

Title: Aggression and hormones are associated with heterogeneity in parasitism and parasite dynamics in the brown mouse lemur

Sarah Zohdy^{a,§*}, Donal Bisanzio^{*b}, Stacey Tecot^c, Patricia C. Wright^d, and Jukka Jernvall^e

^a School of Forestry and Wildlife Sciences, Auburn University,

^b Department of Zoology, University of Oxford

^c School of Anthropology, University of Arizona,

^d Department of Anthropology, Stony Brook University,

^e Institute of Biotechnology, University of Helsinki,

*these authors contributed equally to this work

§Corresponding author: Sarah Zohdy, 602 Duncan Drive, 3231 Forestry and Wildlife Sciences Building, Auburn University, Auburn, AL 36849 USA, ph: (334) 844-8046, fax: (334) 844-1084; zohdy@auburn.edu

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Animal behaviours, like aggression, can directly impact host health by influencing exposure to parasites. Aggressive individuals may experience an increase in agonistic interactions and contact rates with conspecifics, which might increase their probability of acquiring parasites. However, aggression is not the only factor that shapes parasitism; proximate mechanisms like hormone-modulated immunosuppression can also have broad impacts. Here, we hypothesize that high levels of aggression, cortisol (C), and testosterone (T) will be positively associated with parasitism, and that aggressive individuals will play a larger role spreading parasites to conspecifics than docile individuals. Aggression was measured using the level of aggressive response to human handling during capture. The aim of this study was to examine associations between aggression and hormones, C and T, on variation in endo- and ecto-parasitism in a population of wild mouse lemurs (*Microcebus rufus*) over a three-year period. By tracking the movement of lice (directly transmitted parasites) in the population, we also examined the effect of host aggression on population-wide parasite dynamics. We show that animals with high T and C were more likely to exhibit aggressive behaviors, and C was associated with significantly higher ectoparasite infestations. Aggressive individuals were significantly more infested by lice, and also donated significantly more lice to conspecifics in the population. Taken together, our results offer insight into the individual and population health costs of aggression, and empirical support of a trade-off between aggression and ectoparasitism, which may have driven the evolution of aggression and interactions with conspecifics.

Keywords: Madagascar, testosterone, cortisol, primate, behaviour, lice

Highlights:

- High levels of Cortisol and Testosterone are associated with aggression
- Aggressive lemurs have more lice than docile lemurs
- Aggressive lemurs donate more lice to conspecifics, suggesting the population-wide impact of individual aggression
- Aggression places mouse lemur health at risk, which may decrease fitness

1 The drivers of parasitism in host populations can often be complex. For example, changes in host
2 hormone levels can trigger aggressive behaviours that may be advantageous, leading to the
3 exploration and discovery of novel resources or mates (Wilson et al. 1994); however, aggression
4 also has the potential to decrease fitness, by increasing contacts with conspecifics and creating
5 opportunities for exposure to parasites (Jonsson et al. 2000; Biro et al. 2004; Sih et al. 2004;
6 Boon et al. 2008). Hormones may also impact parasitism by decreasing immunity (Folstad &
7 Karter 1992; Klein 2004; Roberts et al. 2004; Cox & John-Adler 2007). Therefore, an
8 understanding of the bidirectional feedback between hormones, aggression, and parasitism is
9 necessary to determine how these factors shape host populations.

10 Androgens such as testosterone (T) have long been associated with behaviour (Koolhaas et al.
11 1999; Carere et al. 2003; Kralj- Fišer et al. 2007; Sellers et al. 2007), for example mediating
12 aggression, reproduction, and agonistic interactions in birds (Wingfield et al. 1987; Klein 2000;
13 Partecke & Schwabl 2008). Research suggests that positive associations between androgens and
14 aggression may also involve heightened HPA-axis activity and elevated glucocorticoid levels, as
15 they can be correlated (Liptrap & Raeside 1978). Glucocorticoids such as corticosterone and
16 cortisol are metabolic hormones that mediate energy in the face of social or physical
17 environmental challenge (Sapolsky et al. 2000; Beehner & Bergman 2017). A study by Muller
18 and Wrangham (2004) found that elevated cortisol levels in dominant male chimpanzees were
19 associated with aggression, and likely due to the high metabolic costs of aggressive displays
20 (though they also acknowledge that aggression may be stressful in and of itself). In turn,
21 aggression can also increase glucocorticoid levels directly, while decreasing T levels, as found in
22 Australian water dragons (*Intellagama lesueurii*) (Baird et al. 2014), possibly reflecting the
23 immunosuppressive effects of HPA-axis energy mobilization. It has thus been suggested that the

24 stress response (as reflected in glucocorticoid levels) and androgen levels may underlie
25 individual variation in aggression (Korte et al. 2005).

26 Hormones may also directly influence heterogeneities in parasitic infections, by altering
27 immunity (Folstad & Karter 1992; Klein 2004; Roberts et al. 2004; Cox & John-Adler 2007).

28 The immunocompetence handicap hypothesis (ICHH) proposes that costly T enhances the
29 expression of sexual traits but suppresses immune function (Folstad & Karter 1992). In several
30 bird species it has also been shown that elevated T can suppress both adaptive and innate
31 immune responses (Navara et al. 2006; Tobler et al. 2010; Müller et al. 2015), increasing the
32 likelihood of parasitism. Cortisol may also influence parasitism by suppressing immune function
33 in a trade-off with other energetic needs (Elenkov & Chrousos 1999; Webster et al. 2002).

34 Not only can host hormones play a role in parasite infestation, but parasites can affect hormone
35 signaling within the host. Studies suggest that protozoan parasites can alter hormone
36 concentrations in their hosts by disrupting reproductive cycles or suppressing androgen
37 concentrations (Aina et al. 1990; Barthelemy et al. 2004). Whether these hormonal changes
38 following infection are mediated by the parasite or the host remains widely unconfirmed (Klein
39 2004). Host hormone manipulation by parasites is reported in both vertebrate and invertebrate
40 hosts and is hypothesized to increase the availability of host resources for parasite growth and
41 development (Baudoin 1975; Larralde et al. 1995; Morales et al. 1996; Romano et al. 2003).

42 The feedback between hormones, aggression, and parasitism are complex and an understanding
43 of the interplay between these components is crucial (Wilson et al. 1994; Natoli et al. 2005;
44 Easterbrook et al. 2007; Boyer et al. 2010). Here, we aim to examine the associations between
45 | hormones, aggression, and parasitism in the hopes of better elucidating these feedbacks -which

46 may be used to determine the directionality of these variables in future studies. One unique
47 component here is that we also examine how individual aggression can affect parasitism in
48 conspecifics in the population.

49 In this study, we evaluated associations between aggression, T and C levels, and ecto- and endo-
50 parasite infestation, to test the hypothesis that aggression and hormones are positively associated
51 with an increase in parasitism. We also hypothesize that aggressive individuals spread more
52 parasites to conspecifics than docile individuals. We conducted this study in a wild population of
53 brown mouse lemurs (*Microcebus rufus*), small, arboreal, trappable primates, in the southeastern
54 rainforests of Madagascar. Previous studies on captive and wild mouse lemur populations have
55 used detailed assays to classify mouse lemur behaviour (Dammhahn 2012; Dammhahn &
56 Almeling 2012; Verdolin & Harper 2013). However, those methods are often time-intensive and
57 therefore limit the number of individuals that can be tested. In this study, we developed a rapid
58 assay to classify behavioural scores from all captured lemurs during all capture events, allowing
59 us to parse out temporal or seasonal trends in aggression in recaptured individuals. Such rapid
60 assessments are a fairly common practice in the animal behaviour literature, and their results are
61 often informative (Réale & Fest-Bianchet 2003; Blumstein et al. 2012; Keiser & Pruitt 2014).
62 The aim of this study was to assess the potential fitness consequences of aggression, through its
63 associations with hormones and parasitism and parasite dynamics.

