

1 **Matrilineal Behavioral and Physiological Changes Following the Death of a Non-alpha**
2 **Matriarch in Rhesus macaques (*Macaca mulatta*)**

3

4 Lauren Wooddell^{1*}, Stefano S.K. Kaburu², Kendra L. Rosenberg³, Jerrold S. Meyer³, Stephen J.
5 Suomi¹, Amanda M. Dettmer¹

6 ¹Eunice Kennedy Shriver National Institute for Child Health and Human Development, National
7 Institutes of Health, Poolesville, Maryland, United States of America

8 ²Department of Population Health and Reproduction, School of Veterinary Medicine, University
9 of California, Davis, California, United States of America

10 ³Department of Psychological and Brain Sciences, University of Massachusetts Amherst,
11 Amherst, Massachusetts, United States of America

12

13 *Corresponding author

14 Email: lauren.wooddell@nih.gov (LW)

15

16 Short title: Changes following the death of a non-alpha matriarch in rhesus macaques

17

18

19

20 **Abstract**

21 In many species, the loss of alpha matriarchs is associated with a number of negative outcomes
22 such as troop fission, eviction, wounding, and reduced vitality. However, whether the dramatic
23 consequences of their loss are due to their role as an old experienced figure or to their alpha
24 status remains unclear. We tested the hypothesis that in a semi-free ranging colony of rhesus
25 macaques (*Macaca mulatta*), the death of a non-alpha matriarch, who had a large set of kin,
26 would lead to changes in behavior and physiological stress within her matriline. Following her
27 death, her matriline increased in aggression, vigilance, and social grooming. Additionally,
28 hierarchical stability, measured by levels of rank changes, decreased within her matriline, and
29 levels of intense aggression by high-ranking animals were more frequent, as well as matrilineal
30 wounding. Although ordinal rank was positively associated with higher chronic hair cortisol
31 concentrations (HCCs) in the months before the matriarch's death, following her death, only
32 those who experienced large increases in rank within her matriline displayed higher HCCs. We
33 conclude that the death of the non-alpha matriarch led to matrilineal instability, characterized by
34 higher levels of aggression and subsequent vigilance, rank changes, physiological stress, and
35 grooming. We suggest that non-alpha matriarchs with a large number of kin and social support
36 can be integral to the stability of family groups.

37 **Keywords:** *Macaca mulatta*; social instability; matriarch; hair cortisol; Elo-rating

38

39

40

41

42 **Introduction**

43 Among long-living animals, old females can play a crucial role for the survival of their
44 family or social group. This support can come indirectly if these females use the experience they
45 have accumulated over the years to lead the group and protect it from external threats [1-3] or
46 directly by providing care to the future generations [4] or, at least in some cases, by controlling
47 intra-group conflicts [5]. For example, in African elephants (*Loxodonta africana*), family units
48 are commonly led by the oldest female, who exhibits the necessary social and ecological
49 knowledge to coordinate her family and better recognize dangers [1, 6-8]. Accordingly, older
50 female elephants were found to be better at discriminating between familiar and stranger groups
51 [6], and at displaying more appropriate responses to predators [1] than young females, which can
52 explain why old females tend to have larger families with more calves [6]. Similarly, among
53 orcas (*Orcinus orca*), menopausal old females use their ecological knowledge to guide their
54 families towards feeding sources, especially during periods of food scarcity [2], a trait that likely
55 increases their offspring's survival [9]. In chimpanzees (*Pan troglodytes*), females have been
56 observed acting as mediators to reconcile individuals (generally males) during aggressive
57 interactions [10-12], while in humans (*Homo sapiens*) the presence of grandmothers enhances
58 grandchildren's survival by increasing their foraging efforts [13-16].

59 Collectively, these studies have highlighted the importance of older females for their
60 social group, and there is evidence that their loss can have dramatic effects on the rest of the
61 group. In elephants, the death of matriarchs results in family unit splitting [17, 18] and increases
62 the likelihood of calf death [19]. In ring-tailed lemurs (*Lemur catta*), the loss of the troop's
63 matriarch led to the eviction of distantly related kin [20], while in captive rhesus macaques
64 (*Macaca mulatta*), matriline with matriarchs received fewer wounds than matriline without

65 matriarchs [21]. However, since in many species matriarchs are often also alpha females, it is
66 unclear whether the disruptive consequences of their loss on the family socio-dynamics are due
67 to their influence as experienced females or rather to the important role of alpha females in the
68 group. This is because high-ranking individuals commonly stabilize the social group through
69 their interventions in ongoing conflicts in the attempt to appease opponents [5, 10, 22, 23].
70 Accordingly, the loss of dominant individuals has been found to increase social instability [11,
71 24, 25], to change social behaviors in the group [11, 26-28] and to affect individuals' stress
72 levels [29]. However, a detailed analysis of the behavioral and physiological consequences of the
73 death of non-alpha matriarchs is currently lacking.

