especially important in regulating vocal production (Floody 1981; Harding
66 1991; Emerson and Boyd 1999; Moore et al. 2005).

67 Since gonadal hormones play a crucial role in animal reproductive behaviour (Nelson
68 2000; Adkins-Regan 2005), these hormones are often involved in modulating the production
69 of calls given in mating contexts. For example, in anuran species T stimulates the production
70 of male advertisement calls (Townsend and Moger 1987; Marler and Ryan 1996; Solís and
71 Penna 1997; Wilczynski et al. 2005). In many birds T regulates the production of songs that
72 function to attract mates or repel sexual rivals (Nowicki and Ball 1989; Harding 1991;
73 Ketterson et al. 1992; McDonald et al. 2001; Boseret et al. 2006). T also affects the
74 production of vocalisations used by rodents in sexual and agonistic interactions (Floody et
75 al. 1979; Floody 1981; Kapusta and Pochroń 2011; Pasch et al. 2011).

76 In some species T can also influence the acoustic properties of a call. For instance,
77 high levels of T lower the fundamental frequency (F0) of songs in zebra finches (Cynx et al.
78 2005), and the pitch of male crowing in Japanese quail (Beani et al. 2000). T implants
79 decrease the minimum frequency, while increasing the duration and frequency range, of

80 male calls in the grey partridge (Fusani et al. 1994; Beani et al. 1995). In singing mice,
81 castrated males treated with T implants sing at lower frequencies than those receiving
82 empty implants (Pasch et al. 2011). In human men, high T levels are associated with low
83 voice pitch (due to lengthening and thickening of the vocal folds), which in turn correlates
84 with perceived social dominance, and may reflect immuno-competence (Dabbs and
85 Mallinger 1999; Archer 2006; Apicella and Feinberg 2009; Hodges-Simeon et al. 2014; Puts
86 et al. 2014).

87 Several bird studies support the idea that T has acute, activational effects on singing
88 motivation, but delayed, chronic effects on the acoustic structure of song (Ritschard et al.
89 2011). For example, Cynx et al. (2005) found that the F0 of arbitrarily chosen harmonic
90 stacks in zebra finch songs was not significantly different following three weeks of T
91 administration. After five weeks, however, the fundamental frequency was significantly
92 lower, and remained so for at least one year. T's effects on acoustic structure may involve
93 long-term changes to anatomical structures of the syrinx (Ritschard et al. 2011) and the
94 brain (Beani et al. 1995). It is unclear, however, whether a similar, long-term effect of T on
95 acoustic characteristics of calls occurs in non-avian species.

96 Literature on non-human primates (hereafter: primates), however, provides mixed
97 evidence for the claim that T influences calling behaviour. Whilst one recent study on white-
98 handed gibbons showed that male androgen levels affects the song pitch (Barelli et al.
99 2013), most studies have failed to find a relationship between T and calling. For example,
100 castration affected mouse lemur calling rates, but variation in natural levels of T did not
101 (Zimmermann 1996). Likewise, the production of long-distance calls in male black howler
102 monkeys was not correlated with T levels (Rangel-Negrín et al. 2011). Ontogenetic changes
103 in T levels did not affect call production in Thomas langurs (Wich et al. 2003). These

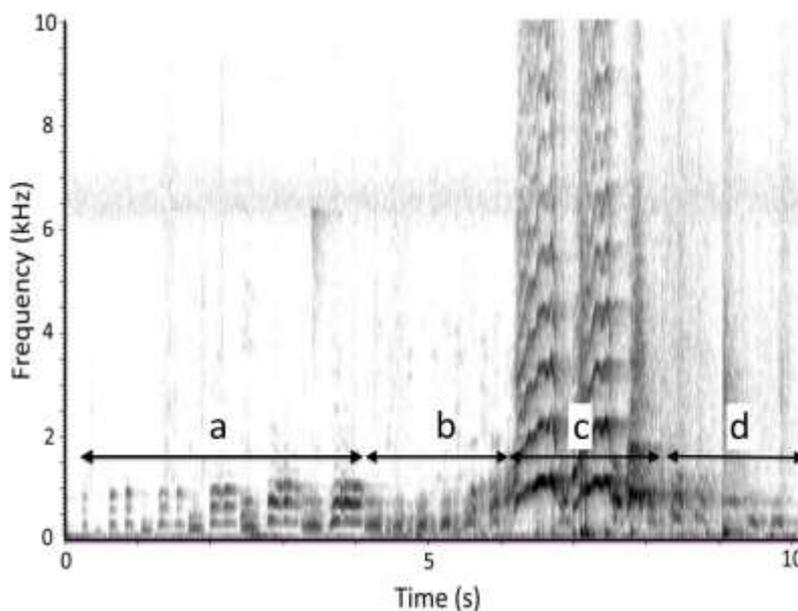
104 predominantly negative results have led some researchers to conclude that primate vocal
105 behaviour is less tightly linked to hormones than that of many animals, and that experience
106 and social factors play a more prominent role in highly intelligent species (Zimmermann
107 1996; Wich et al. 2003). However, such a conclusion is premature given how few primates
108 have been studied, the small sample sizes in these studies, and the diversity of call functions
109 in primates.

110 The purpose of this study was to investigate the relationship between T production
111 and vocal behaviour in wild male chimpanzees. Chimpanzees live in multi-male, multi-
112 female communities in which individuals form temporary subgroups (“parties”) that
113 frequently change in size and composition (Chapman et al. 1995; Aureli et al. 2008).
114 Chimpanzees produce long-distance “pant hoot” vocalizations that play a crucial role in
115 coordinating grouping in their unstable society (Mitani and Nishida 1993; Fedurek et al.
116 2014). Because chimpanzees appear capable of recognizing the calls of specific community
117 members (Mitani et al. 1996; Kojima et al. 2003), pant-hoots allow listeners to locate other
118 individuals.