64

65 **METHODS**

66 *Ethical note*

67 All international, national and institutional guidelines for the care and use of animals were

68 followed. Research adheres to the Animal Behaviour Society Guidelines for the Use of Animals
69 in Research. All research protocols were approved by the government of Madagascar. Sample
70 collection in Ranomafana National Park was approved by Madagascar National Parks under
71 permit numbers #115/10 MEF/SG/DGF/DCB.SAP/ SCBSE 96 and #215/08
72 MEFT/SG/DGEF/DSAP/SSE. Research protocols were also reviewed and approved by the
73 University of Helsinki's institutional animal use rules and regulations board, and the Stony
74 Brook University Institutional Animal Care and Use Committee (IACUC ID #2009-1608 and
75 IACUC #2007-1597). Animals were captured only briefly and returned to the wild immediately
76 after data collection.

77

78 *Study species, site, and trapping*

79 We established a long-term trapping system in the southeastern rainforests of Madagascar to
80 study brown mouse lemurs (*Microcebus rufus*) at Ranomafana National Park (RNP; 47° 18' - 47°
81 37' E and 21° 02' - 21° 25' S) (Atsalis 1999). From 2008-2010 we used a systematic trapping
82 grid, where fifty Sherman traps (XLR, Sherman Traps Inc., FL) were set in pairs along transects
83 in two sites, A and B. Transects were 1.5 km- 2km long and were separated by the Namorona
84 river. Traps were set 2m off the ground, baited with banana, and checked four hours later for
85 captures. Our sampling period spanned the end of the cold season to the beginning of the rainy
86 season, including the mouse lemur breeding season (Aug-Dec), for a total of 239 trap nights (177
87 in 2008 and 2009, and 62 in 2010). Captured mouse lemurs were individually scanned (using an
88 AVID Powertracker VI) for a microchip (FECAVA Eurochips, Vetcare, FI), sexed, weighed,
89 aged (Zohdy et al. 2014), and measured under red light conditions, and then released into the
90 forest the same evening at their capture location. Individuals without a microchip were given one

91 (FECAVA Eurochips, Vetcare, FI). Non-primate captures were released on site.

92 From 2008-2010, aggression assays were conducted on all captured individuals at every capture
93 event to determine scores of aggression and docility.

94

95 We examined hormones, behavioural scores, and parasitic infestation in 104 unique mouse
96 lemurs, of which 63 were males (60.6%). We recorded 549 successful capture events, of which
97 445 (81.1%) were recaptures. The animal with the highest number of recaptures was a male who
98 was captured 32 times during the study period (Figure 1). The lowest trapping success occurred
99 in August (46 captures, 8.3%), and October had the highest trapping success (246 captures,
100 44.8%).

101

102

103 *Behavioural assay*

104 A behavioural assay was developed to quantitatively assign each lemur a score from 0-4,
105 according to their behavioural response to human handling immediately upon removal from a
106 trap bag. Each score on the scale below corresponds to the number of bites and/or audible
107 vocalizations within the first 30 seconds of handling. Only one specific vocalization
108 (Supplementary File 1) was included in this scoring. In this species, this vocalization
109 accompanies biting sequences, and therefore the two measures were used together. Scores were
110 simultaneously recorded by two researchers and compared. Inter-observer reliability was 97.6%.
111 In those cases, the lower score was assigned. Individuals with a score of “0” were considered to
112 be “docile”, while those with a score of “3” or “4” were considered to be “aggressive”.

113

0- no struggle or strange movements or vocalizations

- 114 1- one bite or one vocalization in 30 second time frame
115 2- two bites or vocalizations in 30 second time frame
116 3- three bites or vocalizations in 30 second time frame
117 4- four or more bites or vocalizations in 30 second time frame

118 We recorded 549 behavioural scores during the trapping period. Among all scores, 59.6% (311)
119 had scores of 0 (Figure 1).

120

121 *Hormone collection and analysis*

122 Over a 19-month period during the 2008 and 2009 field seasons, fresh fecal samples for hormone
123 analysis were collected during handling from 56 males (339 samples) and 40 females (201
124 samples). Only one sample per individual per week was used for hormone analyses. Collected
125 fecal samples were heated and desiccated at 70°C for 2-3 days and kept in silica gel at room
126 temperature until extraction, according to (Tecot et al. 2013; Zohdy et al. 2014). Assays were
127 performed at the Wisconsin National Primate Research Center by S. Zohdy and S. Tecot. Fecal T
128 analyses were validated and were performed as previously described (Zohdy et al. 2014). This
129 method uses chromatographic separation because the T antibody (AB156, acquired from Coralie
130 Munro, UC Davis) cross-reacts with DHT (92.4%), 4-androsten 3 β ,17 β -diol (11.2%),
131 dehydroandrosterone (5.44%), androstenediol (3.41%), androstenedione (2.12%), androsterone
132 (0.51%), dehydroepiandrosterone (0.19%), and <0.1% with estradiol, progesterone,
133 desoxycorticosterone, desoxycorticosterone, 17 α hydroxyl-progersterone, estrone pregnalalone,
134 cholesterol, hydrocortisone, and cortisone. Fecal C was analyzed according to similar methods,
135 with the addition of running samples through solvolysis (Ziegler & Wittwer 2005), and
136 excluding chromatographic separation. The C antibody (R4866, acquired from Coralie Munro,

137 UC Davis) cross-reacts with prednisolone (97.5%), prednisone (90%), cortisone (73.8%),
138 corticosterone (2.8%), deoxycorticosterone (1%), 20 β hydroxyprogesterone (1%), 11 α
139 hydroxyprogesterone (0.6%), 17 α hydroxyprogesterone (0.3), 17 α hydroxypregnenone (0.2%),
140 and 0.1% with progesterone, aldosterone, and dihydroandrosterone. To validate the assay,
141 parallelism was determined through serial dilutions of a high fecal extract pool along the
142 standard curve. Pooled samples were parallel to the standard curve ($t=1.48$; $df=24$). Accuracy of
143 the C assay was $98.91 \pm 3.06\%$ standard error of the mean. Fecal sample pools were run in
144 duplicate on each assay in order to establish mean intra- and inter-assay coefficients of variation
145 (CV). Mean intra- and inter-assay CV values were found to be within acceptable limits for both
146 low (C: intra = 6.9%, inter = 34.2%) and high (C: intra = 4.7%, inter = 14.9%) pools. Mean
147 steroid recovery for C was determined by adding exogenous hormone to fecal samples that were
148 then extracted and assayed according to the above protocol, and was 70%.

149

150 *Endoparasite quantification:*

151 Data on endoparasitism was recorded from every lemur at every capture event from 2008-2009.
152 A nematode isolation technique from feces was developed based on Baermann's method used for
153 soil nematodes and larvae (Baermann 1917). Fecal samples were collected and separated into
154 two halves. When fecal wet weight was over 1g ($n=470$), one half was set aside for hormone
155 analysis; the second part was weighed and placed in the center of one quarter of a 1-ply
156 Kimwipe®. The sides of the Kimwipe® were wrapped around the feces and tied together with
157 string. The sample was then placed into a labeled funnel in a modified Baermann's apparatus
158 consisting of a sterile glass funnel with rubber tubing connected to the bottom. In the center of
159 the rubber tubing a clamping device was placed. The sample was then covered with warm

160 distilled water to allow nematodes to hatch and to increase the movement of *Strongyloides* spp.
161 nematodes, causing them to fall through the Kimwipe® into the tubing. Two days later the clamp
162 was released and the liquid decanted into a sterile 2mL Eppendorf ® polypropylene tube. The
163 tubes were then centrifuged for 4 minutes at 2000 rpm and the supernatant was discarded. The
164 pellet was then examined under a microscope and *Strongyloides* spp. larvae and *Taenia* spp. eggs
165 were counted. The total number was recorded and later divided by the fecal wet weight
166 (Baermann 1917).