74 Rhesus macaques are good models for studying the consequences of the death of non-
75 alpha matriarchs. They form large multi-male multi-female groups with an average group size
76 between 10 and 80 individuals [30-32]. Male macaques generally emigrate to neighboring
77 communities around sexual maturity at 4-5 years of age [33], while females remain in the natal
78 group and form linear dominance hierarchies on the basis of their matrilineal kinship. Thus,
79 matriarchs with large families are likely to both have and provide substantial social support (as
80 mothers often aid their offspring in conflicts [34]) as well as to influence social dynamics within
81 their matriline.

82 Here, we use the definition of a matriarch as the oldest living female member of a family
83 (where family is defined as individuals descended from a common female ancestor). As females
84 in our troop have their first offspring around 3-5 years of age, a family's matriarch has at least
85 grandmother status.). Because rank in rhesus macaques is determined by birth rank (i.e. mother's
86 rank) and not age [35], a matriarch is not necessarily the alpha female. We tested the hypothesis
87 that the death of a non-alpha matriarch in a captive colony of rhesus macaques significantly

88 changed behavior and physiology in her matriline but not within the other matriline present in
89 the troop. At the time of this study, this matriarch, Heather, had seven offspring in the
90 population, and had birthed a total of 13 offspring overall, making her the matriarch with the
91 largest number of extant offspring in the troop. Two of Heather's sons were high-ranking males
92 (2nd and 3rd) in the troop, and her two younger sisters were the troop's alpha and beta females
93 (with the beta's eldest daughter, Pepper, outranking Heather, and Pat and Pandora beginning to
94 ascend the hierarchy but not yet ranking above Heather, see Fig 1). Thus, although Heather was
95 not one of the top three ranking females in the troop, it is likely that she exerted a strong
96 influence on matrilineal dynamics.

97

98 **Fig 1 Pedigree of Matriline 3 members from a larger troop of rhesus macaques at the LCE**

99 **field station**

100 Deceased relatives are denoted by parentheses and italicized font

101 Underlines indicate males

102 (#) represents rank within sex (limited to #1-3 to show Heather's relationships to top-ranking
103 members; Alpha male is from matriline 1)

104 * denotes individuals with behavioral data

105

106 We tested this hypothesis by examining dominance stability, vigilance, grooming,
107 aggressive interactions, and hair cortisol concentrations (HCCs) within Heather's matriline,
108 while also examining the other two matriline within the troop and overall troop wounding,
109 before and after her death. We assessed HCC as a measure of chronic hypothalamic-pituitary-
110 adrenocortical (HPA) activity. HCC measurement has several advantages over the collection of

111 other biological samples (i.e. blood, urine, and feces), including the ability to measure chronic
112 HPA activity without the need of multiple sampling and without the confounds of circadian and
113 environmental variability [36-38]. We hypothesized that the matriarch provided stability within
114 the matriline, and predicted that her death would lead to 1) rank changes, and thus higher
115 instability (*prediction 1*), 2) higher aggression rates (*prediction 2*), and 3) higher levels of
116 wounding (*prediction 3*). An increase of rank instability and aggression rates following the
117 matriarch's death should, in turn, increase individuals' vigilant behaviors (*prediction 4*) and
118 should increase HCCs as an index of chronic stress, especially among high-ranking animals
119 (*prediction 5*) as their dominance positions are contested [29]. Finally, previous work has shown
120 how social grooming can decrease social tension among primates [39, 40], and thus we predicted
121 rates of social grooming would increase during the period of social instability (*prediction 6*).
122 Finally, if social grooming can help alleviate chronic stress, we also predicted that monkeys with
123 the highest frequency of grooming would have relatively lower hair cortisol concentrations
124 (*prediction 7*). Because the matriarch is likely the most influential within her matriline, we did
125 not predict any significant changes within the other two matrilines, which were presumably
126 unaffected.

127

128 **Materials and methods**

129 **Subjects and housing**

130 Subjects were 49 rhesus macaques (age range 3-17 years) born and reared at the
131 Laboratory of Comparative Ethology (LCE) field station at the NIH Animal Center in
132 Poolesville, Maryland, USA. Because there is an age-related decline in HCC in infants and
133 juveniles that later stabilizes [41], we included all subjects above three years old. The field

134 station is a 2.0 hectare outdoor open air enclosure consisting of natural vegetation, climbing
135 structures, corncrib shelters, and a central pond measuring 0.09 hectares [42]. Continuous access
136 to three indoor enclosures (2.74 x 5.79 x 4.27m) was also available, where animals had *ad*
137 *libitum* access to water, as well as commercial lab diet (Purina Monkey Chow #5038, St. Louis,
138 MO) and were given fresh fruits and vegetables twice daily. All procedures described below
139 adhered to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the
140 NICHD Animal Care and Use Committee (ACUC). Furthermore, all applicable international,
141 national, and/or institutional guidelines for the care and use of animals were followed.

142

143 **Group structure**

144 The troop consisted of three major matriline, which are related individuals descending
145 from a female line. Matriline 3 has occupied the top rank since 2009, when its major families
146 staged an attack over the previously dominant matriline 1 [42] leading to severe injuries and
147 therefore the permanent removal of most members of matriline 1. Matrilineal hierarchies were
148 ranked in the order, from highest to lowest: matriline 3, matriline 4, and matriline 1 (matriline 2
149 was removed in 2004 for management reasons) [42]. The demographic composition of the troop
150 is shown in Table 1. Subjects were 49 adults (matriline 3: 29; matriline 4: 15; matriline 1: 5).

