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3 Effects of dominance and female presence on secondary sexual characteristics in male tufted
4 capuchin monkeys (*Sapajus apella*)

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20 Abstract

21 Alpha status may lead to physiological changes that enhance secondary sexual characteristics,
22 which may serve as competitive signals to conspecific males, sexual signals to females, or
23 possibly a combination of both. Here we report measurements of secondary sexual
24 characteristics in captive dominant and subordinate male tufted capuchin monkeys (*Sapajus*
25 *apella*) with varying access to females. An adult male (who had previously been subordinate
26 while housed with other males) was paired with an adult female, and then this male-female
27 pair was introduced into a room that housed three other male-male pairs with stable hierarchy
28 arrangements. We analyzed weight, body measurements, facial photographs, and hair cortisol
29 before, during, and after introducing a female into the room. While there were no differences
30 in weight or measurements between alphas and subordinates without physical access to the
31 female prior to or during the female's presence, we found that direct access to the female
32 resulted in dramatic changes in facial appearance, body size, and testicular volume in the male
33 who was paired with her. Overall, we found little evidence to suggest that alpha males
34 advertise their status within all male groups via sexual secondary characteristics. However,
35 direct physical access to females appears to trigger the development of such characteristics in
36 alpha males. It remains of continued interest to identify the endocrine mechanisms responsible
37 for the development, and possible loss, of secondary sexual characteristics.

38

39 Keywords: secondary sexual characteristics; face; testes; weight; alpha male

40 Introduction

41 Studies of development frequently focus on how infants and juveniles mature and become
42 adults, a milestone frequently demarcated by reaching reproductive age (McNamara, 2004).
43 However, in many species, including humans, reproductive capability is often reached before
44 physical development is complete (Daly & Wilson, 1983). Reproductively capable individuals
45 may lack species-typical secondary sexual characteristics, such as male orangutans (*Pongo spp.*)
46 that remain unflanged (Utami, Goossens, Bruford, de Ruiter, & van Hoof, 2002). In some
47 species, sexual secondary characteristics may develop rapidly within just a few months (e.g.,
48 final growth spurt in male long tailed macaques, *Macaca fascicularis*; Van Noordwijk & van
49 Schaik, 2001). Some of these physiological changes in males appear to be linked to
50 reproduction itself and thus triggered by environmental cues, either seasonally or through
51 female behavior (e.g., zebra finches, *Taeniopygia guttata*; Gautier et al., 2008). In group living
52 species, increases in dominance rank, and in particular achievement of alpha male status, which
53 is typically linked to increased reproductive opportunities, may lead to physiological changes
54 such as increases in testicular volume and fattenedness (e.g., mandrills, *Mandrillus sphinx*;
55 Setchell & Dixson, 2001), as well as development of specific adornments (e.g., blue scrotal color
56 in vervet monkeys, *Chlorocebus pygerythrus*, Gerald, 2001; reddening of the sexual skin on the
57 face and genitalia of mandrills, Setchell & Dixson, 2001; stained chests in Verreaux's sifaka,
58 *Propithecus verreauxi*, Lewis & van Schaik, 2007; redness of gelada baboon chest, *Theropithecus*
59 *gelada*, Dunbar, 2014). Loss of alpha status may reverse some of these status indicators
60 (mandrills, Setchell & Dixson, 2001; geladas, Dunbar, 2014; sifakas, Lewis & van Schaik, 2007;
61 see also Georgiev et al., 2016, for effects of loss of alpha status in a male rhesus macaque).

62

63 Changes in appearance may serve as a signal of alpha status to females (intersexual selection)
64 or to other males within the group (intrasexual selection), or potentially both. As sexual signals
65 tend to mirror the health status of the bearer (Lozano, 1994), females may be able to assess a
66 male's genetic quality (e.g., peafowls, *Pavo cristatus*; Petrie, 1994), while males may avoid
67 physical fights that are costly to both parties (e.g., rhesus macaque, *Macaca mulatta*;
68 Petersdorf, Dubuc, Georgiev, Winters, & Higham, 2017; mandrills, Setchell & Dixson, 2001). The
69 utility of signals seems to be more beneficial between individuals who are largely unknown to
70 each other (Setchell & Wickings, 2005; Bergman, Ho, & Beehner, 2009), but signals can also
71 occur in species where individuals have a long history of prior interaction (e.g., wahoo calls in
72 baboons, *Papio cynocephalus ursinus*; Kitchen, Seyfarth, Fischer, & Cheney, 2003) and in large
73 socially complex groups (Grueter, Isler, & Dixson, 2015). A signal that is costly for a male to
74 produce or maintain may be downregulated in the absence of a receiver, i.e., females in the
75 case of intersexual selection or males in the case of intrasexual selection (Gautier et al., 2008).

76

77 Tufted capuchin monkeys (*Sapajus apella*; Figure 1) are moderately sexually dimorphic with
78 males exceeding females in body mass (Fragaszy, Visalberghi & Fedigan, 2004). Physical features
79 of male capuchins may change as a consequence of achieving alpha status, although this has
80 only been observed anecdotally. For instance, alpha male bearded capuchins (*Sapajus*
81 *libidinosus*) are described as bulkier in the head and forequarters than other males (Fragaszy,
82 Izar, Eshchar, Young, & Visalberghi, 2016), and in white-faced capuchins (*Cebus capucinus*),
83 males are described as developing an exaggerated brow ridge and a widened mandibular girth

84 after they achieve alpha status, making alpha status a distinct life-history stage (Jack et al.,
85 2014) with keystone functions (Jack & Fedigan, 2018). Since female capuchins actively solicit
86 males for mating (Alfaro, 2005; Falotico & Ottoni, 2013) with the alpha male being the
87 preferred mating partner in approximately 70% of matings (Fragaszy, Visalberghi, & Fedigan,
88 2004), distinct appearance may be one of several possible signals that could advertise alpha
89 status to females. Although capuchin males rarely compete directly with other males over
90 copulations with females, they compete indirectly by vying for alpha rank within a group
91 (Schaebs, Perry, Cohen, Mundry, & Deschner, 2016). Appearance could therefore also be a
92 useful signal for other males, leading to low in-group male-male aggression in capuchins (Lynch,
93 Ziegler, & Strier, 2002). In addition, male bearded capuchins gain body mass after they achieve
94 alpha status and may lose body mass when alpha position is lost (Fragaszy et al., 2016). It is less
95 clear if this change in body mass is a consequence of physiological changes associated with
96 achieving alpha status (such as an increase in testosterone production; Mendonca-Furtado et
97 al., 2014), or whether it is a consequence of the benefits of being alpha (e.g., being able to
98 monopolize food sources; di Bitetti & Janson, 2001).