167

168 *Ectoparasite quantification:*

169 All captured lemurs were thoroughly examined for the presence of ticks and lice, as described in
170 (Durden et al. 2010). Hairs were separated using a flea comb during the examination. The
171 number of ectoparasites, and the location on the body (ears, face, etc.) on which they were found
172 were recorded.

173

174 *Louse tracking*

175 In 2010 (Aug-Nov), in addition to the standard capture procedures, sucking lice, obligate
176 ectoparasites found on the ears (previously found to be the most heavily parasitized location on
177 the body) (Durden et al. 2010) were marked with a host-specific color dot code using nail
178 lacquer while on the host (Zohdy et al. 2012). During recaptures, lemurs with marked lice from
179 conspecifics were documented to note the movement of lice between lemurs in the population
180 (Zohdy et al. 2012). To examine the effect of aggression on patterns of parasite exchanges, we
181 tested for correlations between aggression and louse donations. We also calculated donor scores,
182 a metric to determine whether individuals engaging in parasite transfers acted predominantly as

183 donors or receivers, calculated by subtracting the total number of lice received from the total
184 number of lice donated by an individual.

185

186 *Consistency of behavioural score in response to handling*

187 Repeatability was calculated to investigate consistency in subject behavior during study period
188 (Dean et al. 2011). Repeatability is the fraction of variance explained by difference between
189 individuals. Repeatability was calculated using cumulative link mixed models (CLMM) models
190 applied to animals trapped during the entire study period (Nakagawa & Schielzeth 2010). Only
191 animals that were recaptured more than five times during the entire study period were used.
192 Applying CLMM to capture data from 2008 and 2009, we obtained a repeatability of behavioural
193 score. Repeatability was adjusted accounting for sex, age and capture date.

194

195 *Statistical analysis*

196 Generalized linear mixed models (GLMMs) were applied to investigate the effect of sex and
197 month on hormone levels. The full model used was:

$$198 \quad T \text{ (ng/g)} = \text{Weight} + \text{Age} + \text{Month} + \text{Sex} + \text{Site} + C + \text{Rand(Individual)} + \text{Rand(Capture day)}$$

$$199 \quad C \text{ (ng/g)} = \text{Weight} + \text{Month} + \text{Age} + \text{Sex} + \text{Site} + T + \text{Rand(Individual)} + \text{Rand(Capture day)}$$

200 The model included individual identification and capture day as random effects to account for
201 differences among subjects and possible effect of capture day. Other variables of the models
202 were added as control effects (confounders).

203 Associations between sex, hormones, and month on behavioural score were assessed using a
204 cumulative logit model, which is a model that can be used for ordinal data from score 0 to score

205 4 (Lee 1992). The model was built using a cumulative link mixed model (CLMM). CLMM is a
206 generalization of mixed logit models to order response categories, more details on its formulation
207 can be found in McCullagh (1980) and Ivy and Agresti (2005).

208 Full model:

209 *Behavioural Score* = *Sex* + *Weight* + *Age* + *Month* + *Sex* + *Site* + *C* + *T* + *Recapture times* +
210 *Rand(Individual)* + *Rand(Capture day)*

211 This model included as covariate the number of time an individual was collected during the
212 study period ('*Recapture times*'). We applied GLMMs to investigate the association between
213 capture month, hormone levels, age, sex, and parasitism. Given the high frequency of zero in
214 parasite counts, zero-inflated distributions were used to perform the GLMMs. Zero-inflated
215 negative binomial distribution was used to describe count of ticks and lice, and zero-inflated log-
216 normal distribution was applied for endoparasite intensity (endoparasites/100mg feces). Full
217 model:

218 *Parasite load* = *Weight* + *Age* + *Recapture times* + *Month* + *Sex* + *Site* + *C* + *T* + *Recapture*
219 *times* + *Rand(Individual)* + *Rand(Capture day)*

220 Each model included animal identification as a random effect to account for pseudo-replication
221 resulting from high recapture rates and possible individual variations not captured by the chosen
222 predictors. To account for non-independency of animals trapped in during same days we added
223 in each model the capture date as random effect. Model selection was performed using Akaike
224 Information Criteria (AIC). The importance of each model variable was evaluated by estimating
225 the relative importance. Model selection procedures and relative importance calculation are

226 described in Burnham and Anderson (2004) (Burnham & Anderson 2003). All models were
227 performed using BayesX software (Brezger et al. 2003).

228 **RESULTS**

229 Results from the CLMM showed that only hormone levels had a significant impact on lemur
230 aggression (Table1). Animals that had high levels of T and C had a higher chance of showing an
231 aggressive behavioural score during processing (Table 1). C was a significant predictor of
232 increased aggression (Table 1). No association between aggression and the number of trapping
233 events was found. The probability of obtaining an aggressive score was significantly lower than
234 obtaining a docile score (Table 1). Most of the subjects showed docile behavior during the study
235 period (Figure 2). However, subjects captured during 2008 showed higher aggression scores
236 compared with those collected in 2009 (Figure 2). An increase in aggressive scores was seen in
237 September and October. The CLMM best model included months as covariates, but the effect
238 was not significant (Table 1). Repeatability of behavioural score was equal to 0.37 (95% CI:
239 0.17-0.58) indicating variability in animal behavior as shown in Figure 2. Animals captured in
240 2008 showed higher repeatability ($R=0.25$; 95% CI: 0.09-0.51) than those captured in 2009 ($R=$
241 0.32 ; 95% CI: 0.16-0.49), with no significant difference.

242

Table 1. Results from cumulative link mixed models (CLMM) on associations between sex, hormones, trapping month, body mass (g), and behavioural score.

| <i>Covariate</i> | <i>OR (95% CI)</i> | <i>Importance weight</i> |
|------------------------------------|----------------------------|--------------------------|
| Behavioural Score (Ref.: Score 0): | | |
| Score 1 | 0.02 (0.01; 0.14)** | |
| Score 2 | 0.03 (0.01; 0.23)** | |
| Score 3 | 0.02 (0.01; 0.31)** | |
| Score 4 | 0.03 (0.01; 0.51)** | |
| Sex M (Ref.: F) | 1.1 (0.7; 1.71) | 0.41 |
| Testosterone (ng/g) | 1.08 (0.87; 1.33) | 0.67 |
| Cortisol (ng/g) | 1.22 (1.05; 1.51)** | 0.95 |
| Site B (Ref.: Site A) | 0.91 (0.57; 1.47) | 0.35 |
| Year 2009 (Ref.: 2008) | - | 0.11 |
| Weight (g) | - | 0.05 |
| Age | - | 0.07 |
| Number of recaptures | - | 0.15 |
| Month | - | 0.12 |
| <i>Random effect</i> | | |
| | <i>Variance</i> | |
| Subject ID | 0.56 | |
| Trapping date | 0.02 | |

243 *Hormone levels and behaviour*

244 Of all individuals, the median level of T was 9.7 ng/g (interquartile range (IQR) = 4.9-15.9ng/g).
 245 Females had a higher median level of T (MD=10.3, IQR=5.6-16.8ng/g) than males (MD=8.8,
 246 IQR=4.8-15.4ng/g) (Figure 2). GLMM results showed a significant effect of sex on T levels
 247 (Table 2) with higher levels in males. No difference in testosterone was found comparing months
 248 of the same year (Figure 2). The best model did not included months as covariate. Lemurs
 249 captured at Site A had higher T levels than those captured at Site B (Figure 2). However, site was
 250 not included in the best model. Individuals captured in 2008 had higher hormone levels than
 251 those captured in 2009, but capture year did not have a significant effect (Figure 2, Table 2).