151

152 **Table 1: Troop composition at the LCE field station in 2014.**

	Adults (3+)	Juveniles/infants (<2)	Total
Matriline 3	29 (38%)	14 (18%)	43 (57%)
Matriline 4	15 (20%)	11 (15%)	26 (34%)

Matriline 1	5 (7%)	2 (3%)	7 (9%)
Total	49 (64%)	27 (36%)	76

153 Numbers in parentheses represent proportion out of entire troop (%)

154

155 **Demographic change: Death of matriline 3 matriarch**

156 On October 10, 2014, a veterinary decision was made to humanely euthanize the troops’
 157 eldest (but non-alpha) matriarch, Heather, at 18 years of age, due to chronic gastrointestinal
 158 issues that resulted in chronic diarrhea which were unresolved with medical intervention. A
 159 licensed veterinarian performed the euthanasia with 2.0 cc pentobarbital intravenously to induce
 160 a humane, painless, and rapid death. All institutional requirements in the euthanasia and disposal
 161 were followed.

162 Heather’s offspring remained in the troop after her death, including six adults (five
 163 females, one male) and one juvenile female. However, the two youngest females were
 164 permanently removed from the population at the bi-annual health exam in February 2015 as part
 165 of routine population management.

166

167 **Data collection and analysis**

168 **Behavioral data**

169 Behavioral data were collected using modified frequency data sheets [43] from eight
 170 females and two males (see Fig 1), representing every major lineage within matriline 3, through
 171 continuous 5-minute focal animal interval sampling [44]. Data were also collected from the three
 172 highest-ranking females from matriline 4 and the two highest-ranking adults in matriline 1 (the

173 troop's alpha male and one female). Due to limited researcher availability, focal behavioral data
174 were only collected on this subset of the population.

175 The 5-min samples were divided into 20, 15-second intervals. For each interval, every
176 behavior that occurred within the 15 seconds was recorded. The frequency of intervals in which
177 the behaviors occurred was then calculated. The maximum number of intervals an animal could
178 perform a behavior therefore in one session was 20 intervals. Focal subjects received one
179 weekly AM session (9:00-12:00) and one weekly PM session (12:00-17:00) for ten consecutive
180 weeks from August to December 2014 by three observers (inter-rater reliability >85%). Data
181 used were part of a long-term data collection procedure for unrelated projects, and therefore,
182 coders were blind to the hypotheses of the study.

183 The focal animals within matriline 3 included: 1) the top three ranking females
184 (Heather's sisters and niece: Hermione, Haley, Pepper; see Fig 1); 2) a female who underwent a
185 major rank change and rose above the matriarch's offspring (Cilix); 3) a matriarch ranking
186 immediately below Heather's family (Babette); 4) one son of the matriarch (2nd in the male
187 hierarchy, Kip), 5) one high-ranking male (Charley, distant cousin to Heather), 6) and three
188 intermediate ranking females within the matriline (Bella, Yvonne, Yvette). From the focal
189 animals, we recorded data on both social (e.g. aggression, social grooming) and non-social (e.g.
190 feeding, vigilance) behaviors (only behaviors in Table 2 were analyzed for this study). In order
191 to calculate rank changes and dominance stability, we noted all instances of dominance
192 interactions (i.e. supplant, threat, chase, attack, and submissive) within the entire troop through
193 *ad libitum* observations [44]. We collected 600 focal observations (40 sessions/focal animal, 20
194 before and 20 after), for a total of 50 hours of observations (25 hours before and 25 hours after
195 Heather's death), as well as 1,258 *ad libitum* troop dominance interactions [44].

196 Behavioral observations thus included the ten focal subjects from matriline 3 and the five
 197 focal subjects from matriline 4 and 1, while *ad libitum* aggressive interactions included all 49
 198 subjects from across the three matriline.

199

200 **Table 2: Behaviors and definitions collected during focal observations**

Behavior	Definition
Initiation of aggression	Displacements (taking the place of another), chases (aggressive approach at least 3 strides), threats (open mouth, ears back, direct eye contact, includes lunges), attack (pin down and bite)
Grooming	Move hair apart, and visual inspection, removal of debris; combination of given and received bouts
Vigilance	Scan the environment visually at least 3 seconds

201

202 **Dominance rank and social instability**

203 Dominance hierarchies were calculated via Elo-rating, a numerical system that tracks
 204 rank changes over time by constantly updating values based on wins and losses [45-49]. We
 205 generated Elo-ratings using the *elo.sequence* function provided by Neumann et al. [45] in R
 206 software (v 3.1.2), by setting animals' initial rating at 1,000, and the k factor, which is weighted
 207 based on the probability of winning, at 200. Two main advantages of the use of Elo-rating over
 208 conventional matrix-based analyses include the ability to track rank changes over time, and
 209 accommodating variations in social dynamics due to a fluctuations in the study population [45,
 210 47], making Elo-rating ideal for this study.