99

100 While anecdotal evidence suggests that alpha capuchin monkeys develop secondary sexual
101 characteristics, empirical work on this topic is limited and it remains unclear whether these
102 changes are partially explained by the social benefits of alpha status (e.g., increased food
103 access). Here, we sought to determine whether alpha male status in tufted capuchin monkeys is
104 associated with distinct secondary sexual characteristics, and whether the expression of these
105 characteristics is dependent on the presence of an adult female, by measuring the physical

106 condition and facial appearance of captive male tufted capuchin monkeys with varying access
107 to a female. The current study was conducted with the tufted capuchin monkey colony at the
108 Laboratory of Comparative Ethology, National Institutes of Health Animal Center near
109 Poolesville, Maryland, USA, which experienced changes to their housing arrangements
110 necessitated by facility management decisions. These changes resulted in an adult female being
111 paired with an adult male (JR), who was previously low-ranking in an all-male group. The newly-
112 established male-female pair was housed in a colony room with visual, auditory, and olfactory
113 contact to three other male-male capuchin pairs with stable dominance relationships. JR
114 therefore became the only male to have direct access to the female while all other males had
115 visual/auditory/olfactory contact, but no physical contact, with the female. As part of a
116 longitudinal, unrelated research project, we had collected physical measurements and facial
117 photographs of all male monkeys prior to this change in housing arrangements. We thus used
118 these measurements to retrospectively document physical changes exhibited by JR following
119 his pairing with the female, and to explore how these changes compared to the physical
120 characteristics exhibited by the other males (both alpha and subordinate). Since food and other
121 environmental factors were held constant throughout the study period, any changes in physical
122 appearance in JR can be attributed to changes in the social environment, not to a secondary
123 effect of access to higher quality or quantity of food.

124

125 We hypothesized that males advertise their alpha status primarily to other males, based on
126 previous findings that male, but not female, tufted capuchins are sensitive to facial symmetry in
127 other male capuchins (Paukner, Wooddell, Lefevre, Lonsdorf, & Lonsdorf, 2017), and male

128 capuchins spent more time looking at pictures of male capuchin faces compared to female
129 capuchin faces (Lonsdorf, Engelbert, & Howard, 2019). However, the presence of a female
130 might further enhance the expression of secondary sexual characteristics, especially in alpha
131 males who might gain mating opportunities. These gains could be dependent on the presence
132 of the female, i.e., without female presence and potential mating opportunities, alpha males'
133 secondary sexual characteristics could be costly to maintain and might revert to previously seen
134 levels. We were also able to gain insights into some of the underlying physiology of these
135 changes by analyzing glucocorticoids in hair samples collected after the introduction and later
136 removal of the female.

137

138 We predicted that:

139 1. Prior to the introduction of a female, alpha males housed in same-sex pairs would be bigger
140 (in weight, facial measurements, body measurements) than subordinate males.

141 2. Introduction of a female would result in increased weight, face measurements, and body
142 measurements in all alpha males. However, the male with direct access to the female (JR)
143 would show a significantly larger increase in measurements relative to the males without direct
144 access.

145 3. If alphas experienced a gain in their weights, measurements, and facial appearance following
146 the introduction of the female, then removal of the female might reverse some these gains.

147

148 Unfortunately we were only able to investigate the last prediction in the same-sex pairs as JR
149 remained housed with the female for the duration of the study.

150

151 Methods

152 All data were collected at the Laboratory of Comparative Ethology, National Institutes of Health
153 Animal Center, near Poolesville, Maryland, USA, between April 2012 and April 2016. All
154 applicable national and institutional guidelines for the care and use of animals were followed.
155 All procedures were in accordance with the ethical standards of the institution at which the
156 studies were conducted (*Eunice Kennedy Shriver* National Institute of Child Health and Human
157 Development Animal Care and Use Committee, ASP #12-015 and #15-015).

158

159 *Subjects.*

160 We studied seven males (aged between 5y1m-9y9m in April 2012; Table 1) in conjunction with
161 one adult female (aged 12y9m in April 2012). All monkeys were born and reared in captive
162 social groups. All monkeys were housed indoors for the duration of the study and received their
163 regular diet of commercial monkey biscuits (Purina Monkey Chow, #5054, St Louis, MO) as well
164 as twice daily enrichment (scatter feed of grains or seeds in the mornings, fruit or nuts in the
165 afternoon). All monkeys received a set amount of monkey biscuits per group, determined by
166 the number and size of monkeys in each group. It was facility practice that at feeding time, a
167 small amount of monkey biscuits from the previous feeding should still be present in the cage,
168 thus ensuring that all monkeys had plentiful access to food. Water was available *ad libitum*.

169

170 At the start of the study, monkeys were housed in two pairs (Group 1: IC, JA; Group 2: SH, LE)
171 and one group of 3 (HO, MR, JR) in the same room with visual/auditory/olfactory access to each

172 other. All monkeys came from multi-male / multi-female social groups, and none were alpha
 173 males prior to being housed in all male groups. Dominance ranks were evident as observed
 174 through displacements and other dyadic aggression within groups and remained stable for all
 175 pair-housed monkeys for the duration of the study. For the group of 3 monkeys, the two lower-
 176 ranking monkeys (JR, MR) switched dominance ranks several times during the first data
 177 collection phase prior to introduction of the female; HO remained the highest-ranking monkey
 178 of the group throughout.