119 Although male chimpanzees often cooperate with each other, and form strong social
120 bonds (Watts 2002; Mitani 2009), aggressive interactions are also important to the
121 achievement and maintenance of male status (Muller and Mitani 2005). It has been
122 suggested that chimpanzee pant hoots might be involved in male status competition. For
123 example, high-ranking individuals pant hoot more often than low-ranking ones, suggesting
124 that the call signals social status (Mitani and Nishida 1993; Clark and Wrangham 1994;
125 Fedurek et al. 2014). Males often pant hoot in choruses, and such chorusing may facilitate
126 coalition formation against other males (Fedurek et al. 2013a). In addition, pant-hooting

127 rates are elevated on days when between-male competition is high, for example when
128 valuable resources such as oestrous females or high-quality food are available (Fedurek et al
129 2014). Consequently, male pant hoots, apart from coordinating movements of community
130 members, may play an important signalling role in male-male competition. Although this
131 hypothesis has not been explored in detail, a positive relationship between pant-hooting
132 and T levels would be consistent with this function, since in many animals, ranging from
133 amphibians to mammals, the production of calls associated with mating or territorial
134 behaviour is regulated by T (Floody 1981; Van Duyse et al. 2002; Wilczynski et al. 2005).

135 A pant hoot has a distinct and complex acoustic structure (Fig. 1; Marler and Hobbett
136 1975). The call starts with a short introduction phase consisting of low-frequency elements.
137 The introduction is followed by the build-up phase, comprising short inhalations and
138 exhalation. The build-up grades into the climax, which is the loudest part of the call,
139 comprising one or several “screams” (climax elements) produced in succession.



140

141 **Fig. 1** A spectrogram showing the four phases of a pant hoot. a = introduction, b = build-up,
142 c = climax, d = let-down (from Fedurek et al. 2013b)

143 The call might be finalised by a short low-frequency let-down, which has similar acoustic
 144 features to the build-up. Although pant hoots may play an important role in male-male
 145 competition, it is unclear whether or how T may affect the acoustic features of this call. We
 146 sought to test the following three hypotheses of how T may affect the loudest part of the
 147 pant hoot call: the climax element (see Table 1). First, high levels of T may be correlated
 148 with low pitched vocalisations. As reviewed above, previous research has shown several
 149 species, including humans, to demonstrate this pattern and indicated that low pitched
 150 vocalisations are signals of good health or condition (Beani et al. 2000; Pasch et al. 2011;
 151 Puts et al. 2014). In addition, decreasing vocal tract resonant frequencies likely helps to
 152 exaggerate perceived body size, making an animal sound more formidable to competitors
 153 (Fitch and Reby 2001). In male chimpanzees, T levels appear to mediate aggressive
 154 behaviour towards other males (Muller and Wrangham 2004) and potentially reflect
 155 physical condition or health (Muehlenbein and Watts 2010), which in turn might be
 156 signalled by producing pant hoots with low F0. If this were the case, high T levels should be
 157 associated with pant hoot climaxes with low mean F0s.

Table 1 An outline of the two hypotheses regarding the relationship between T levels and acoustic features of the pant-hoot climax (the loudest element of this long-distance call)

| Hypothesis | Prediction |
|----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| High T levels are associated with pant-hoot climaxes with low pitch | T levels correlate negatively with mean pitch of the climax |
| High T levels are associated with pant-hoot climaxes with high pitch | T levels correlate positively with mean and maximum pitch of the climax or peak frequency at the start of the climax |

158

159 An alternative hypothesis, however, is that high levels of T may correlate with high
160 frequency vocalisations. For instance, male gibbons with elevated androgen levels produce
161 songs with higher pitch (Barelli et al. 2013). In several mammalian species, production of
162 calls with high rather than low F0 has been shown to reflect good physical condition (Taylor
163 and Reby 2010). For example, in baboons dominant individuals give calls with higher F0 than
164 low-ranking ones (Fischer et al. 2004) and, in red deer, stags with higher F0 are preferred by
165 oestrous females and have higher reproductive success (Reby and McComb 2003; Reby et
166 al. 2010). It has been suggested that high frequency calling may be an effective signal of an
167 individual's condition (Garcia et al. 2013). For example, the production of high F0 requires a
168 high sub-glottal pressure and large muscular effort that in turn may reflect an individual's
169 physical capacity or endurance (Titze 1989; Titze and Riede 2010). It is possible, therefore,
170 that males producing pant hoots with high rather than low frequency climax elements are
171 associated with high T levels. If this were the case, T levels should be positively associated
172 with the mean and maximum pitch of the climax of the call. In a similar vein, Riede et al
173 (2007) suggested that producing climaxes with high frequencies is physiologically costly
174 (e.g., Zahavi 1975, 1977) since it requires the caller to immediately switch from the low-
175 frequency build-up phase to the high-frequency climax phase, and that males in good
176 physical condition are more likely to produce climaxes with high initial frequencies that
177 contrast the low-frequency build-up elements. This "calling at the edge" model predicts
178 that T level should be most closely associated with F0 at the beginning of the climax, which
179 directly follows the low-pitched build-up. Consequently, we predicted that T levels should
180 be associated with the peak frequency at the start of the first climax.

181 In this study, we examined the relationship between T levels and different aspects of
182 male pant hooting behaviour in the Kanyawara community of chimpanzees, Kibale National
183 Park, Uganda. In particular, we examined whether there was both within- and between-
184 individual variation in terms of the relationship between T levels and pant hooting
185 behaviour. T levels in male chimpanzees fluctuate over time, on both a daily and monthly
186 basis, and diurnal variation in T levels is independent from seasonal or monthly variation
187 (Muller and Lipson 2003; Muller and Wrangham 2004). Therefore, we investigated whether
188 temporal changes in T production corresponded to temporal changes in both pant hoot
189 rates and key acoustic parameters of the pant hoot climax, such as peak frequency and
190 pitch. First, we examined whether annual T levels were correlated with annual rates of pant
191 hooting or acoustic features of the call. Second, we examined whether mean monthly levels
192 of T were associated with monthly rates of pant hooting or acoustic features of the call.
193 Since factors such as male fission-fusion rates with other males, time spent travelling, the
194 type of food consumed, the presence of a parous female in oestrus, and the rank of the
195 caller are known to substantially influence male pant hoot rates in chimpanzees (Mitani and
196 Nishida 1993; Fedurek et al. 2014), we examined whether T levels interacted with these
197 factors to influence the rate of call production. It is important to note, however, that the
198 purpose of this work was not to explore the function of pant hooting, but to provide insight
199 on a possible regulating mechanism by examining its association with T. Third, considering
200 that diurnal variation in T levels affects F0 of male voices (Evans et al. 2008) and that in
201 chimpanzees T levels decline steadily over the course of the day (Muller and Lipson 2003),
202 we investigated whether acoustic features of the pant hoot or rate of calling were
203 associated with mean hourly levels of T. With regard to the potential association between T
204 levels and the acoustic features of the call, we specifically examined whether high T levels

205 were associated with high or low F0 (pitch) of the call climax so as to test the two
206 hypotheses outlined above (summarised in Table 1).