211 Social instability was calculated through the *stability.index* function derived from
212 Neumann et al. [45]. This function measures the ratio of rank changes per individuals present
213 over a given time period based on the derived Elo-ratings. As Elo-ratings can be arranged to
214 reflect ordinal ranks, fluctuations in Elo-ratings reflect higher levels of instability. According to
215 Neumann et al. [45], S values typically range from 0 to 0.5, where higher S values reflect higher
216 levels of instability, and lower values reflect a relatively stable hierarchy. Stability measures
217 were taken for the three months before and after Heather’s death for each matriline. Dominance
218 ranks and stability measures thus included data from all 49 subjects across the three matriline.

219

220 **Wounding rates**

221 Archival data from veterinary records from 2014 to 2015 were examined for incidences
222 of fight wounds from all troop members above three years of age. We analyzed only major
223 wounds, indicative of severe aggression [41, 42], which required medical treatment, recorded in
224 the three months before and after the matriarch’s death. Fight wounds were analyzed to
225 determine whether Heather’s death resulted in an increase in wounds for her matriline compared
226 to matriline 4 and 1.

227

228 **Hair sample collection and cortisol assay**

229 Hair samples were collected by shaving the back of the animals’ necks [36] during
230 routine health exams in August 2014 and February 2015 for all 49 subjects across the three
231 matriline. Because these animals had been shaved as part of a longitudinal study every six
232 months beginning in Fall 2012, the samples in August 2014 reflected hair cortisol concentrations
233 (HCCs) that had accumulated since February 2014, and the February 2015 samples reflected

234 HCCs that had accumulated since August 2014 (thus reflecting the timing of Heather's death in
235 October 2014). Samples were stored in a foil pouch at -80°C until shipment to the Hormone
236 Assay Core Laboratory at the University of Massachusetts Amherst. Following Dettmer et al.
237 [41], samples were weighed, washed twice with isopropanol and dried for 5-7 days under a fume
238 hood. Samples were then ground to a fine powder with a ball mill grinder (MM200; Retsch,
239 Newtown, PA) and incubated in methanol for 24 hours to extract cortisol from the samples.
240 Aliquots of the methanol extract were dried down and reconstituted with assay buffer, then
241 analyzed via enzyme immunoassay (EIA) using a salivary cortisol kit (#1-3002; Salimetrics,
242 State College, PA). Resulting values ($\mu\text{g/dL}$) were converted to pg/mg for analysis. Inter- and
243 intra-assay coefficients of variation were $<8\%$ based on aliquots of the same extracted pooled
244 hair sample analyzed repeatedly across assays.

245

246 **Data Analysis**

247 Due to the small sample size of matriline 1 ($N=5$), data for matrilines 4 and 1 were
248 analyzed together for all analyses.

249 To assess whether focal animals changed frequencies in their behaviors following
250 Heather's death, we compared rates of each behavior collected approximately three months
251 before and after her death using paired-sample t-tests for both matriline 3 and the combined
252 matrilines 4 and 1.

253 Subjects were classified as high or low ranking (within their respective matriline) based
254 on a median split of the Elo-ratings. Levels of intense aggression (i.e. chases and attacks) were
255 compared using both focal and *ad libitum* data using paired-sample t tests to examine whether
256 high ranking monkeys increased in intense aggression.

257 Wounds were analyzed as the proportion of wounds that occurred across each matriline,
258 (i.e. out of all reported wounds, how many were from each matriline) and Chi square tests were
259 used to compare changes in proportion of matrilineal wounds before and after Heather's death.

260 HCC values were log transformed to ensure normality prior to analysis. Seven subjects
261 were missing a HCC measurement in August (three from matriline 3, three from matriline 4, one
262 from matriline 1), as they could not be captured during routine exams. Two subjects were also
263 missing a HCC measurement in February (both from matriline 4; one of which did not have any
264 HCC data for either time point). One subject from matriline 3 was also excluded from the August
265 HCC analysis (as well as the HCC change analysis), as the HCC measurement in August was
266 well above six standard deviations from the mean. We tested the association between
267 individuals' HCCs and their Elo-rating using Pearson's correlation test for both matriline 3 and
268 matrilines 4/1. Additionally, we ran Spearman's correlation test (owing to the smaller sample
269 sizes) between HCC changes (from August to February, in pg/mg) and Elo-rating changes for
270 high and low ranking monkeys. We predicted that an increase in rank would be associated with
271 an increase in physiological stress among high-ranking monkeys in matriline 3 as their
272 dominance positions were contested.

273 To examine whether frequencies of social grooming were associated with HCCs after the
274 matriarch's death, we calculated a grooming frequency across the entire 6 months (from August
275 to February; the total amount of time that HCC accumulated) for the 15 subjects of whom
276 behavioral data was collected (ten from matriline 3, five from matrilines 4 and 1). We then tested
277 the association between grooming frequency and February 2015 HCC (which reflected chronic
278 activity since August) using Spearman's correlation test.