179

180 Table 1. Overview of ages, rank, and housing arrangements of monkeys.

Monkey	Group	Rank	<u>Date of Birth</u>	Age at start of study in 2012	Age when female was introduced in 2014
IC	1	alpha	April 2006	6 years	7 years 10 months
JA	1	subordinate	November 2006	5 years 5 months	7 years 3 months
SH	2	alpha	March 2003	9 years 1 month	10 years 11 months
LE	2	subordinate	March 2007	5 years 1 month	6 years 11 months
HO	3	alpha	July 2002	9 years 9 months	13 years 7 months
MR	3	subordinate	October 2004	7 years 6 months	9 years 4 months
JR	4	subordinate -> alpha (paired with female)	May 2004	7 years 11 months	9 years 9 months

181

182 In February 2014, facility management decisions necessitated that the then lowest-ranking
 183 monkey (JR) was paired with an adult female from another group (LY). JR was removed from
 184 his group and successively introduced (visual contact only at first, then contact through a mesh
 185 panel, then full contact) to LY in another room without any visual/auditory/olfactory contact
 186 with other monkeys. After approximately two weeks, they were moved back into the room
 187 where the other male pairs were housed. Thus, there were three male pairs (Pair 1: IC, JA; Pair

188 2: SH, LE; Pair 3: HO, MR) and one male-female pair (Pair 4: JR, LY), each with a stable
189 dominance hierarchy (dominant monkeys were IC, SH, HO, and JR). Each pair had
190 visual/auditory/olfactory access, but not physical access, to the other pairs. To avoid
191 unintended pregnancies, JR was vasectomized in April 2014. LY was treated with the injectable
192 contraceptive medroxyprogesterone acetate (MPA; Depo-Provera, 20mg/kg) between February
193 and May 2014 and cycled naturally thereafter until July 2015, when she was put back on to
194 Depo-Provera. In November 2015, facility management decisions resulted in JR and LY being
195 moved to another building; the three male pairs remained housed together in the same room
196 (but in separate enclosures).

197

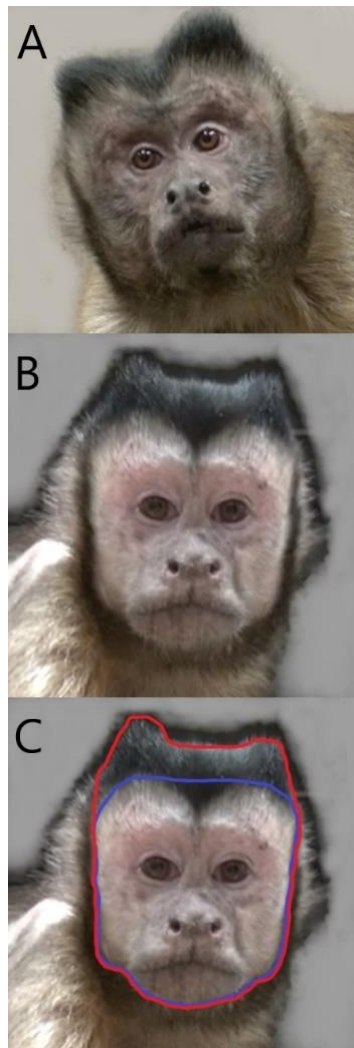
198 *Procedure.*

199 We collected body measurements, weights, and facial photographs from all monkeys during
200 routine quarterly veterinary health exams from 2012 to 2016. We were able to collect hair
201 samples between 2014-2016. All monkeys were sedated with a 10:1 mixture of ketamine and
202 acepromazine at 0.1 ml/kg (9 mg/kg ketamine and 0.1 mg/kg acepromazine), IM. We collected
203 body measurements and weights on eleven occasions (three prior to introduction of the
204 female, six while the female was present in the room, two after removal of the male-female
205 pair), facial photographs on ten occasions (two prior to introduction of the female, six while the
206 female was present in the room, two after removal of the male-female pair), and hair samples
207 on 6 occasions (four while the female was present in the room, two after the removal of the
208 male-female pair).

209

210 *Body measurements.* We collected a range of body measurements from monkeys. Some
211 measurements were related to soft tissue (testicle size, circumference, skinfold) and therefore
212 we hypothesized these measurements could be amenable to changes in the social
213 environment. Other measurements were based on hard tissue (bone lengths), and while it is
214 possible that certain social circumstances may trigger an increase in these measurements, they
215 are less likely to show decreases based on social factors. Measurements included: crown-rump
216 length (top of the skull to the most distal part of the ischial tuberosity), ulna length (wrist bone
217 to end of ulna with elbow bent), femur length (top of femur to tip of bent knee), neck
218 circumference (narrowest part), chest circumference (just below nipples), abdominal
219 circumference (at umbilical level), biceps circumference (widest part with elbow bent), scapula
220 skinfold (1cm below scapula), upper abdominal skin fold (2cm above umbilicus), lower
221 abdominal skinfold (2cm below umbilicus), foot length (distal end of calcaneus to tip of third
222 digit), and testicle size (one testicle isolated in upper portion of scrotum and compared to
223 testicle mass while scrotal skin stretched taut). All measurements were made on the right side
224 with the monkey in left lateral recumbency. Body and body-segment lengths were measured
225 with sliding calipers (in cm). Circumferences were measured with a tape measure to the nearest
226 0.5cm. Skinfold was measured using medical skinfold calipers (in mm). Testicle size was
227 estimated using an orchimeter (in ml). Each measurement was taken twice by the same
228 experimenter (AP) and had to be within 0.5 measurement units of each other; an average of
229 both measurements was used for analysis.
230