252 The median level of C was 67.7 ng/g (interquartile range (IQR) = 40.8-135.8ng/g). Males
 253 showed a higher median level of C (MD=72.0, IQR=37.5-164.5ng/g) than females (MD=65.6,
 254 IQR=46.6-100.9ng/g). However, we did not find a significant effect of sex on C levels (Figure 2,
 255 Table 2). We found an association between body mass and C levels (Table 2). Lemurs captured
 256 in 2009 had higher C levels than those in 2008 (Figure 2, Table 2). The two hormones, C and T,
 257 were positively associated (Table 2).

Table 2. Results from GLMM investigating the relationship of hormone levels with sex, weight, and capture month.

| <i>Covariate</i> | <i>Testosterone in ng/g^a</i> <i>Effect (95% CI)</i> <i>Importance weight []</i> | <i>Cortisol in ng/g^a</i> <i>Effect (95% CI)</i> <i>Importance weight []</i> |
|-------------------------------|--|--|
| (Intercept) | 0.21 (-1.23; 1.62) | 6.61 (4.72; 8.51) |
| Sex (Ref.: Female) | -0.28 (-0.49; -0.07)**[1.00] | 0.08(-0.16; 0.32) [0.58] |
| Cortisol | 0.45 (0.4; 0.51)**[0.98] | |
| Testosterone | - | 0.86 (0.75; 0.96)** [1.00] |
| Weight (g) | - | -0.02 (-0.03; -0.01)* [0.92] |
| Age | - | - |
| Month (Ref.: August) | - | - |
| Transect A (Ref.: Transect B) | - | - |
| Year 2009 (Ref.: 2008) | -0.20 (-0.34; 0.12) [0.43] | -0.48 (-0.68; -0.28)** [1.00] |
| <i>Random effect</i> | <i>Variance</i> | <i>Variance</i> |
| Individual | 0.04 | 0.12 |
| Capture day | 0.07 | 0.03 |

*p<0.05; **p<0.01; - covariate not included in the best model; ^alogarithmic scale

258

259 *Behaviour, hormones, and parasite infestation*

260 The GLMM model showed that sex, aggression, and hormones had a relationship with parasite
 261 infestation of mouse lemurs (Table 3), where parasite infestation refers to the number of
 262 ectoparasites, and the number of endoparasites per 0.1 g feces. Males harbored more ectoparasites

263 and endoparasites than females. High levels of C were associated with increased ectoparasite
 264 infestation, but did not show a relationship with endoparasites.

265
 266 Lemurs with aggressive behavioural scores were positively associated with ectoparasitism and
 267 had higher infestations by lice, in particular (Table 3). Subjects with docile behavioural scores
 268 showed significantly lower infestation of endoparasites. Lemur weight was positively associated
 269 with tick burden.

270

Table 3. Results from GLMM models applied to investigate the effects of behavioural score, hormones, and trapping month on parasite infestation.

| <i>Covariate</i> | <i>Ticks Effect (95% CI)</i> | <i>Lice Effect (95% CI)</i> | <i>Endoparasite Effect (95% CI)</i> |
|---|-------------------------------------|--------------------------------------|---|
| Intercept | -5.11 (-8.21; -2.02)** | -4.96 (-8.07; -1.86)** | 0.15 (-1.74; 2.05) |
| <i>Behavioural Score (Ref=Score 0):</i> | | | |
| Score 1 | -0.15 (-1.14; 0.83) | 0.05 (-0.8; 0.91) | 0.18 (-0.33; 0.69) |
| Score 2 | -0.2 (-1.51; 1.1) | 0.76 (-0.14; 1.65) | 0.01 (-0.57; 0.59) |
| Score 3 | 0.63 (-0.32; 1.57) | 1.66 (0.79; 2.53)** | -0.3 (-0.88; 0.29) |
| Score 4 | 0.59 (-0.33; 1.51) | 2.02 (1.09; 2.95)** | -0.34 (-0.89; 0.21) |
| Importance weight | [0.42] | [0.98] | [0.49] |
| Male | 1.04 (0.14; 1.94)* [0.97] | 2.07 (1.05; 3.09)** [1.00] | 0.69 (0.16; 1.22)* [0.94] |
| Testosterone (ng/g) ^a | 0.12 (-0.4; 0.64) [0.57] | 0.37 (-0.85; 0.1) [0.61] | 0.21 (-0.08; 0.5) [0.39] |

| | | | |
|---------------------------------------|------------------------------|--|-----------------------------|
| Cortisol (ng/g) ^a | 0.15 (-0.21; 0.51) [0.65] | 0.34 (0.06; 0.62)* [0.96] | 0.01 (-0.19; 0.2) [0.78] |
| Weight (g) | | | |
| Age | | | |
| <i>Month</i> (Ref= August) [0.59]: | | | |
| September | -0.2 (-1.33; 0.92) | 0.57 (-0.57; 1.71) | -0.33 (-1.06; 0.41) |
| October | 0.01 (-1.09; 1.11) | 0.96 (-0.15; 2.08) | 0.12 (-0.62; 0.85) |
| November | 0.28 (-0.96; 1.52) | 0.59 (-0.68; 1.85) | 0.23 (-0.58; 1.04) |
| Importance weight | [0.37] | [0.65] | [0.52] |
| Site A (Ref.: Site B) | - | - | - |
| Number of recaptures | - | - | - |
| Year 2008 (Ref.: 2009) | - | - | - |
| <i>Random effect</i> | <i>Variance</i> | <i>Variance</i> | <i>Variance</i> |
| Subject | 0.01 | 0.13 | 0.16 |
| Trapping date | 0.01 | 0.02 | 0.02 |

*=p<0.05, **=p<0.01, - covariate not included in the best model, ^a logarithmic scale

271

272 *Lemur behaviour and louse movement*

273 After having had their lice marked and being released, fourteen lemurs from site A, all male,
274 donated and/or received lice from conspecifics. Aggressive individuals transferred significantly
275 more lice to conspecifics than docile individuals in terms of number of lice transferred (U= 41,
276 N1= 7, N2= 25, P < 0.05, P=0.01) and number of transfer events (U= 43, N1=7, N2=25, P <
277 0.05, P=0.01) (Fig 4, Fig 5). No significant difference was found between aggressive and docile
278 lemurs in terms of the number of lice received from other individuals (U=60, N1=7, N2=25, P >

279 0.05, $P=0.1$). Aggressive lemurs donated 71% of the lice marked on them to conspecifics, while
280 only docile lemurs donated only 30% of the lice marked on them to conspecifics. The mean
281 donor score for aggressive lemurs was 2.3, and the mean donor score for docile individuals was -
282 1.5.

283

284 **DISCUSSION**

285 Through the implementation of a field behavioural assay for the classification of aggression or
286 docility, our findings support the hypothesis that hormones (T and C) are associated with
287 aggression, that C is associated with ectoparasitism (with lice specifically), and that aggression
288 can predict parasite spread in a population.

289

290 Similar to other studies (Wingfield et al. 1987; Korte et al. 2005), we found T and C to be
291 associated with aggression. Androgen elevations during the mating season and mating
292 competition may explain the associations between T and aggression. In our study system, we
293 were also able to identify an increase in aggression during the mating months of September and
294 October, which may be a result of hormonal shifts during this period (Balthazart 1983; Wingfield
295 et al. 1990; Exner et al. 2003). Similarly, environmental effects like temperature, or food
296 availability that can affect hunger, may explain the aggression seen during the mating period. A
297 shift towards aggression during the mating season may provide polygynandrous species, such as
298 *M. rufus*, with exposure to additional mates as well as other conspecifics with whom they come
299 into contact. The aggressive behavioural scores found here during the mating period may also
300 explain the increase in ectoparasites seen in males during the mating season (as reviewed in
301 Roberts et al. 2004), because lice are obligate ectoparasites that can only be transmitted via host-

302 host contact. Therefore, males may be acquiring lice during agonistic male-male contests during
303 the mating season.