279 All tests were two tailed with the significance level set at $p < 0.05$. SPSS 22 was used for
280 analyses.

281

282 **Results**

283 **Social stability and behavioral changes**

284 As expected (*prediction 1*), we found lower social stability after Heather's death compared
285 to the three months before: the stability index for matriline 3 increased from 0.039 to 0.128.
286 Matrilines 4&1 had little change in stability (from .017 to .018). Furthermore, our focal
287 observations revealed that in the months immediately following Heather's death, there was a
288 significant increase in the initiation of aggression (paired-sample t-test: $t_{(9)} = -3.20$, $P = 0.01$, Fig
289 2a), in the time spent in vigilance ($t_{(9)} = -3.15$, $P = 0.01$, Fig 2b), and in grooming time ($t_{(9)} = -2.74$,
290 $P = 0.02$, Fig 2c) within matriline 3, supporting *predictions 2, 4* and *6*. No significant changes
291 were found for the initiation of aggression ($t_{(4)} = 0.10$, $P = 0.93$), vigilance ($t_{(4)} = -1.68$, $P = 0.17$), or
292 grooming ($t_{(4)} = -1.33$, $P = 0.25$) for matrilines 4 and 1.

293

294 **Fig 2 Frequencies of behaviors within matriline 3 before and after Heather's death**

295 Frequencies represent the average number of intervals (out of 400) the behavior occurred for
296 each focal subject

297 **Fig 2a Aggression frequency** ** $p = 0.01$

298 **Fig 2b Grooming frequency** ** $p = 0.01$

299 **Fig 2c Vigilance frequency** * $p = 0.02$

300

301 The *ad libitum* and focal data revealed that high-ranking monkeys from matriline 3 also
302 exhibited an increase in the levels of intense aggression (i.e., chases and attacks; mean \pm SE:
303 0.67 ± 0.33 before to 7.60 ± 1.81 after; $t_{(14)}=-3.86$, $P=0.002$) whereas low ranking animals did
304 not show a significant change ($t_{(13)}=-1.629$, $P=0.13$). There was no significant change in intense
305 aggression for high ranking monkeys ($t_{(9)}=-1.65$, $P=0.13$) or low ranking monkeys ($t_{(9)}=-2.25$,
306 $P=0.05$) within matriline 4 and 1 .

307

308 **Wounding rates**

309 Contrary to our expectation (*prediction 3*), there was a reduction in overall troop
310 wounding (from 13 to 8) compared to the period prior to Heather's death. However, when
311 compared to the number of wounds experienced by the other matriline, matriline 3 suffered
312 proportionally more wounds after Heather's death. In the three months prior to her death,
313 matriline 3 and 4 reported approximately equal numbers of wounds (out of 13 wounds, six were
314 from matriline 3 monkeys, and seven wounds were from matriline 4 monkeys). However, in the
315 three months after her death, matriline 4 and 1 reported no wounds, while matriline 3 received
316 100% of recorded wounds (8 wounds total; chi-square test, $X^2=6.46$, $P=0.01$).

317

318 **Rank changes and hair cortisol concentrations**

319 There was no significant change in HCCs from August 2014 to February 2015 for either
320 matriline 3 ($t_{(24)}=-1.05$, $P=0.31$) or matriline 4 and 1 ($t_{(13)}=0.15$, $P=0.89$). For matriline 3, there
321 was a significant positive correlation between Elo-rating and HCCs prior to Heather's death in
322 August (Pearson correlation: $r=0.63$, $N = 25$, $P=0.001$; see Fig. 3a), but there was no correlation
323 after her death ($r =0.27$, $N = 28$, $P=0.16$, see Fig 3b). No significant correlations between Elo-

324 rating and HCC were evident for matriline 4 and 1 for either August (Pearson correlation: $r=$
325 0.23, $N=16$, $P=0.39$) or February (Pearson correlation, $r=-0.42$, $N=18$, $P=0.08$).

326

327 **Fig. 3 Relationship between rank and HCCs within matriline 3 before (3a:left panel) and**
328 **after (3b:right panel) Heather's death**

329

330 When comparing individuals based on high or low ordinal rank in matriline 3, the change
331 in Elo-rating was positively correlated with the change in HCC (from August to February;
332 Spearman, $r_s = 0.71$, $N = 13$, $P < 0.01$, Fig 4a) for high-ranking monkeys, indicating that as top
333 animals increased in dominance rank (and thus dominance assertion/increase in intense
334 aggression), they experienced an increase in physiological stress, supporting *prediction 5*. There
335 was no significant correlation for low-ranking animals (Spearman, $r_s = -0.434$ $N = 12$, $P = 0.16$,
336 Fig 4b) within matriline 3. No relationship between Elo-rating change and HCC change was
337 found for matriline 4 and 1 for either high-ranking monkeys (Spearman, $r_s = -0.32$, $N = 7$, $P = 0.48$)
338 or low-ranking monkeys (Spearman, $r_s = -0.04$, $N = 8$, $P = 0.93$; see S1 File for data used in this
339 current study).

340

341 **Fig 4 Relationship between rank change and HCC change in high-ranking (4a: left panel)**
342 **and low-ranking (4b: right panel) rhesus macaques in matriline 3**

343 Rank and HCC changes reflect changes from August 2014 to February 2015

344

345 **Hair cortisol concentrations and grooming frequency**

346 While the total grooming frequency was not significantly associated with February HCC
347 for matriline 3 (Spearman, $r_s=-0.56$, $N=10$, $P=0.15$, failing to support *prediction 7*), a significant
348 negative association was evident when analyzing all subjects from which grooming data was
349 collected (including the other two matriline; Spearman, $r_s= -0.52$, $N=15$, $P=.049$; see Fig. 5).