231 *Photographs.* Facial photographs were collected with a digital video camera (Sony HDR-
232 CX560V). All pictures were frontal and included the face and all hair tufts. Prior to
233 measurement, photographs were aligned and scaled according to interpupilar distance. Since
234 monkeys were sedated (eyes closed) when pictures were taken, we determined interpupilar
235 distance following Pryor (1969) by measuring the distance between the outer angles of the eyes
236 (A), the distance between the inner angles of the eyes (B), and calculating $(A-B)/2 + B$.
237 Photographs were then uploaded into Tobii Studio (Tobii Technology, Sweden) and pixel sizes
238 of the face and the head were measured by drawing areas of interest (AOIs). The head
239 measurement consisted of the face area (from jaw line to top of forehead) as well as the hair
240 tufts on top of the head, and the face measurement consisted of the just face area without any
241 hair tufts (Figure 1). A subset of photographs (N=16, 20%) were measured by a second coder,
242 and reliability measures were good (ICC = 0.99 for full head, 0.80 for face). Both coders were
243 blind to the hypotheses under investigation at the time of measurement.



245 Figure 1. Male tufted capuchin monkeys (*Sapajus apella*). 1A: 12-year-old alpha male housed
 246 with a female (JR, photo from 2016). 1B: 12-year-old subordinate male housed with another
 247 male (MR, photo from 2016). 1C: outline of indicative facial measurements. Blue line – face
 248 measurement, red line – head measurement.

249

250 *Hair cortisol analysis.* Hair was shaved from the back of the neck following a standardized
 251 protocol developed for rhesus macaques (Davenport, Tiefenbacher, Lutz, Novak, & Meyer,
 252 2006). Samples were placed in an aluminum foil pouch and stored in a -80C freezer. Hair

253 samples were weighed, washed twice with isopropanol, allowed to air dry for 5-7 days, then
254 ground to a fine powder and incubated in methanol for 18-24hrs. Aliquots of the methanol
255 extract were then dried down and reconstituted with assay buffer at a dilution of 1:8 prior to
256 being analyzed via enzyme immunoassay (EIA) using a salivary cortisol kit (#1-3002; Salimetrics,
257 State College, PA). Resulting values ($\mu\text{g}/\text{dL}$) were converted to pg/mg for analysis. Intra-assay
258 coefficients of variation were $<10\%$.

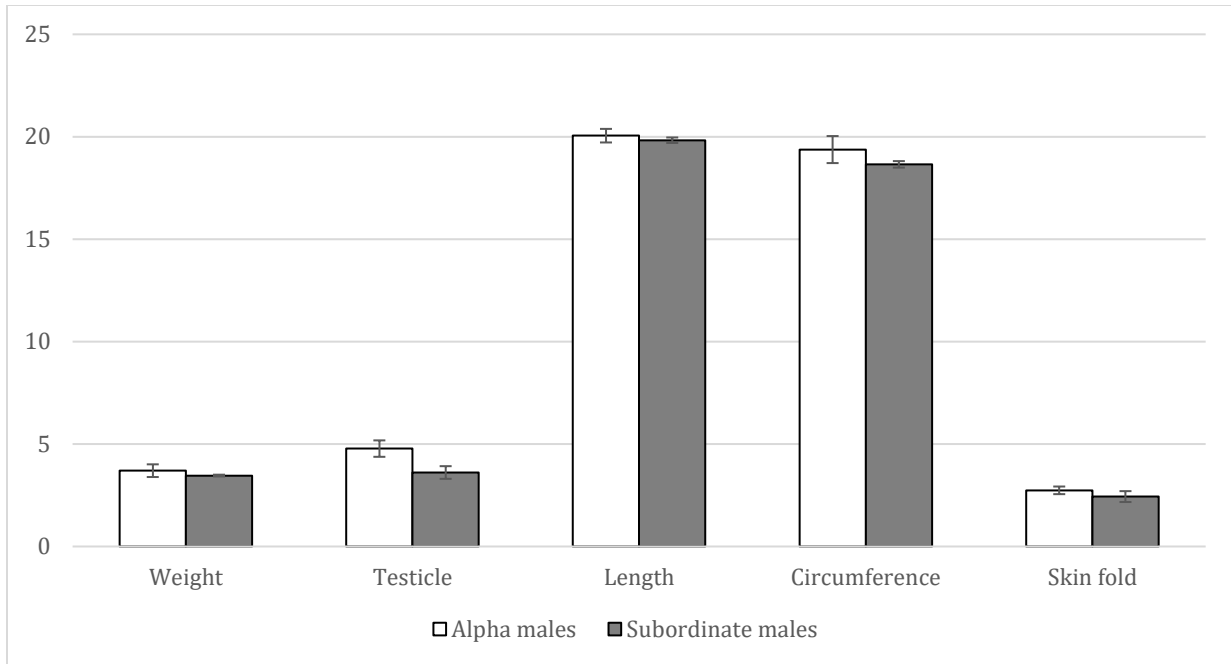
259

260 Results

261 *1. Prior to the introduction of a female, alpha males housed in same-sex pairs would be bigger*
262 *(in weight, facial measurements, body measurements) than subordinate males.*

263 We used randomization tests to evaluate this prediction. We averaged measurements from
264 2012 to 2014 (prior to introduction of the female) for all monkeys whose dominance rank
265 remained stable (HO, IC, JA, LE, MR, SH). In randomization tests, the proportion of 1,000
266 randomly sampled data divisions giving differences in the predicted direction
267 (alpha>subordinate) at least as large as the experimentally obtained difference across weights,
268 face measurements, and individual body measurements were $p > 0.05$ and therefore not
269 statistically significant (Todman & Dugard, 2001; see Figure 2). In the absence of females, alpha
270 males were not significantly larger than subordinate males on any individual measurement.

271



272

273 Figure 2. Summary of average weight and body measurements of alpha and subordinate males
 274 in the absence of any females. Length = average of crown-rump, ulna, femur, and foot
 275 measurements, circumference = average of neck, chest, abdomen, and biceps measurements,
 276 skin fold = average of scapula, upper abdomen, and lower abdomen measurements. Error bars
 277 depict standard errors. Weight in kg, testicle size in ml, length and circumference in cm, skin
 278 fold in mm.