304 In our data, animals with aggressive behavioural scores were significantly more infested by
305 ectoparasites, and had higher infestation by lice, in particular. In addition to ecto-parasitism, lice
306 have been found to be important vectors of pathogens (Raoult & Roux 1999), and are vectors of
307 pathogens *Anaplasma* spp. and *Rickettsia* spp. (Hornok et al. 2010), which may cause fatal
308 infections. These findings suggest that aggression may in fact place vertebrate health at risk,
309 which may decrease fitness.

310 In contrast to ectoparasites, animals with aggressive behavioural types were found to harbor
311 significantly fewer endoparasites than docile individuals. Since endoparasites are often
312 transmitted using routes other than direct contact, such as through the environment, our results
313 suggest that docile individuals may be more prone to environmental contamination. Spiegel et al.
314 (2015) reported that aggressive individuals are less responsive to ecological properties of the
315 habitat and share less space with conspecifics, which supports our finding that docile individuals
316 harbor more endoparasites.

317 We also found a population-level trend towards docility, with the majority of animals in our
318 study system exhibiting a docile score (Figure 1). Since aggressive individuals have increased
319 parasitism, aggression could be selected against. The selective advantage of an aggressive
320 behavioural type is as yet unknown, but could be linked to enhanced competitiveness during
321 male-male competition (DiRienzo et al. 2012; McDermott et al. 2014). Aggression may also,
322 however, confer benefits to an individual by providing access to novel resources, habitats, and
323 potential mates, explaining why it is maintained in the population.

324 The Immunocompetence Handicap Hypothesis (ICHH) proposes that the immunosuppressive
325 effects of testosterone enable testosterone-linked traits to signal the health of the immune system
326 to potential mates (Folstad & Karter 1992). Yet, we found T to have no relationship with an
327 increase in either endo- or ecto-parasitism. If “health” is determined by females, then
328 ectoparasites, not endoparasites or other pathogens, may provide an honest visual signal due to
329 the visibility of the parasites on the head (the most parasitized region of the body in this species
330 (Durden et al. 2010), and damage to the fur. We therefore propose that future studies testing the
331 ICHH highlight the effect on ectoparasitism in particular. As many ectoparasites are also vectors
332 of disease, an increase in ectoparasitism may also contribute to a suite of other vector-transmitted
333 pathogens in the host. In order to have increased ectoparasitism, an animal must not only have
334 decreased immunity, but also an increase in exposure to ectoparasites. Therefore, we suggest that
335 future studies examining the ICHH take into consideration behaviour, hormones, and social
336 contacts with conspecifics to account for T-linked parasite dynamics. In contrast, C, the anti-
337 stress hormone that acts as an immunosuppressant (Roberts et al. 2007), appears to be directly
338 linked with an increase in ectoparasites (though it did not show a relationship with
339 endoparasites). Our findings do not support the ICHH; however, the association between C and
340 parasites do provide support for cortisol-mediated immune function.

341 In a previous study (Zohdy et al. 2012), lice were tracked to monitor the movement of parasites
342 within a population. Here, we combined phenotypic behavioural scoring with this method to gain
343 a better understanding of the role that behaviour may play in parasite dynamics. During
344 experiments monitoring louse movement in this system, we found that aggressive individuals

345 donated significantly more lice to conspecifics than docile individuals (in terms of number of lice
346 and louse transfer events). Aggressive individuals transferred a larger number of their marked
347 lice to conspecifics, and had positive donor scores, suggesting that they donate more lice than
348 they receive; docile individuals had negative donor scores, suggesting that they receive more lice
349 than they donate. Our results indicate that regardless of spatial capture location, aggressive
350 individuals are less likely to contribute to other aggressive individuals, even if they are close by
351 (Figure 5). These findings also suggest that aggression may not only influence an individual
352 animal's health, but also an entire population's health. This could be particularly true for
353 exceedingly aggressive individuals, which may act as keystone individuals for emergent
354 population dynamics (Meyers et al. 2005; Modlmeier et al. 2014). Therefore, our finding of the
355 low ratio of aggressive to docile individuals and their spatial configuration may maintain the
356 delicate host-parasite dynamics and co-evolution in populations. Any alteration in these ratios
357 may therefore alter population-wide disease transmission dynamics.

358 *Conclusions*

359 This study reveals relationships between aggression and hormones on the heterogeneity of
360 parasite infestations. Our findings highlight the associations between animal aggression,
361 androgens and glucocorticoids, and ectoparasitism at the individual and population levels, and
362 the potential impact of animal behaviour on health and host fitness. We suggest that future
363 studies place emphasis on key behaviours that lead to parasite exposure and neuroendocrine
364 states that contribute to peak immune susceptibility. Further, parasitism itself has been shown to
365 play a role in behavioural modulation according to host manipulation theory (as reviewed in
366 Poulin & Maure 2015), and empirical evidence may further elucidate its role in endocrine
367 modulation. Many ecological factors, including environmental conditions, may also play a role

368 in endocrine and behavioural modulations, and therefore should also be included in studies
369 examining host exposure and susceptibility to parasites. Integrative approaches and perspectives
370 from diverse disciplines will provide essential new insights on parasite-host dynamics and can
371 have an impact on individual and population health in human and animal communities.

372

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381 University of Helsinki, and Stony Brook University.

TABLE LEGENDS

Table 1. Results from cumulative link mixed models (CLMM) on associations between sex, hormones, trapping month, body mass (g), and behavioural score.

Table 2. Results from GLMM to investigate relationship of hormone levels with sex, weight, capture months.

Table 3. Results from GLMM models applied to investigate the effects of behavioural score, hormones, and trapping month on parasite infestation.

FIGURE LEGENDS

Figure 1. Frequency of captures and behavioural scores (0-4) of captured animals.

Figure 2. Variation of behavioural scores during the study period. This figure shows behavioural scores of animals recaptured more than 5 times. Tables in the left column report frequency and behavioural score for each animal. The color of the table cells represents the number of captures (darker red= more captures). The dot plots on the right show distribution of behavioural scores during sampling months. Each dot represents a subject-score pair recorded in the capture month, and the size of dot is the frequency with which the pair was recorded.

Figure 3. Boxplots of levels of T and C shown by sex (A), month (B), site (C), and behavioural score (D) for 2008 and 2009. Solid black lines indicate the medians, white boxes indicate interquartile ranges, dotted bars indicate extreme values of data (1.5 times interquartile ranges), and shows outlier measurements. Every boxplot in the graph is composed of two boxes (2nd and 3rd interquartile range) and two lines which represent 1st and 4th interquartile range.

Figure 4. Aggressive individuals made significantly more louse donations to conspecifics than docile individuals. Whiskers indicate standard error.

Figure 5. Aggressive lemurs donate more lice to conspecifics than docile lemurs. Diagram of movement of lice between the lemurs in site A, where circles with black outlines represent aggressive individuals and circles without an outline represent docile individuals. The color of the circle corresponds to the location the lemur was most frequently captured at along the transect (seen as a dotted black line in the legend). Black arrows indicate louse donations from aggressive individuals. Grey lines indicate all other louse transfer events. Arrow thickness represents the number of lice transferred.