350

351 **Fig. 5 Total grooming frequency and HCC following Heather's death**

352 Frequency represents total number of intervals grooming that occurred from August to February
353 (when the HCC samples were taken)

354

355 **Discussion**

356 Our data indicate that a non-alpha matriarch exerted a strong influence on her matriline;
357 with direct ties to the dominant females and a large set of kin, her social ties were significant
358 enough to influence dominance stability within her matriline, although she was not the alpha.
359 Accordingly, in the period following Heather's death, her matriline endured a period of social
360 instability, with increased aggression and higher rates of both vigilance and social grooming.
361 Interestingly, we also found that large increases in Elo-rating after Heather's death were
362 associated with large increases in HCC. These findings suggest that individuals within her
363 matriline may have experienced higher levels of chronic stress, probably because in a period of
364 frequent rank changes, each individual struggled to exert dominance over others (hence the
365 increase of chase and physical attacks). The lack of any significant relation between rank
366 changes and hair cortisol for the other matriline suggests that only Heather's matriline was
367 affected by her death. We found that her matriline experienced both behavioral and physiological
368 changes that resemble the consequences of the loss or takeover of alpha individuals described in

369 both this [25] and other mammalian species (e.g. chacma baboons, *Papio ursinus* [26, 50] naked
370 mole-rat, *Heterocephalus glaber* [24]; chimpanzees, *Pan troglodytes* [11]).

371 Our results are consistent with findings reported from a variety of species (chacma
372 baboons, *Papio ursinus* [51-54]; wild dogs, *Lycaon pictus* [55]; long-tailed macaques, *Macaca*
373 *fascicularis* [56]; African cichlid fish, *Haplochromis burtoni* [57]) showing that social stress in
374 dominant individuals can be linked to social instability and the use of intense aggression by
375 dominants to affirm their position. Interestingly, we found a positive association between
376 dominance rank and hair cortisol before Heather's death, suggesting that high-ranking
377 individuals may experience more chronic physiological stress than subordinates under stable
378 conditions. This result is at first puzzling since in the period preceding the matriarch's death, we
379 found stable rank relationships within the matriline, and social stability is commonly associated
380 with higher stress levels in *subordinates*, who receive continuous harassment from dominants
381 (chacma baboons [29]; but see [Meyer and Hamel, 2014] for a discussion of moderating factors
382 that influence the relationship between social rank and physiological stress in nonhuman
383 primates). However, this relationship might be explained by the relatively new, but stable,
384 hierarchy present in the troop (circa 1 year) due to a social overthrow within the matriline in late
385 December 2013/early January 2014 [47]. It is possible therefore that there might have been some
386 degree of social tension within the matriline even in the period *before* Heather's death, that her
387 presence might have helped to hold in check, through, for instance, policing interventions [22].
388 Consequently, after the death of the matriarch, and the threat to this relatively new hierarchy, this
389 social tension was no longer under control, leading to higher levels of social instability and
390 stress. Unfortunately, we do not have data on policing behavior to test this hypothesis directly in
391 this study. However, we do know that Heather managed conflict among her offspring and

392 following her death, there was a surge in offspring conflicts, as well as challenges to their
393 dominance positions (e.g. Cilix).

394 Notably, we did not find any association between dominance rank and HCC in the
395 months following Heather's death, probably because only a restricted number of individuals
396 experienced heightened levels of stress during social instability, namely those high-ranking
397 monkeys who increased in Elo-rating and intense aggression. Furthermore, since grooming has
398 been shown to decrease animal stress levels [58-61] it is plausible that the increase in social
399 grooming we found in Heather's matriline after her death might have been a strategy monkeys
400 used to counter-act the increased instability, and this, in turn, minimized the surge in social and
401 physiological stress in this period. In wild chacma baboons, females who experienced increased
402 stress levels following the death of close relatives displayed higher rates of grooming which
403 likely helped them to return cortisol concentrations to baseline levels [62]. While we did not find
404 a significant relationship between total grooming frequency and HCCs specifically for Heather's
405 matriline, we did find a significant negative relationship when analyzing all subjects for whom
406 we had behavioral data recorded, indicating that social grooming may be an important
407 mechanism to reduce chronic physiological stress. It is possible that with more subjects from her
408 matriline, we would have found a significant relationship between hair cortisol and grooming.
409 Importantly, our work is the first to relate social rank to HCCs in primates, though one study
410 found a positive correlation between rank and HCCs in hyraxes (*Procavia capensis* [63]). Our
411 study suggests that HCCs may be useful in assessing how social dynamics influence chronic
412 stress in group-living mammals.