279

280 *2. Introduction of a female would result in increased weight, face measurements, and body*
 281 *measurements in all alpha males. However, the male with direct access to the female (JR) would*
 282 *show a significantly larger increase in measurements relative to the males without direct access.*

283

284 The following results compare the last measurement prior to introduction of the female
 285 (January 2014) to the average measurements collected while co-housed with the female (April

286 2014 to October 2015). By April 2014, all males were considered full adults (ranging in ages
287 from 7 years 1 month to 11 years 1 month).

288

289 *Male (JR) paired with female*

290 1. *Weight*

291 JR increased his weight from an average of 3kg prior to introduction of the female to an
292 average of 3.92kg after introduction of the female, a gain of 30.56%.

293 2. *Body measurements*

294 Most of JR's body measurements increased modestly but consistently after being paired
295 with the female. His limb measurements showed an average increase of 2.65%, and his
296 circumference measurements showed an average increase of 14.71%. JR also gained in
297 body fat with his skinfold measurements increasing on average 60.41%. The most dramatic
298 change was observed in testicle size from 3 prior to introduction to an average of 11.33
299 after introduction, an increase of 277.78%.

300 3. *Face measurements*

301 Facial measures also showed an increase after introduction of the female: on average, the
302 head size (head and tufts) increased by 12.78% and face size increased by 17.53%.

303

304 *Alpha males not paired with female (HO, IC, SH)*

305 1. *Weight*

306 On average, the established alpha males only gained a modest amount of weight following
307 the introduction of the female to the room (averages from 3.6kg to 3.79kg, +5.4%).

308 2. *Body measurements*

309 The established alphas' body measurements showed no consistent pattern of gain or loss.

310 On average, alphas lost -0.98% in limb measurements, -1.04% in circumference

311 measurements, and gained 11.46% in skinfold measurements. Testicle measurements

312 showed a small increase from 5.00 to 5.06, or 1.11% increase.

313 3. *Face measurements*

314 Face size of alpha males slightly changed after introduction of the female into the room. On

315 average, head size decreased by 2.70% and face size increased by 0.22%.

316

317 *Subordinate males not paired with female (JA, LE, MR)*

318 1. *Weight*

319 Weight of subordinate males slightly increased after introduction of the female into the

320 room, from an average of 3.48kg to 3.53kg, or 1.44%.

321 2. *Body measurements*

322 Subordinate monkeys' body measurements showed a slight increase in limb measurements

323 (average 0.14%) but decreases in circumference measurements (average -0.47%) and

324 skinfold measurements (average -6.09%). Testicle sizes showed an increase from averages

325 3.67 to 3.82, or 4.28%.

326 3. *Face measurements*

327 Facial measurements showed inconsistent changes after introduction of the female into the

328 room. Head size increased by an average of 2.48% and face size decreased by an average of

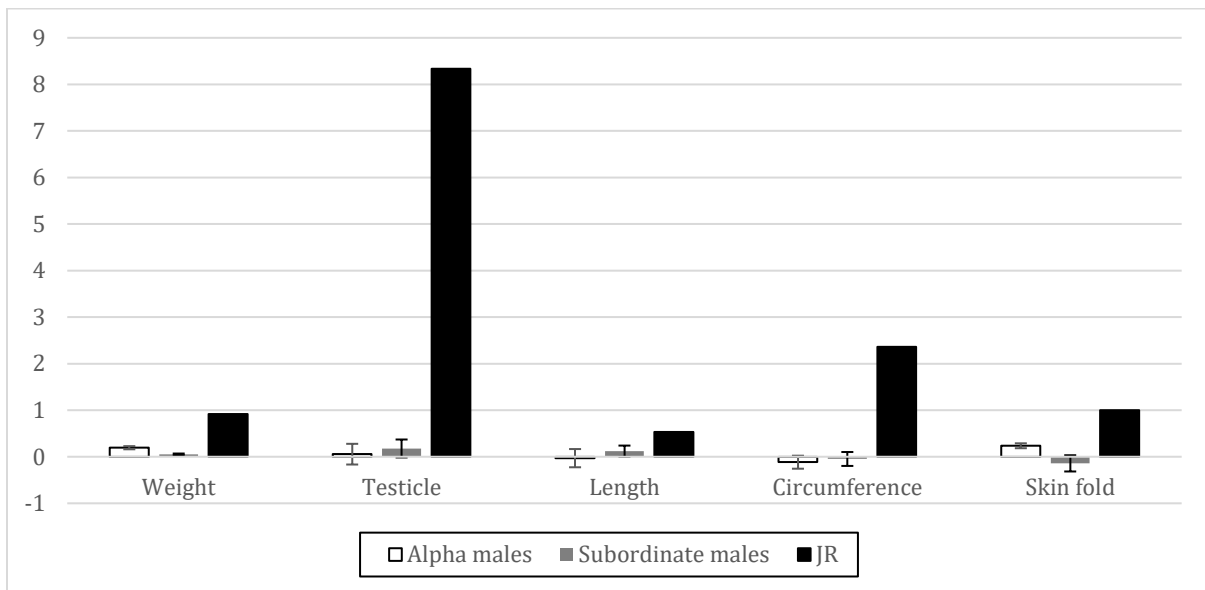
329 1.44%.

330

331 *Comparison of physical changes between alpha and subordinate males*

332 We used randomization tests to evaluate this prediction. We calculated an average difference
333 score between the last measurement prior to introduction of the female (January 2014) and the
334 average measurements taken while co-housed with the female (April 2014-October 2015) for
335 all alpha males (IC, SH, HO) and subordinate males (JA, LE, MR). In randomization tests, the
336 proportion of 1,000 randomly sampled data divisions giving differences in the predicted
337 direction (alpha>subordinate) at least as large as the experimentally obtained difference across
338 weights, face measurements, and individual body measurements were $p > 0.05$ and therefore
339 not statistically significant (Todman & Dugard, 2001; Figure 3). Alpha males did not gain
340 significantly more in size or facial appearance compared to subordinates.