207 Methods

208 *Study subjects and study site*

209 We collected the data between October 2010 and September 2011. The study was
210 conducted on the Kanyawara community in Kibale National Park, located in south-western
211 Uganda (0° 13'–0° 41' N and 30° 19'–30° 32' E). At the beginning of the study, in October
212 2010, the community included 54 individuals (including 10 adult males and 14 adult
213 females). Their home range covered approximately 14 km² of the park in 2006 (Wilson et al.
214 2012). Kibale is transitional between lowland rainforest and montane forest (Struhsaker
215 1975). The area occupied by the Kanyawara chimpanzees is a mosaic of semideciduous
216 primary forest, regenerated forest, grasslands, and swamps (Chapman and Wrangham
217 1993). The community is well habituated, and has been studied continuously since 1987 by
218 the Kibale Chimpanzee Project and also from 1983 to 1985 (Isabirye-Basuta 1987;
219 Wrangham et al. 1992). The study subjects were eleven adult males (i.e., ≥15 years old;
220 estimated mean age at the beginning of the study in October 2010=31.18 (SD=14.25 years;
221 Table 2)).

Table 2 Age (estimated age of the male) and dominance rank (rank; 1–11) of the focal males

| Male ID | Age (years) | Rank |
|---------|-----------------|------|
| AJ | 36 ^a | 3 |
| BB | 44 ^a | 4 |
| ES | 16 | 6 |
| KK | 25 | 1 |
| LK | 28 ^a | 2 |
| PB | 15 | 10 |
| PG | 22 | 7 |
| ST | 55 ^a | 8 |
| TJ | 15 | 5 |
| TU | 50 ^a | 9 |
| YB | 37 ^a | 11 |

^aEstimated age of the male

222

223 *Data collection*

224 a) Vocalisations

225 A randomly chosen male was followed by PF for a whole day (i.e., from nest to nest)
226 and all vocal behaviour of the focal male was noted including the context in which
227 vocalizations were produced (i.e., travelling, feeding and resting). Vocalisations were audio-
228 recorded from both the focal male and males present in his party where possible, using a
229 Marantz Professional PMD661 solid state recorder and a Sennheiser ME67 directional
230 microphone. Instantaneous scan samples were also conducted at 5-min intervals to record
231 (i) the behaviour of the focal male (i.e., travelling, feeding, and resting or grooming), (ii) the
232 type of any food consumed by the focal animal (see below), and (iii) the composition of the
233 focal male's party. In line with established protocols at this field site, the focal individual's
234 party was defined as all individuals within 50 m of him (Clark and Wrangham 1994). An
235 individual that had not been seen in the party within 15 min was excluded from the party
236 (Clark and Wrangham 1994). Data on all pant grunts received and given by the focal male
237 and all dyadic agonistic interactions (i.e., physical attack, chase, charge, displacements, etc.
238 (Bygott 1979; Goodall 1986; Muller and Wrangham 2004)) involving the focal were also
239 recorded. In total, 185 days of focal observations were collected. Only days of data
240 collection in which the focal was followed for at least 6 h were incorporated in the analyses
241 ($N=168$; mean=549.98 min of direct focal observation/day; see Supplementary Material for
242 the number of days of data collection per male per month). Since our study involved focal
243 animals in the field, it was not possible to record data blind.

244

245

246 b) Testosterone

247 Urinary T levels in chimpanzees show a clear diurnal pattern with the highest levels
248 in the early morning (from 5:00-9:00), followed by a steady decline through the day (Muller
249 and Lipson 2003). To control for this effect, in the analyses concerning annual and monthly
250 relationships between pant hooting and T, we incorporated only samples collected before
251 9:00 am ($N=141$, Mean=12.82 samples per focal male, $SD=7.13$; see Supplementary Material
252 for the number of T samples per male per month).

253 Urine samples were collected opportunistically from both focal individuals and
254 others in the focal party by four Kibale Chimpanzee Project field assistants. We regularly
255 collected first-morning samples, as chimpanzees predictably urinate upon waking. When a
256 chimpanzee urinated from a tree, we trapped the urine in a disposable plastic bag attached
257 to a two-meter pole. Urine samples were also collected opportunistically throughout the
258 day. Whenever possible, we captured samples on plastic; if a bag could not be placed in
259 time, then urine was pipetted from leaves in the ground layer of vegetation (Muller and
260 Wrangham 2004). After collection, we recorded the identity of the chimpanzee, the date,
261 and the time of urination. Specific gravity of the samples was assayed with a hand-held
262 refractometer (Atago PAL-10S). Samples were stored in a propane-powered freezer and
263 transported frozen to the Hominoid Reproductive Ecology Laboratory at the University of
264 New Mexico, where they were assayed for T.

265 Prior to analysis, we deconjugated urine samples with beta-glucuronidase (*Helix*
266 *pomatia*, Calbiochem, <2% aryl sulfatase activity) to recover the principal metabolite of
267 testosterone and performed an ether extraction (as in Muller and Wrangham 2004).
268 Samples were then assayed with a widely used enzyme immunoassay provided by the

269 University of California at Davis Clinical Endocrinology Laboratory (R156/7). The antibody
270 has strong cross-reactivity (57%) with 5alpha-dihydrotestosterone, which itself derives
271 exclusively from testosterone. Cross-reactivities with the adrenal androgen
272 androstenedione (0.3%) and other androgens/derivatives are minimal (< 0.04%). Inter-assay
273 coefficients of variation (CVs) were 13.9% and 11.9%, respectively, for a low and high urine
274 pool. Intra-assay CV, assessed from the mean CV of sample duplicate determinations, was
275 4.9%. Testosterone concentrations (in pg/ml) were standardized to specific gravity.

276

277 *Data collected and definitions*

278 Vocalisations

279 a) Pant hoot rates

280 We defined a call as a pant hoot only if it included the climax (Mitani and Gros-Louis
281 1998). We recorded, in total, 1320 pant hoots (see Supplementary Material for the number
282 of pant hoots recorded per male per month). We calculated a focal male's daily pant hoot
283 rate by dividing the total number of pant hoots he produced on a given day by the duration
284 of focal observations that day (Fedurek et al. 2014). We calculated monthly pant hoot rates
285 by averaging a male's daily pant hoot rates in a given month, which resulted in 12 data-
286 points per focal male, corresponding to the 12 months of data collection. Similarly, a focal
287 male's hourly pant hoot rates were calculated by averaging daily values for each one-hour
288 period (i.e., between 7am and 7:59, between 8am and 8:59, etc. up to 6pm).