413 Following Heather's death, major fight wounds were only reported for her matriline,
414 indicating that high intensity aggression was more frequent within her matriline. The increase in

415 dominance instability, physical aggression, and the infliction of major fight wounds were the
416 likely consequences of monkeys trying to reaffirm their rank. In support of this, one month after
417 the matriarch's death, Cilix had undergone a major rank increase and ranked above all seven of
418 Heather's offspring. During this time, she also received a large tear wound. This wound was
419 likely a byproduct of Cilix attempting (successfully) to increase in rank, as well as the
420 unwillingness of the others to sacrifice their positions (those attempting to reaffirm their
421 position). Unfortunately, wounds are rarely ever seen occurring, so it is impossible to know
422 exactly who inflicted this wound. The fact that no wounds occurred within the other matriline
423 suggests that they were likely not struggling to assert dominance over one another, a notion
424 supported by their relatively stable hierarchies. Furthermore, the wounding and increased levels
425 of intense aggression may have resulted from changes in submissive signaling. Submissive
426 signals (such as the silent bared teeth display) have been related to lower levels of severe
427 aggression [64] and are associated with greater dominance relationship certainty [65]. While
428 submissive displays were recorded, they were originally not a focus of *ad libitum* sampling and
429 occurred too infrequently during focal observations, and therefore direct comparisons were not
430 available during this study. Therefore, an interesting opportunity exists in the future to study the
431 role and changes in submissive signaling and resultant levels of aggression during a time of
432 social instability such as after the loss of a matriarch.

433 As a likely result of the increased levels of aggression and wounding, there was also an
434 increase in the frequency of vigilance. In a time of rank changes and monkeys attempting to
435 reaffirm their rank, vigilance may have been a buffering mechanism from both directed and
436 redirected aggression. Additionally, monkeys may have increased in vigilance to monitor the
437 social interactions of others. Indeed, female mountain gorillas (*Gorilla gorilla beringei*) spend

438 more time monitoring individuals whom they had aggressive interactions [66] and may play a
439 role in conflict-avoidance in tufted capuchins (*Cebus apella*; [67]).

440 Importantly, it is worth noting that the matriarch's death happened in the midst of the
441 breeding season and thus the variation in aggression rates and possibly wounding rates we found
442 in our study might be related to increased competition during the breeding season [68, 69].
443 However, we did not find any evidence for changes in wounding in the previous 2013 breeding
444 season: with matriline 3 and 4 both receiving approximately five wounds in each time period
445 identical to this study. In addition, we found a reduction in the overall number of severe fight
446 wounds in 2014 (from before the start of the breeding season to after), indicating that high levels
447 of aggression and wounding may not be limited to the breeding season. Furthermore, because our
448 findings (grooming, vigilance, aggression, HCC, rank, instability, etc.) were only significant for
449 the matriarch's matriline (matriline 3), and not the other matrilines (matrilines 4 and 1), it is
450 unlikely that the findings are solely a result of the breeding season alone. However, data from
451 matrilines 4 and 1 should be taken with caution due to the small sample sizes.

452 More generally, our results emphasize the importance of careful consideration in the
453 management of a captive colony when deciding whether some individuals should be removed or
454 euthanized. Group members who play a key role for the stability of the social group should be
455 identified, even if they are not among the alpha individuals. Our study shows how the removal of
456 even non-alpha matriarchs significantly impacts matrilineal dynamics: their absence can trigger a
457 period of social instability and heightened intense aggression. In the case in which socially
458 significant individuals must be removed, extra monitoring may be important in detecting
459 potential changes in stability. Managers of captive colonies may therefore be able to use data
460 generated via Elo-rating and rank changes (i.e. social instability) to examine how certain

461 characteristics (age, rank, number of kin, rate of policing behavior, etc.) of an individual may be
462 important in maintaining stability. This will then in turn allow managers to predict the effects of
463 the removal of certain individuals in the future and guide future management decisions.

464 In conclusion, this study provides evidence that old-aged matriarchs may play a
465 substantial role in family stability in group-living primates. When considering the stability of
466 large family groups, it may be important to recognize the critical role that non-alpha matriarchs
467 provide. Collectively, our findings demonstrate the power of specific individuals, including non-
468 alpha group members, to exert strong influences within their group and the consequences
469 following their loss.

470

471 **Supporting Information**

472 **S1 Dataset. Dataset for the current study in excel format**

473

474 **Acknowledgments**

475 We thank Kristen Byers for assistance with pedigree construction and Ryan McNeill for
476 assistance with data collection. We are also grateful for the veterinary and animal care staff at the
477 NIH Animal Center for their dedicated care.

478

479 **References**

480 1. McComb K, Shannon G, Durant SM, Sayialel K, Slotow R, Poole J, et al. Leadership in
481 elephants: the adaptive value of age. . Proc R Soc B. 2011:rsjb20110168.