341



342

343 Figure 3. Summary of average change in weight and body measurements of alpha males,
344 subordinate males, and JR after being co-housed with the female. Length = average of crown-

345 rump, ulna, femur, and foot measurements, circumference = average of neck, chest, abdomen,
346 and biceps measurements, skin fold = average of scapula, upper abdomen, and lower abdomen
347 measurements. Error bars depict standard errors. Weight in kg, testicle size in ml, length and
348 circumference in cm, skin fold in mm.

349

350 *Comparison of physical changes in JR to all other males*

351 To assess whether the changes observed in JR were significantly different to the changes
352 observed in the males housed in same-sex pairs, we calculated an average difference score
353 between the last measurement prior to introduction of the female (January 2014) and the
354 average measurements taken while co-housed with the female (April 2014-October 2015) for
355 each monkey and each measure. We then converted JR's measurements into z-scores using the
356 means and standard deviations of the males (n=6) housed in same sex pairs to establish
357 population means and standard deviations. Using two-tailed tests ($\alpha = 0.05$), JR gained
358 significantly more weight ($z = 8.84, p < .0001$), testicle size ($z = 24.96, p < .0001$), neck
359 circumference ($z = 4.83, p < .0001$), chest circumference ($z = 5.22, p < .0001$), abdominal
360 circumference ($z = 2.90, p = .004$) and bicep circumference ($z = 4.31, p < .001$), as well as scapula
361 skinfold ($z = 4.00, p < .001$), upper abdominal skinfold ($z = 2.18, p = .029$), and lower abdominal
362 skinfold ($z = 1.97, p = .049$) than the other males housed in same-sex pairs. In addition,
363 comparisons in facial measurements showed that JR's face size ($z = 2.02, p = .044$) increased
364 significantly more than those of the males housed in same sex pairs. Similar results were
365 obtained when comparing JR's data to just the alpha males. In sum, JR's physical gains were

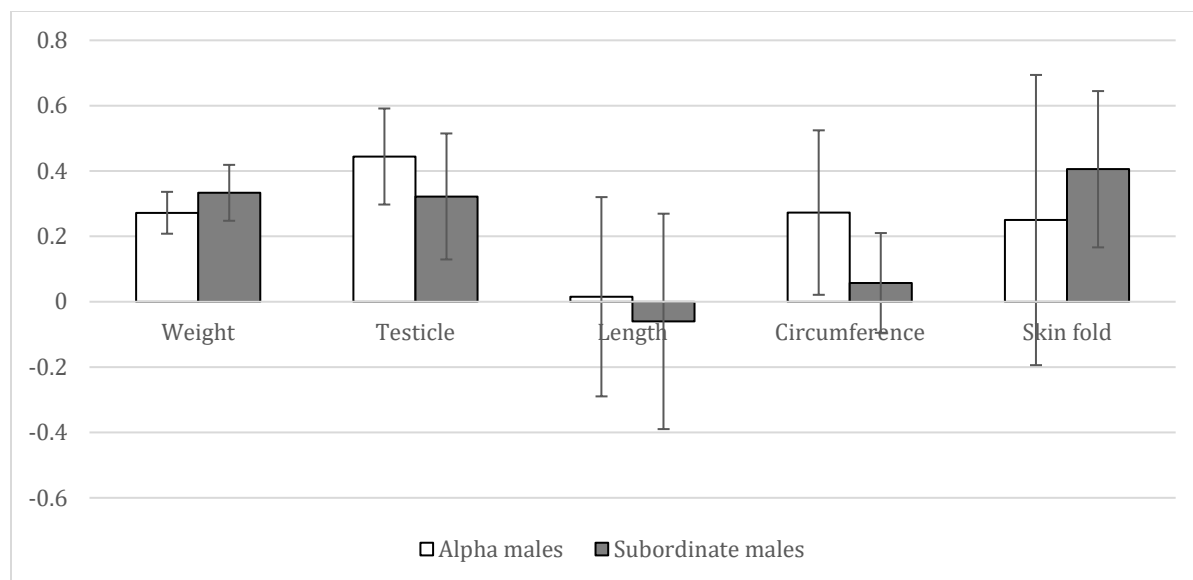
366 larger than those of the other males, most notable in his face, circumference measurements,
367 skinfold measurements, testicles, and weight (Figure 3).

368

369 *3. If alphas experienced a gain in their weights, measurements, and facial appearance following*
370 *the introduction of the female, then removal of the female might reverse some these gains.*

371 We compared the average from all measurements during the time the female was housed in
372 the room (April 2014 to October 2015, six measurements) with the average from
373 measurements taken after the male-female pair (JR-LY) was removed from the room (January
374 2016 to April 2016, two measurements). We found only slight changes, with alpha males on
375 average gaining 0.27kg in weight, or 7.05%; increase in testicle size by 0.44, or 5.44%; 0.25%
376 loss in length measurements; 1.26% gain in circumference measurements; and 9.18% gain in
377 skin fold measurements. Head sizes increased by 1.22% and face size increased by 1.23% on
378 average (Figure 4). These changes were small and since they indicate gains rather than the
379 decreases that we expected, we did not test for statistical significance, as it is obvious that the
380 hypothesis is not supported.