289

290 b) Acoustic parameters

291 Only audio-recorded solo pant hoots were incorporated into the acoustic analyses
 292 ($N=144$ calls, Mean= 13.09 calls per focal male, $SD=8.93$; see Supplementary Material for the
 293 number of acoustically analyzed pant hoots per focal male per month), and acoustic
 294 parameters were taken from the climax of the pant hoot. Pant hoots were recorded in
 295 travelling ($N=67$ calls) and feeding ($N=77$ calls) contexts. If more than one climax was
 296 present in a call (which occurred in 47.30% of the pant hoots recorded), we measured the
 297 first climax in the sequence. We took three acoustic measurements from the climax (Table
 298 3).

Table 3 Acoustic measures taken from a pant-hoot climax

| Measure | Definition |
|----------------------|---------------------------------------------------------------------------------------------------|
| Peak frequency start | The frequency at which most acoustic energy was present in the F0 at the start of the climax (Hz) |
| Mean pitch | Mean pitch of the climax (Hz) |
| Maximum pitch | Maximum pitch of the climax (Hz) |

299

300 All acoustic measurements were conducted using Praat software (version 5.2.19).
 301 Peak frequency was determined by taking a spectral slice (frequency vs amplitude) at the
 302 start of the climax call. Pitch range was set to 75 -2500 Hz and pitch measurements (see
 303 Table 3) for the selected climax call were calculated automatically by Praat. As Praat was
 304 designed for the analysis of human speech, rather than chimpanzee calls, the tracking of
 305 pitch, as depicted by the pitch line on the spectrogram, was also visually checked to ensure
 306 that measurements were accurate.

307

308 Each focal male's mean monthly values for these three acoustic parameters were
 309 calculated by averaging these values from a given month. Similarly, each focal male's hourly
 310 values of these parameters were calculated by averaging daily values, across the entire

311 study period, for each one-hour period. Annual values of particular pant hoot characteristics
312 were calculated by averaging values over the whole study period.

313

314

315 Testosterone levels

316 A focal's hourly T levels were calculated by averaging values from each one-hour
317 interval between 07:00 and 18:00, across the entire study period. For each focal, monthly T
318 levels were calculated by averaging values from all samples collected prior to 9:00 am in a
319 given month. A focal's annual T levels were calculated by averaging values from all samples
320 collected prior to 9:00 over the entire study period.

321

322 Fission-fusion rates with males

323 The focal animal's fission–fusion rates with males were based on the number of
324 changes in the presence of males in the focal's party, as recorded during the instantaneous
325 scan samples of party composition over one day of data collection (Fedurek et al. 2014). For
326 example, if one or more males left or joined the party in one party composition scan,
327 compared with the previous scan, this was recorded as a single change in the composition of
328 males in the focal's party. The total number of scans containing changes in the composition
329 of males (mean=6.33 changes/day, SD=3.95) in the focal's party on a given day was then
330 divided by the number of hours of focal observation on that day. This represented the
331 focal's overall daily rate of fission–fusion with male community members. We then
332 calculated each male's monthly fission–fusion rates with males by averaging his daily
333 fission–fusion rates with males in a given month.

334

335 Time spent travelling

336 We established the proportion of time spent travelling by the focal male during a
337 given day of data collection by dividing the number of scans in which the focal was observed
338 travelling by the total number of scans on that day (Fedurek et al. 2014). We then calculated
339 monthly time spent travelling by averaging the focal male's daily time spent traveling in a
340 given month.

341

342 Social rank

343 Each male was assigned an ordinal linear rank (1–11, where 1 is the highest ranking).
344 The linear hierarchy was based on the outcomes of win–lose interactions combining pant
345 grunt and agonistic interactions recorded during the whole study period using focal animal
346 sampling (Muller and Wrangham 2004). The analysis was carried out using Matman
347 Software Package (version 1.1, Noldus Information Technology; de Vries, 1993). Male
348 dominance hierarchies were significantly linear using a two-step randomization procedure
349 with 10000 iterations (de Vries 1993, 1995).

350

351 Food type consumed

352 If, during the scan, the focal male was observed feeding, the species and plant part
353 consumed were recorded. Chimpanzees tend to form larger groups during seasons of fruit
354 abundance (Conklin-Brittain et al. 1998; Basabose 2002; see also Wrangham 2002), and the
355 consumption of fruits, especially non-fig fruits, correlates positively with energetic status in
356 the Kanyawara chimpanzees (Emery Thompson et al. 2009). We established the proportion
357 of time spent feeding on non-fig fruits by the focal male during a given day of data collection

358 by dividing the number of scans in which he was observed consuming non-fig fruits by the
359 total number of scans during which he was observed feeding (Fedurek et al. 2014). We then
360 calculated monthly proportion of time spent feeding on non-fig fruits by averaging his daily
361 time spent feeding on this food type in a given month.

362

363 The presence of an oestrous female

364 Females were considered to be in oestrous when their genital swellings were
365 maximally swollen. For each day of data collection, we recorded whether ($N=61$) or not
366 ($N=107$) a parous female ($N=12$) in oestrous was present (i.e., during at least one party
367 composition scan) in the focal male's party. For each focal individual, we calculated the
368 monthly proportion of days in which a parous oestrous female was present in his parties by
369 dividing the number of focal days in which such females were present in his party in a given
370 month by the total number of days in which that male was a focal during that month.

371

372 *Statistical analyses*

373 Linear mixed-effect models (LMM) were used in the analyses as the main statistical
374 tools. LMM deals effectively with the problem of non-independence of data by
375 incorporating into the model data on entities from which repeated measurements were
376 taken as 'random effects'. In all of our models we put the ID of the caller as a random effect.
377 There was no collinearity between the examined independent variables (variance inflation
378 factors (VIF) of the independent variables were below the value of 2 (Mean=1.34)), which
379 allowed us to include all of the independent variables in the same model. Before running
380 the analyses, the values of all quantitative variables were z-transformed to a mean of 0 and

381 standard deviation of 1. We used Stata 12.0 (StataCorp LP) and SPSS 22 (IBM Corp) software
382 for all statistical analyses.