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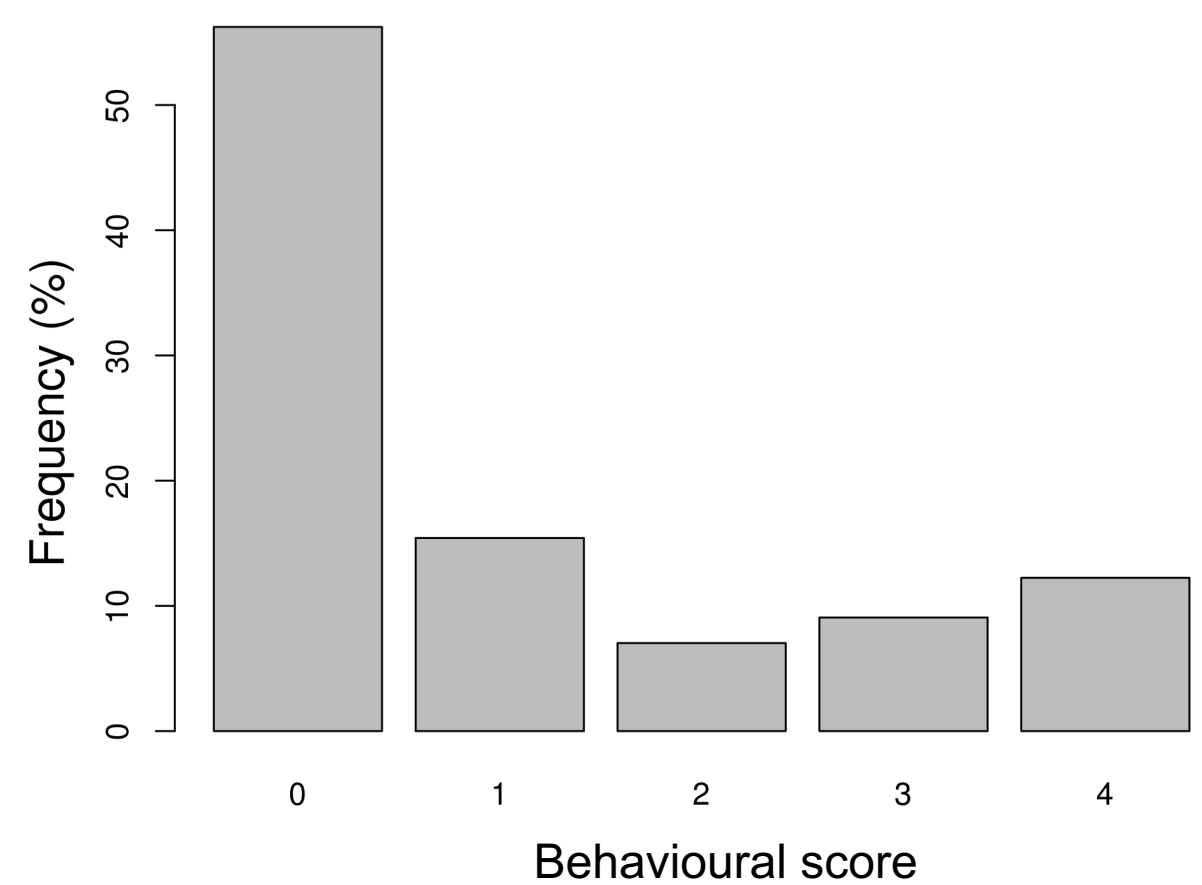
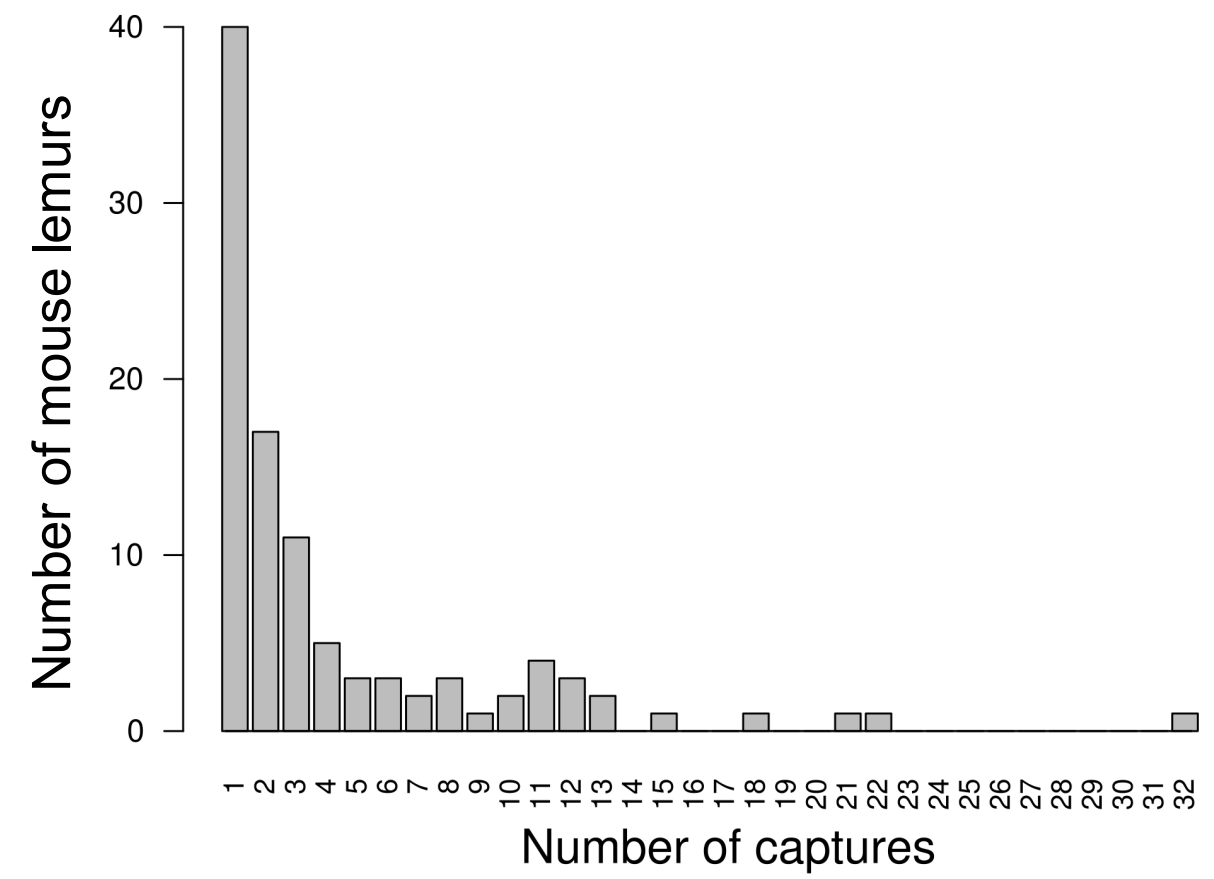
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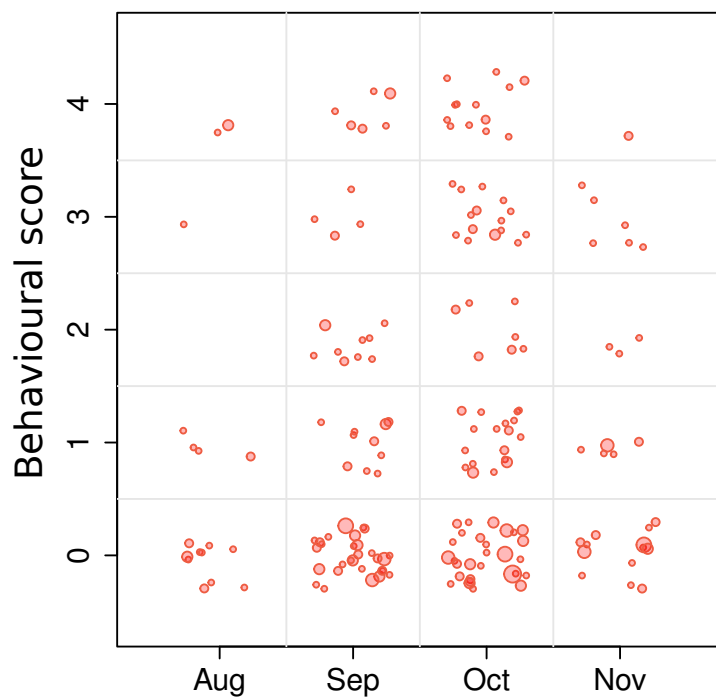
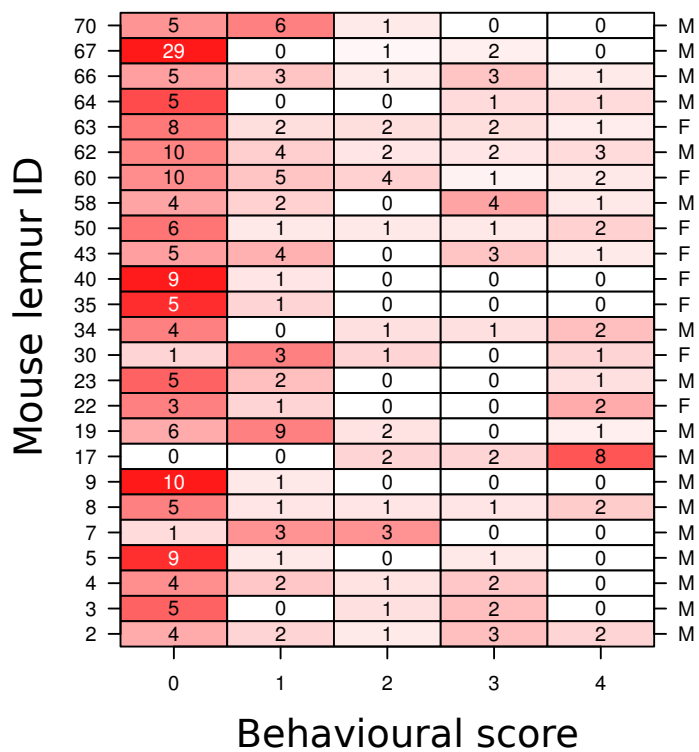
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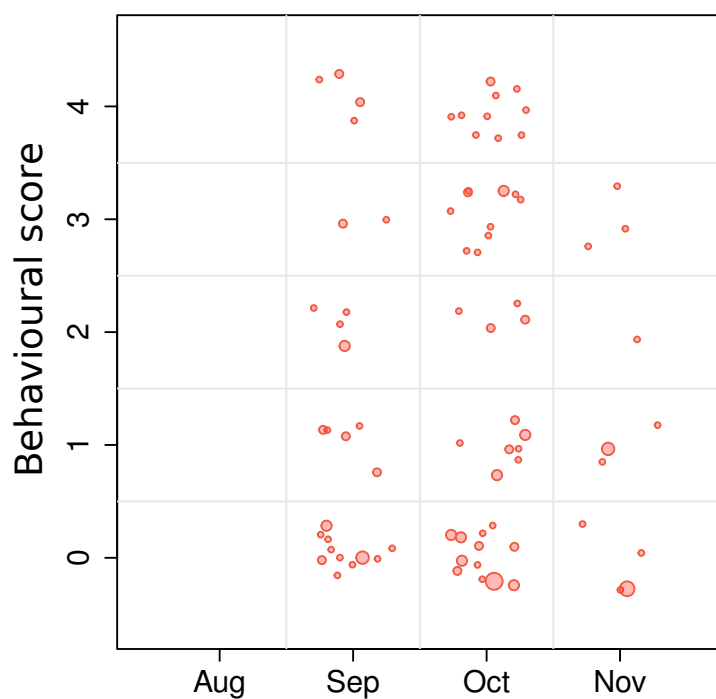
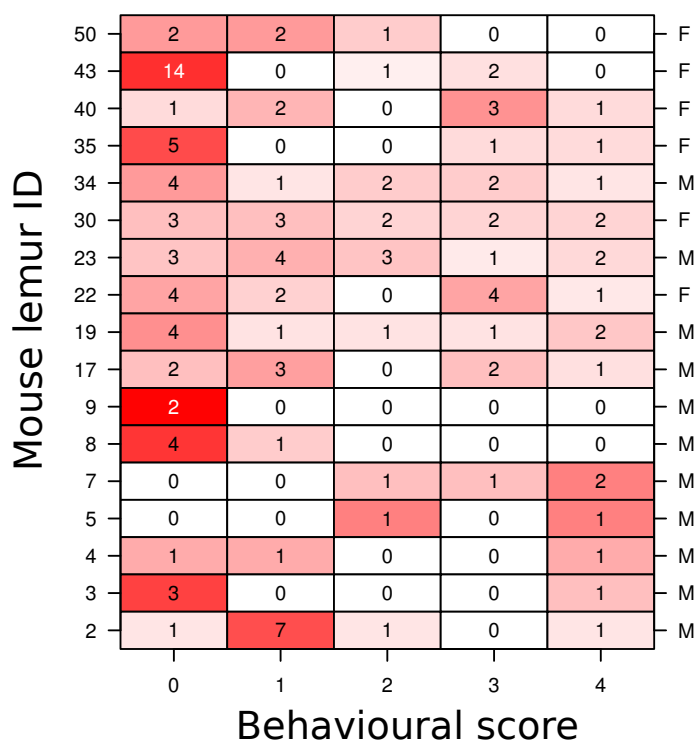
Figure