381

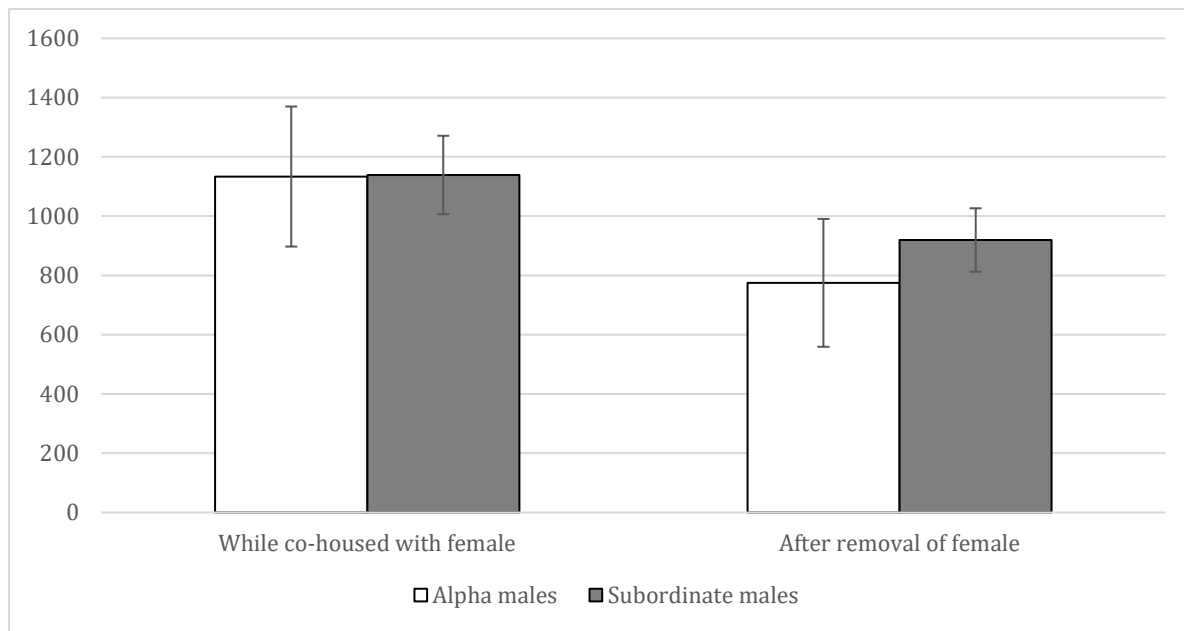


382
 383 Figure 4. Average percentages of change in measurements in alpha males between presence of
 384 female and removal of female. Length = average of crown-rump, ulna, femur, and foot
 385 measurements, circumference = average of neck, chest, abdomen, and biceps measurements,
 386 skin fold = average of scapula, upper abdomen, and lower abdomen measurements. Error bars
 387 depict standard errors.

388
 389 *Hair cortisol values*

390 We unfortunately did not have hair samples prior to the introduction of the female, as hair
 391 collection for an unrelated longitudinal study began in October 2014. Hair cortisol values for all
 392 monkeys ranged between 532.48 – 2755.02 pg/mg. While the female was housed within the
 393 same room, the three alpha males' hair cortisol levels were similar to the three subordinate
 394 males' hair cortisol levels (1134 pg/mg vs. 1139 pg/mg, respectively; Figure 5). During the same
 395 period, JR's cortisol levels averaged 1678.93 pg/mg. Converting JR's cortisol values into z-scores
 396 using the means and standard deviations of the other males (n=6) as population means and

397 standard deviations and using two-tailed tests ($\alpha = 0.05$), we found that JR's average hair
398 cortisol value was not significantly different from the other males' hair cortisol values ($z = 1.83$,
399 $p > .05$). However, all males' hair cortisol values dropped after JR and the female were removed
400 from the colony room; this drop was significantly different from 0 on a one-sample t -test (two-
401 tailed, $t(5) = 5.12$, $p = .004$). We further compared hair cortisol values after the removal of JR
402 and the female between alphas and subordinates, as well as the magnitude of decrease in
403 cortisol values between alphas and subordinates. However, none of these comparisons showed
404 any significant differences ($ps > 0.05$).



405
406 Figure 5. Hair cortisol values (in pg/mg) for alpha and subordinate males while co-housed with
407 the female and after removal of the female.

408
409 Discussion

410 In this study, we measured physical appearance in relation to dominance rank in captive male
411 tufted capuchin monkeys before, during, and after the introduction of a female. We

412 hypothesized that secondary sexual characteristics related to alpha status would primarily serve
413 as a signal to other males, not females; we therefore expected that alpha males would be larger
414 than subordinate males when housed in all-male groups. However, we anticipated that the
415 presence of a female would further enhance secondary sexual characteristics, particularly for
416 the male who had direct physical access to her. We also predicted that, due to energetic costs,
417 gains achieved as a result of female presence would be lost again after the female was
418 removed.

419
420 Contrary to our hypothesis, we did not find that alpha males were generally larger than
421 subordinate males prior to the introduction of the female, suggesting that alpha status on its
422 own may not be sufficient to lead to the development of secondary sexual characteristics in
423 male capuchin monkeys. Two of the studied male pairs showed consistent differences in the
424 predicted direction for weight and body measurements, but the third pair did not. However, it
425 is worth noting that in the two former pairs, the alpha males had likely reached adult body
426 mass (being at least nine years old at the start of the study) whereas for the latter pair, the
427 alpha might not have (being six years old at the start of the study when full maturity is generally
428 considered seven years old; Fragaszy et al., 2004). Two of the subordinate monkeys were also
429 under 7 years old at the start of the study, suggesting that they may not have reached their full
430 body size at that point. Thus, it remains possible that physical gains associated with male alpha
431 status are further dependent on having reached adult body mass. Alternatively, it is possible
432 that body mass may affect alpha status in that males with the potential to achieve larger body
433 mass also more easily achieve alpha status. The causal direction of alpha status and body size is

434 not determinable from the present data, but these ideas could be addressed in future studies
435 using males of various ages and dominance status.