383

384 Models created

385 To investigate the relationship between monthly pant hoot rates and monthly T
386 levels, we created a LMM with monthly pant hoot rate as the dependent variable, and
387 monthly T levels as the independent variable. In this model we also included the following
388 independent variables: the rank of the caller, focal's monthly rates of fission-fusion with
389 other males, focal's monthly proportion of time spent traveling, focal's monthly proportion
390 of time spent feeding on non-fig fruits, and focal's monthly proportion of days in which
391 there was an oestrous parous female present in his party. To investigate the relationship
392 between pant hoot rates and the above independent variables within a specific period of
393 the study, we included in the model only data-points from the months of interest. We then
394 created five additional models with monthly pant hoot rates as the dependent variable. In
395 these models we included interactions between monthly levels of T and the above
396 independent variables, to investigate whether T level interacted with these variables in
397 terms of its influence on pant hoot rates.

398 The acoustic structure of a pant hoot may depend on the context in which it is
399 produced (Notman and Rendall 2005). To examine whether acoustic parameters differed
400 between pant hoots given in travelling and feeding contexts, we created three LMMs. The
401 dependent variables in these models were the average monthly values for the following
402 acoustic measures: 1) peak frequency at the start of the climax or 2) mean pitch of the
403 climax or 3) maximum pitch of the climax, and the independent variable was whether the
404 pant hoot was produced in the travelling (0) or feeding (1) context.

405

406 To investigate the relationship between monthly values of particular acoustic
407 parameters and T levels, we created three models. The dependent variables in these models
408 were the average monthly values for the following acoustic measures: 1) peak frequency at
409 the start of the climax or 2) mean pitch of the climax or 3) maximum pitch of the climax.
410 Mean monthly T level was entered as the independent variable. Since peak frequency or
411 pitch of the climax might be mediated by social rank and age (Riede 2007), we included
412 these attributes as additional independent variables. We then created additional models
413 with interactions between significant independent variables to investigate whether these
414 factors exerted independent influence on pant hooting.

415 To investigate the relationships between hourly pant hoot rates or hourly values of
416 particular pant hoot acoustics and hourly levels of T, we created four LMMs. The dependent
417 variables in these models were the average monthly values for the following measures: 1)
418 pant hoot rates or 2) peak frequency at the start of the call or 3) mean pitch of the climax or
419 4) maximum pitch of the climax. Independent variables were the hourly level of T, and the
420 age and social rank of the individual.

421 To investigate the relationship between annual T levels and annual values of pant
422 hoot rates or particular acoustic features of the call for each male, we used Spearman's rank
423 correlations ($N=11$ males).

424 In situations where multiple post hoc tests were conducted on the same dataset, the
425 α -level for significance was corrected using Sidak's adjustment equation (Sidak 1967) to
426 control for family-wise error.

427

428 Results

429 *Pant hoot rates and testosterone levels*

430 Annual testosterone levels and pant hoot rates

431 There was no significant correlation between yearly individual pant hoot rates and T
 432 levels ($r_s=0.236$, $P=0.484$, $N=11$).

433 Monthly testosterone levels and pant hoot rates

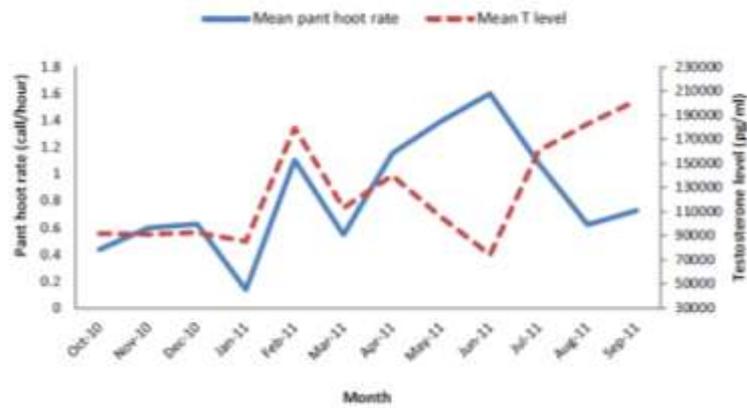
434 There was a positive relationship between a male’s monthly levels of T and his
 435 monthly pant hoot rates (Table 4, Fig. 2). Male monthly proportion of time spent travelling,
 436 fission-fusion rates with other males, proportion of time spent on feeding on non-fig fruits,
 437 and his social rank were also significantly associated with monthly pant hoot rates (Table 4).
 438 The variable “fission-fusion rates with other males” was most strongly associated with pant
 439 hoot rates (Table 4). There was no interaction between male monthly T levels and monthly
 440 proportion of days in which there was a parous oestrus female in his party ($P=0.616$),
 441 fission-fusion rate with other males ($P=0.281$), proportion of time spent travelling ($P=0.828$),
 442 proportion of non-fig fruits consumed ($P=0.818$), or male social rank ($P=0.801$), suggesting
 443 that these variables predicted pant hoot rates independently from T.

Table 4 The relationship between monthly pant-hoot rates and the investigated (independent) variables (LMM; dependent variable: monthly pant-hoot rates; random effect: focal ID)

| Independent variables | Coefficient | Standard error | z value | P value | 95 % confidence interval | |
|---------------------------------|-------------|----------------|---------|---------|--------------------------|-------|
| Testosterone level | 0.19 | 0.09 | 2.08 | 0.037 | 0.01 | 0.36 |
| Time travel | 0.26 | 0.09 | 2.98 | 0.003 | 0.09 | 0.43 |
| Rank | -0.27 | 0.11 | -2.50 | 0.012 | -0.48 | -0.06 |
| Fission-fusion rates with males | 0.41 | 0.08 | 4.64 | ≤0.001 | 0.24 | 0.58 |
| Presence of oestrous female | 0.18 | 0.09 | 1.91 | 0.056 | -0.00 | 0.37 |
| Non-fig fruit consumption | 0.25 | 0.09 | 2.55 | 0.011 | 0.06 | 0.45 |