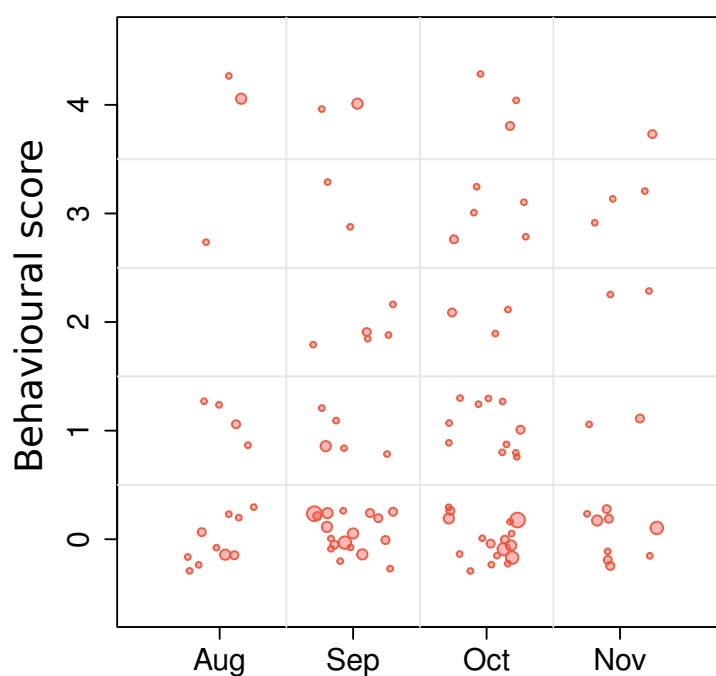
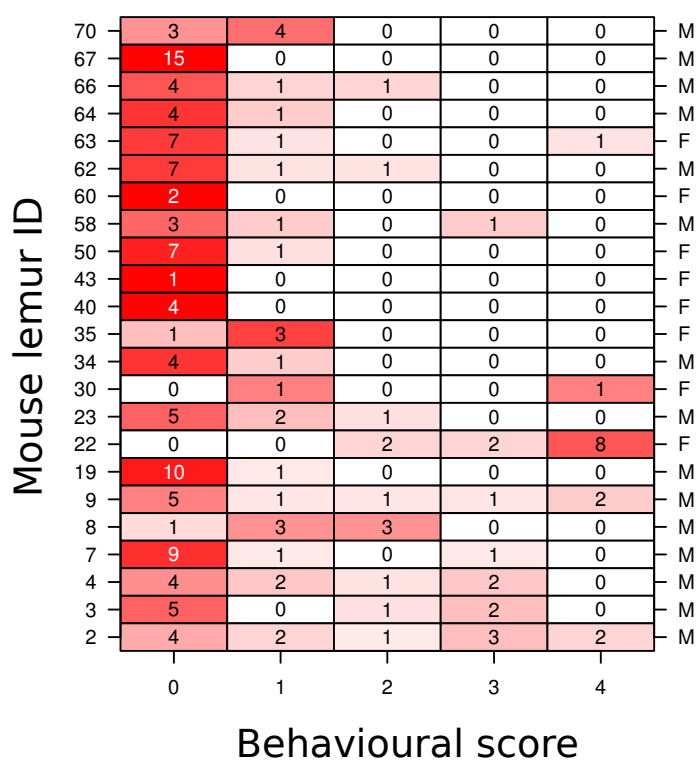
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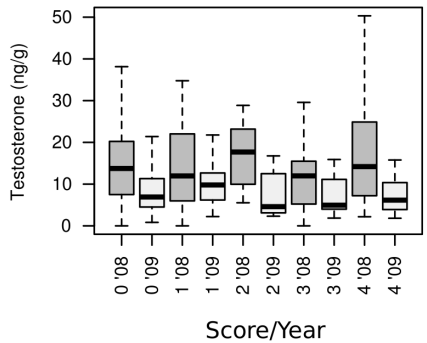
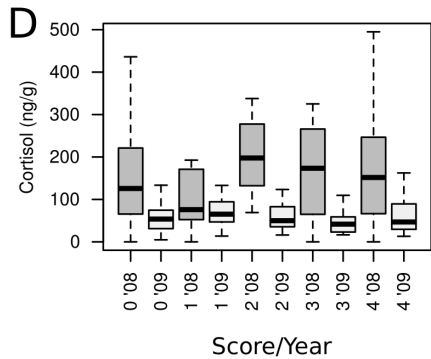
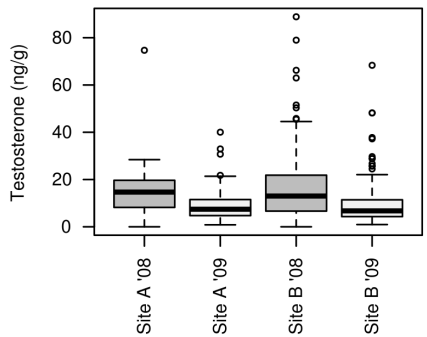
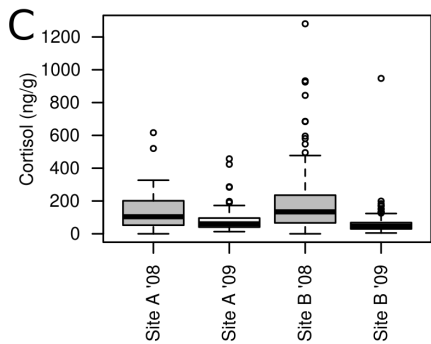
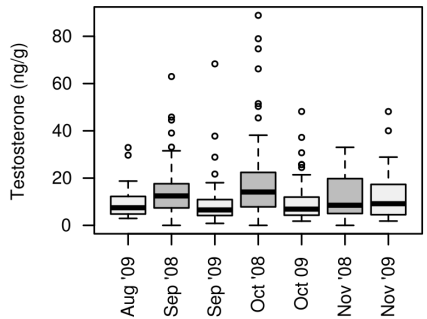
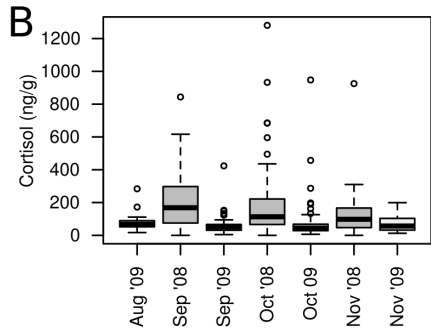
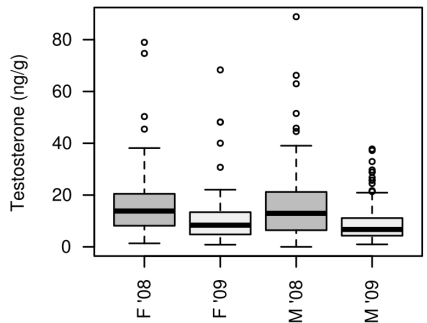
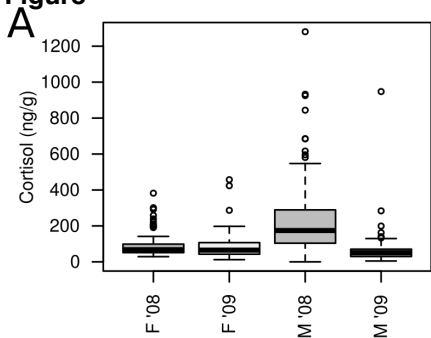