436

437 Introduction of a female had a dramatic effect on JR, the male who was paired with her and
438 given full contact. JR showed significant gains in weight and physical appearance, in particular in
439 the face, circumference measurements (soft tissue), and testicles, leading to an overall weight
440 gain following her introduction. These gains were significantly larger than those experienced by
441 the other adult males without direct access to the female, who exhibited small and inconsistent
442 changes. During the course of the study, the female was observed sexually soliciting males from
443 the all-male pairs, but we did not observe any matings with JR. Thus, all males within her
444 vicinity may have been affected by her behavioral and/or physiological cues of sexual
445 availability, but JR, who had mating opportunities with her (whether realized or not), appears to
446 have changed in the most dramatic fashion. In other species, female presence or behavior can
447 directly influence male behavior, such as intensity of displays in Siamese fighting fish (*Betta*
448 *splendens*; Doutrelant, McGregor, & Oliveira, 2001) or singing in starlings (*Sturnus vulgaris*;
449 Gwinner Van't Hof, & Zerman, 2002). Presence of females can also affect physiological traits,
450 such as facial redness in male mandrills (Setchell, Smith, Wickings, & Knapp, 2008). Our results
451 further clarify that it is not just the presence of females, but also males' direct access to them,
452 that influence male physique. The observed changes may be the result of increased levels of
453 testosterone, which can increase in the presence of fertile females in white-faced capuchin
454 monkeys (Jack et al., 2014), although other studies have reported that tufted capuchin monkey
455 alphas do not show higher testosterone levels than other adult males (Lynch et al., 2002).

456 Future studies that directly measure testosterone and/or dihydrotestosterone in relation to the
457 physical presence of, and access to, females may shed further light on this issue.

458

459 Finally, we predicted that removal of the female from the room would reverse some secondary
460 sexual characteristics in alpha males housed in same-sex pairs. When the female was first
461 introduced in April 2014, all males had achieved adult male status (7+ years old; Fragaszy et al.,
462 2004). Presence of the female only inconsistently affected males housed in same-sex pairs who
463 did not have direct access to her, resulting in some small gains in weight but also losses in
464 several body and face measurements. After removal of the female, alpha males modestly
465 gained rather than lost weight (possibly as body fat as shown through skin fold measures).

466 Generally, it remains unclear when and how secondary sexual characteristics are maintained.

467 In some species, secondary sexual characteristics are strongly tied to alpha status and/or
468 females (e.g., mandrills; Setchell & Dixson, 2001) whereas in others, secondary sexual
469 characteristics are retained even in the absence of females (e.g., flanged orangutans; Utami et
470 al., 2002). Future studies are required to understand whether secondary sexual characteristics
471 signal a temporary 'state' or true 'genetic quality' (Setchell & Dixson, 2001) in capuchin
472 monkeys.

473

474 The results of the hair cortisol measurements further suggest that, similar to rhesus macaques
475 (Bernstein, Weed, Judge, & Ruehlmann, 1989), the presence of a female may be a significant
476 stressor to male tufted capuchin monkeys. During breeding season, rhesus macaque males may
477 lose weight although they tend to regain it during the non-breeding season (Bernstein et al.,

478 1989), making breeding a possible source of energetic stress for males (Lynch et al., 2002).
479 During the female's presence in the present study, alpha males' hair cortisol levels were
480 comparable to subordinate males' levels, and JR's hair cortisol levels did not differ from those
481 monkeys who did not have direct access to her. Unlike white-faced capuchin monkeys, where
482 the alpha male shows significantly higher levels of fecal glucocorticoids in the presence of
483 fertile females (Schoof, Jack, & Ziegler, 2014) or after intergroup encounters (Schoof & Jack,
484 2013), tufted capuchin monkey alphas and non-alpha males do not seem to differ in fecal
485 glucocorticoid levels (Lynch et al., 2002), a finding replicated in our captive group. However,
486 after removal of the male-female pair, hair cortisol levels significantly decreased across all
487 monkeys housed in male-male pairs. There are two potential explanations for the observed
488 decrease. Males may experience a significant level of frustration in the presence of a sexually
489 receptive female, who may even sexually solicit them, but whom they cannot physically access.
490 This frustration may then be re-directed at subordinate males in the form of aggression, leading
491 to elevated hair cortisol levels in both alpha and subordinate males. Alternatively, the mere
492 presence of additional monkeys in the room, potentially increasing perceived population
493 density (Dettmer, Novak, Meyer, & Suomi, 2014) and agonistic inter-group interactions, may
494 have increased hair cortisol levels. It is worth noting that actual population density did not
495 change between study phases as the monkeys' enclosure size remained constant throughout
496 the study. Future studies could investigate to what degree presence of other monkeys (male or
497 females, with and without direct physical contact to each other) affects cortisol and behavioral
498 indicators of stress in tufted capuchin monkeys.
499

500 Overall, we found little evidence to suggest that in all male groups, alpha males advertise their
501 status to other males via sexual secondary characteristics. Instead, alpha status while having
502 direct physical access to females appears to trigger the development of such characteristics,
503 most notable in the male's size and face. It does not necessarily follow that males' secondary
504 sexual characteristics serve as signals primarily to females; it is equally possible that signaling to
505 males only becomes relevant in the presence of females. Given the small sample size and
506 opportunistic nature of the present study, the findings presented here may be considered
507 preliminary and in need of further careful experimental investigations including more frequent,
508 and therefore more robust, measurements of males during the different phases of the
509 experiment. It also remains of continued interest to identify the mechanisms responsible for
510 the development, and possible loss, of secondary sexual characteristics. Changes in endocrine
511 function promoted by social factors have been established in relation to reproductive
512 suppression (e.g., cotton top tamarins, *Saguinus oedipus*; Ziegler, Scheffler, & Snowdon, 1995),
513 and we strongly encourage future studies to investigate endocrine functions as well as behavior
514 in relation to secondary sexual characteristics.

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615 Data associated with this manuscript can be found here: Dryad

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617

618 Competing Interest Statement

619 The authors declare no competing interests.

620

621 Author Contributions

622 AP conceived and designed the study; AP and LW collected data; ES coded data; AP analysed

623 the data and drafted the article; all authors revised the article critically for intellectual content

624 and approved of the final version to be published.