444

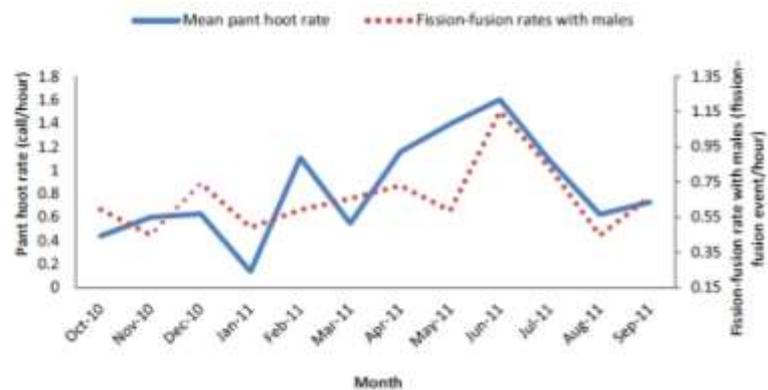
Fig. 2 Mean monthly pant-hoot rates and testosterone levels



445

446 The relationship between pant hoot rates and T levels was strongest between October 2010
 447 and April 2011 ($\beta \pm SE = 0.32 \pm 0.10$, $z = 3.22$, $P = 0.001$), and weakest between May and
 448 September 2011 (Fig. 2; $\beta \pm SE = 0.09 \pm 0.11$, $z = 0.83$, $P = 0.405$). In contrast, there was a positive
 449 relationship between pant hoot rates and fission-fusion rates with males between May and
 450 September 2011 ($\beta \pm SE = 0.23 \pm 0.10$, $z = 2.25$, $P = 0.024$), but not between October 2010 and
 451 April 2011 (Fig. 3; $\beta \pm SE = 0.14 \pm 0.10$, $z = 1.39$, $P = 0.163$).

Fig. 3 Mean monthly pant-hoot rates and fission-fusion rates with males



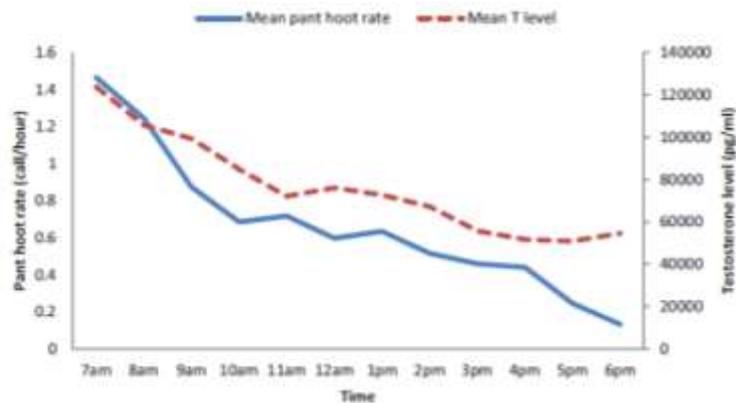
452

453 Hourly testosterone levels and pant hoot rates

454 There was a positive relationship between male hourly pant hoot rates and hourly T
 455 levels (Fig. 4; $\beta \pm SE = 0.47 \pm 0.09$, $z = 5.35$, $P \leq 0.001$). There was also a negative relationship
 456 between male hourly pant hoot rates and social rank, with high-ranking individuals calling
 457 more often than low-ranking ones ($\beta \pm SE = -0.29 \pm 0.14$, $z = -2.10$, $P = 0.036$). There was a

458 significant interaction between hourly T levels and male social rank in predicting pant hoot
 459 rates, with high-ranking males showing a stronger relationship between T levels and pant
 460 hoot rates ($\beta \pm SE = -0.15 \pm 0.08$, $z = -1.98$, $P = 0.047$). There was no significant relationship
 461 between male hourly pant hoot rates and his age ($\beta \pm SE = 0.09 \pm 0.14$, $z = 0.65$, $P = 0.517$).

Fig. 4 Mean hourly levels of pant-hoot rates and testosterone levels



462

463 *Pant hoot acoustic features and testosterone levels*

464 Acoustic features of pant hoots produced in travelling and feeding contexts

465 The acoustic features of pant hoots recorded in travelling and feeding contexts did
 466 not differ significantly in terms of the peak frequency at the start of the climax ($P = 0.313$),
 467 and the mean ($P = 0.534$) and maximum ($P = 0.231$) pitch of the climax, which allowed us to
 468 include acoustic values from these two contexts in the same models.

469 Annual testosterone levels and acoustic parameters of calls

470 Table 5 shows that there was both within- and between-individual variation in terms
 471 of the examined acoustic parameters. There was a significant positive correlation between
 472 mean annual T levels and mean peak frequencies at the start of the climax ($r_s = 0.782$,
 473 $P = 0.004$, $N = 11$, Sidak-corrected α level for significance = 0.006, Fig. 5). Age ($r_s = -0.469$,
 474 $P = 0.145$, $N = 11$) and rank ($r_s = 0.273$, $P = 0.446$, $N = 11$) did not correlate significantly with peak

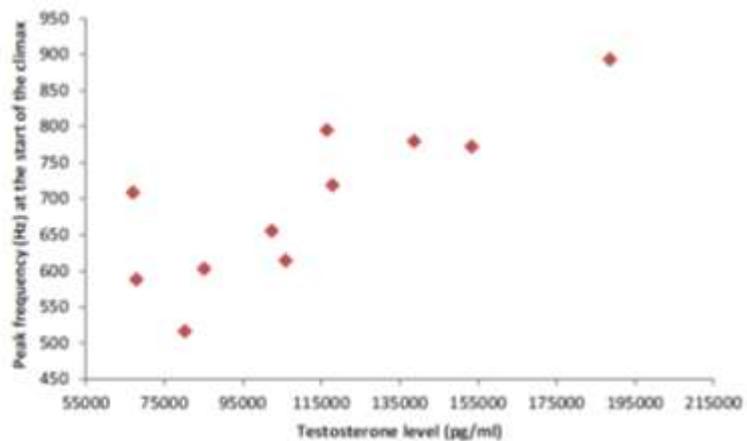
475 frequency at the start of the climax. There was no significant correlation between annual T
 476 level and annual mean ($r_s=0.445$, $P=0.170$, $N=11$) or maximum ($r_s=0.364$, $P=0.272$, $N=11$)
 477 pitch of the climax.