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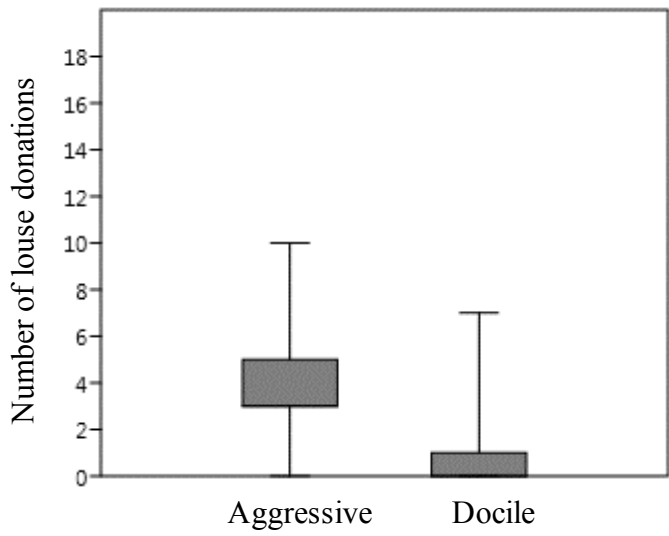


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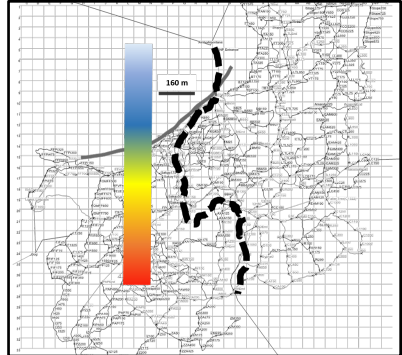
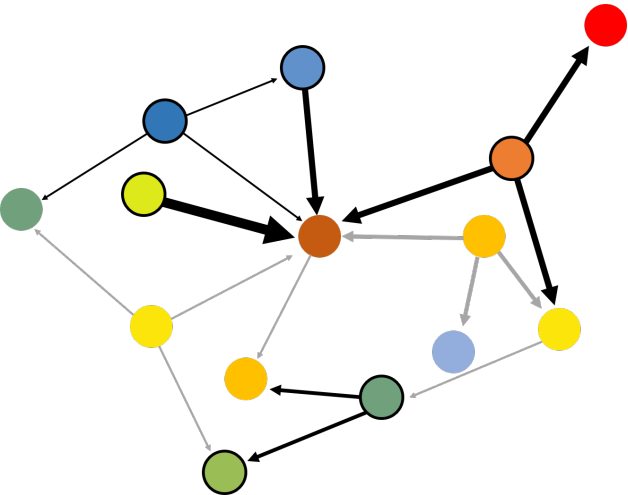


Figure

Figure



Figure



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