Table 5 Mean (\pm SD) values of the examined acoustic parameters of the pant-hoot climax for each male: peak frequency start, mean pitch and maximum pitch

| Male ID | Peak frequency start (Hz) | Mean pitch (Hz) | Maximum pitch (Hz) |
|---------|---------------------------|----------------------|----------------------|
| AJ | 655.33 \pm 131.56 | 1012.35 \pm 151.28 | 1250.02 \pm 113.25 |
| BB | 708.50 \pm 122.80 | 1178.41 \pm 119.65 | 1391.76 \pm 160.77 |
| ES | 779.64 \pm 174.76 | 1065.50 \pm 176.04 | 1379.69 \pm 135.81 |
| KK | 772.00 \pm 145.69 | 1231.01 \pm 257.53 | 1470.59 \pm 243.31 |
| LK | 516.40 \pm 81.49 | 662.44 \pm 206.03 | 801.75 \pm 252.59 |
| PB | 718.50 \pm 207.18 | 1150.67 \pm 103.21 | 1359.34 \pm 12.86 |
| PG | 614.09 \pm 164.96 | 1023.48 \pm 167.09 | 1259.19 \pm 170.32 |
| ST | 602.77 \pm 120.40 | 739.36 \pm 126.44 | 904.59 \pm 70.80 |
| TJ | 794.97 \pm 152.57 | 1161.92 \pm 202.17 | 1354.48 \pm 162.74 |
| TU | 588.00 \pm 80.61 | 853.20 \pm 203.54 | 986.03 \pm 190.50 |
| YB | 892.71 \pm 214.73 | 1095.85 \pm 217.60 | 1249.21 \pm 188.68 |

478

Fig. 5 The relationship between mean annual levels of testosterone and mean peak frequencies at the start of the climax



479

480 Monthly testosterone levels and acoustic parameters of calls

481 There was a positive relationship between monthly values of peak frequency at the
 482 start of the climax and monthly T levels ($\beta\pm SE=0.37\pm 0.15$, $z=2.47$, $P=0.014$). Age ($\beta\pm SE=-$
 483 0.47 ± 0.15 , $z=-3.29$, $P=0.001$), but not rank ($\beta\pm SE=0.11\pm 0.15$, $z=0.70$, $P=0.481$) of the focal
 484 was also significantly associated with peak frequency at the start of the climax, with younger
 485 individuals having higher peak frequency. There was a non-significant negative interaction
 486 between T levels and age in predicting peak frequency ($\beta\pm SE=-0.24\pm 0.13$, $z=-1.82$, $P=0.069$),

487 with the relationship between T levels and peak frequency at the start of the climax being
488 stronger for younger individuals.

489 There was no relationship between monthly T levels and mean ($\beta \pm SE = 0.21 \pm 0.13$,
490 $z = 1.56$, $P = 0.119$) or maximum ($\beta \pm SE = 0.22 \pm 0.14$, $z = 1.58$, $P = 0.113$) pitch of the climax.

491 Hourly testosterone levels and acoustic parameters of calls

492 There was no relationship between hourly T levels and hourly values of peak
493 frequency at the start of the climax ($\beta \pm SE = -0.05 \pm 0.13$, $z = -0.37$, $P = 0.712$), mean ($\beta \pm SE = -$
494 0.17 ± 0.10 , $z = -1.73$, $P = 0.085$) or maximum ($\beta \pm SE = 0.21 \pm 0.13$, $z = 1.56$, $P = 0.119$) pitch of the
495 climax.

496

497 Discussion

498 Our results are consistent with the hypothesis that T levels in male chimpanzees
499 mediate pant hooting behaviour. Temporal changes in T levels corresponded with temporal
500 changes in pant hoot rates, at both hourly and monthly scales. Monthly and annual mean T
501 levels were correlated with the peak frequency at the start of the pant hoot climax.
502 Therefore, on a proximate level, pant hoot production in male chimpanzees seems to be
503 mediated by T. In this respect, our results are consistent with studies of vocal behaviour in
504 many non-primates showing that T influences long-distance calling (Floody 1981; Nowicki
505 and Ball 1989; Marler and Ryan 1996). The positive relationship between chimpanzee pant
506 hooting and T levels therefore implies that, even though an important function of long-
507 distance calls concerns contact maintenance, pant-hoots are also associated with male-male
508 competition (Fedurek et al. 2014).

509 Although monthly pant hoot rates were associated with monthly T levels, this
510 relationship was not as clear as in the hourly relationship between these two variables.
511 Extrinsic factors such as a male's fission-fusion rates with other males, time spent travelling
512 and the type of food consumed also predicted monthly pant hoot rates independently from
513 T (see also Fedurek et al. 2014). Indeed, the relative influence of monthly T levels and
514 extrinsic factors such as fission-fusion rates with other males varied in different seasonal
515 time periods. It is clear that multiple factors influence pant hooting rates and this may
516 reflect the numerous social functions of this call (Mitani and Nishida 1993; Fedurek et al.
517 2014). It is likely, therefore, that the relationship between long calling and T in chimpanzees
518 is not as strong as in animals that perform long calls solely as mating or territorial displays.
519 Our study, however, is not consistent with the view that primate vocal behaviour is fully
520 independent from the influence of gonadal hormones (Zimmermann 1996). Our results
521 shows that the strength of the relationship between T levels and particular call parameters
522 depends on the time scale investigated. It is thus possible that in the studies on primate
523 species where no relationship between call production and testosterone was found
524 (Zimmermann 1996; Wich et al. 2003; Rangel-Negrín et al. 2011), exploring this relationship
525 on different time scales would produce different results.

526 Our study has several limitations. Most notably, this was an opportunistic study in
527 which we utilised two datasets, one on vocal behaviour, and another on T production by the
528 Kanyawara chimpanzees. Although collected over the same period, these data were
529 associated with independent projects. Consequently, we were unable to investigate the
530 association between pant hooting and T levels on shorter timescales, which arguably would
531 be more informative regarding the cause-effect nature of this association. Having data on T

532 levels of the caller during the time of calling would be especially informative about the
533 cause-effect relationships between calling and T production. Considering the multiple
534 functions of pant hooting, it is possible that the strength of the relationship between T and
535 pant hooting depends on contextual details of emission. We also note that, even though we
536 found a positive relationship between calling and T, with our data it is not possible to
537 investigate whether T influences calling rates directly, or indirectly through some other
538 behaviour or mechanism associated with T.

539 Our results provide some evidence that T levels are related to some acoustic
540 features of the call. Individuals with the highest T levels over the study period produced, on
541 average, pant hoots with the highest peak frequency at the beginning of the climax.
542 Similarly, although there was considerable individual variation in terms of the peak
543 frequency of the pant hoot climax, during months of elevated T production, males
544 produced pant hoot climaxes with higher initial peak frequencies. Our results contrast with
545 studies on humans and other animals where high T levels are associated with low F0 in
546 males (e.g., Titze 1994; Cynx et al. 2005; Puts et al. 2014). Our study, however, lends some
547 support to T levels being associated with higher frequency calls, and is consistent with a
548 recent study on male gibbons showing a positive association between androgen levels and
549 song pitch (Barelli et al. 2013). It is important to note, however, that we only found a
550 positive relationship between peak frequency at the start of the climax and T levels, not the
551 mean or max pitch. Thus, the elevated frequencies produced by individuals with high T
552 levels were not sustained over the climax element of the call. None the less, our result
553 provides some support for the 'calling at the edge' hypothesis (Riede et al. 2007), suggesting
554 that T levels should correlate with F0 especially at the start of the climax.

555 The effect of T on peak frequency of the climax might be partially confounded by
556 age. Age correlated negatively with F0 at the start of the call, suggesting that younger
557 individuals are more capable of producing calls with high frequencies than older ones. Age is
558 negatively correlated with F0 in many other mammals, mainly because thinner and shorter
559 vocal folds in young individuals generate higher pitched sounds than thicker and longer
560 vocal folds characterising older individuals (Taylor and Reby 2010). Our results suggest that
561 monthly changes in T levels also accounted for the variance in peak F0 of the climax, even
562 though this process was stronger for younger individuals. This suggests that monthly
563 fluctuations in T levels indeed relate to the F0 of the climax, with age modulating rather
564 than driving this process. We suggest that future research uses playback experiments to test
565 whether chimpanzee listeners are sensitive to the subtle acoustic differences we have
566 identified here. Moreover, it is crucial to test whether this acoustic parameter is an effective
567 signal of condition or dominance. Whether chimpanzee males that produce calls containing
568 higher frequencies are perceived as more dominant by other males remains to be
569 empirically tested.

570 We found no evidence that hourly T levels affect the acoustic structure of the call.
571 This finding seems to contrast with studies on male voice in humans, which show that F0 is
572 sensitive to daily circulating levels of T (Evans et al. 2008). Pant hoot rates, by contrast, were
573 associated with T levels at the hourly but not yearly scale. This is consistent with the
574 suggestion that the motivation to call is mediated by T (e.g., Taylor and Reby 2010). Thus, in
575 line with previous studies on birds (Beani et al. 1995; Cynx et al. 2005; Ritschard et al. 2011),
576 overall vocal activity may be sensitive to short-term changes in T levels, whereas the
577 acoustic structure of the call may be influenced by T in the long term. This interpretation is

578 consistent with studies on birds showing that while short-term changes in T levels affect call
579 production, the acoustic structure of calls is influenced by T on a long-term basis. This may
580 be because, for example, long-lasting high levels of T induce anatomical changes in the
581 structures associated with the vocal tract, thereby allowing greater sub-glottal pressure. It is
582 possible, therefore, that the chronic effect of T on the acoustic structure of calling is not an
583 avian-specific process, but also occurs in chimpanzees and other mammals. Alternatively,
584 the delay between T production and urine excretion might have obscured the results
585 concerning the hourly effect of T on the subtle acoustic features of the call. Although more
586 research is needed to explore the influence of T on the acoustic structure of primate calls,
587 our study is consistent with studies on anuran and avian species showing that some aspects
588 of the acoustic structure of calling are related to T levels (Solis 1994; Cynx et al. 2005).

589 Pant hoots seem to play an important role in signalling the social status of the caller
590 and facilitating coalitions against other males (Clark and Wrangham 1994; Fedurek et al.
591 2013a), and therefore likely contribute to male fitness. However, little is known about the
592 features that would make pant hoots an effective signal in this respect. In addition to
593 containing salient acoustic cues to individual identity (Mitani et al. 1996), our data suggest
594 that the rate of calling and initial peak frequency of the climax may reflect social status and
595 hormonal state, as we have shown these are related to T levels. It is possible that these two
596 T-related properties of pant hooting signal the likelihood with which the caller will be
597 involved in agonistic interactions or coalitions against other males when challenged (e.g.,
598 Fedurek et al. 2013a, b). Playback experiments examining the listener's responses to
599 manipulations of call rate and the initial peak frequency of the climax element are needed
600 to test this hypothesis directly. Similarly, considering that the initial peak frequency of the

601 climax element of the call is associated with T levels and that high-ranking males often have
602 higher T levels than low-ranking ones (Muller and Wrangham 2004), it would be interesting
603 to examine whether a change in the male hierarchy brings about changes in this feature of
604 the call. There is anecdotal support for the hypothesis that in chimpanzees, descent in the
605 status hierarchy corresponds with a drop in the dominant frequency of the climax of the call
606 (Riede et al. 2007).

607 Although we did not incorporate females in our study, it would be interesting to
608 examine the relationship between T levels and female vocal behaviour. Female chimpanzees
609 pant-hoot less frequently than males (Notman 2003), which on a proximate level may be the
610 result of lower T levels in females. In other apes, call inhibition of unflanged orang-utan
611 males might be mediated by the low T levels recorded in these males (Knott 2009). Similarly,
612 the elevated long-calling behaviour of high-ranking compared to low-ranking males
613 observed in baboons and black-crested macaques (Fischer et al. 2004; Neumann et al. 2010)
614 might also be partly explained by differences in T levels. More studies are needed to
615 investigate short-term and long-term effects of T on vocal behaviour in apes and other
616 primates.

617 In conclusion, our data show that temporal changes in T levels correlate with aspects
618 of male chimpanzee pant hoot behaviour, including the rate at which the calls are produced
619 and the initial frequency of the climax element of the call. Our study is consistent with the
620 hypothesis that, on a proximate level, male chimpanzee vocal behaviour is mediated by T
621 levels, and thus pant hoots likely play a role in male-male competition.

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635

636 Ethical standards

637 Permission to conduct the study was granted by the Uganda Wildlife Authority and
638 the Uganda National Council for Science and Technology. The study complied with the
639 current laws of Uganda. This study was approved by, and carried out in accordance with, the
640 Department of Psychology Ethics Committee at the University of York.

641

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