- 482 2. Brent LJ, Franks DW, Foster EA, Balcomb KC, Cant MA, Croft DP. Ecological knowledge,
483 leadership, and the evolution of menopause in killer whales *Curr Biol.* 2015;25(6):746-50.
- 484 3. Davenport LC. Aid to a declining matriarch in the giant otter (*Pteronura brasiliensis*). *PLoS*
485 *One.* 2010;5(6):e11385.
- 486 4. Kachel AF, Premo LS, Hublin J-J. Grandmothering and natural selection. *Proc R Soc B.*
487 2011;278(1704):384-91.
- 488 5. Beisner BA, McCowan B. Policing in nonhuman primates: Partial interventions serve a
489 prosocial conflict management function in rhesus macaques. *PloS One.* 2013;8(10):e77369.
- 490 6. McComb K, Moss C, Durant SM, Baker L, Sayialel S. Matriarchs as repositories of social
491 knowledge in African elephants. *Science.* 2001;292(5516):491-4.
- 492 7. Moss CJ. The demography of an African elephant (*Loxodonta africana*) population in
493 Amboseli, Kenya. *J Zool.* 2001;255(02):145-56.
- 494 8. Wittemyer G, Douglas-Hamilton I, Getz WM. The socioecology of elephants: analysis of
495 the processes creating multitiered social structures. *Anim Behav.* 2005;69(6):1357-71.
- 496 9. Foster EA, Franks DW, Mazzi S, Darden SK, Balcomb KC, Ford JK, et al. Adaptive
497 prolonged postreproductive life span in killer whales. *Science.* 2012;337(6100):1313-.
- 498 10. De Waal F. Chimpanzee politics: Sex and power among apes. London, UK: Jonathan Cape.
499 1982.
- 500 11. Kaburu SS, Inoue S, Newton-Fisher NE. Death of the alpha: within-community lethal
501 violence among chimpanzees of the Mahale Mountains National Park. *Am J Primatol.*
502 2013;75(8):789-97.
- 503 12. Nieuwenhuijsen K, de Waal F. Effects of spatial crowding on social behavior in a
504 chimpanzee colony. *Zoo Biol.* 1982;1(1):5-28.

- 505 13. Hawkes K, O'Connell JF, Blurton Jones NG. Hadza women's time allocation, offspring
506 provisioning, and the evolution of long postmenopausal life spans. *Curr Anthropol.*
507 1997;38(4):551-77.
- 508 14. Sear R, Mace R, McGregor IA. Maternal grandmothers improve nutritional status and
509 survival of children in rural Gambia. *Proc R Soc B.* 2000;267(1453):1641-7.
- 510 15. Sear R, Mace R. Who keeps children alive? A review of the effects of kin on child survival.
511 *Evol Hum Behav.* 2008;29(1):1-18.
- 512 16. Croft DP, Brent LJ, Franks DW, Cant MA. The evolution of prolonged life after
513 reproduction. *Trends Ecol Evol.* 2015;30(7):407-16.
- 514 17. Douglas-Hamilton I, Douglas-Hamilton O. *Among the elephants.* London: Collins & Harvill
515 Press; 1975.
- 516 18. Douglas-Hamilton I, Bhalla S, Wittemyer G, Vollrath F. Behavioural reactions of elephants
517 towards a dying and deceased matriarch. *Appl Anim Behav Sci.* 2006;100(1):87-102.
- 518 19. Foley C, Pettorelli N, Foley L. Severe drought and calf survival in elephants. *Biol Lett.*
519 2008;4(5):541-4.
- 520 20. Soma T, Koyama N. Eviction and Troop Reconstruction in a Single Matriline of Ring-
521 Tailed Lemurs (*Lemur catta*): What happened when “grandmother” died? In: Masters J, Gamba
522 M, Génin F, editors. *Leaping ahead.* Springer; 2012. p. 137-46.
- 523 21. Beisner BA, Jackson ME, Cameron AN, McCowan B. Detecting instability in animal social
524 networks: genetic fragmentation is associated with social instability in rhesus macaques. *PloS*
525 *One.* 2011;6(1):e16365.
- 526 22. Flack JC, Girvan M, De Waal FB, Krakauer DC. Policing stabilizes construction of social
527 niches in primates. *Nature.* 2006;439(7075):426-9.

- 528 23. Jennings DJ, Carlin CM, Gammell MP. A winner effect supports third-party intervention
529 behaviour during fallow deer, *Dama dama*, fights. *Anim Behav.* 2009;77(2):343-8.
- 530 24. Clarke F, Faulkes C. Dominance and queen succession in captive colonies of the eusocial
531 naked mole-rat, *Heterocephalus glaber*. *Proc R Soc B.* 1997;264(1384):993-1000.
- 532 25. Oates-O'Brien RS, Farver TB, Anderson-Vicino KC, McCowan B, Lerche NW. Predictors
533 of matrilineal overthrows in large captive breeding groups of rhesus macaques (*Macaca*
534 *mulatta*). *J Am Assoc Lab Anim Sci.* 2010;49(2):196-201.
- 535 26. Barrett L, Henzi SP, Lusseau D. Taking sociality seriously: the structure of multi-
536 dimensional social networks as a source of information for individuals. *Phil Trans R Soc B.*
537 2012;367(1599):2108-18.
- 538 27. Kaburu SS, Newton-Fisher NE. Egalitarian despots: hierarchy steepness, reciprocity and the
539 grooming-trade model in wild chimpanzees, *Pan troglodytes*. *Anim Behav.* 2015;99:61-71.
- 540 28. Franz M, McLean E, Tung J, Altmann J, Alberts SC. Self-organizing dominance hierarchies
541 in a wild primate population. *Proc R Soc B*; 2015.
- 542 29. Sapolsky RM. The influence of social hierarchy on primate health. *Science.*
543 2005;308(5722):648-52.
- 544 30. Lindburg DG, Rosenblum L. The rhesus monkey in North India: an ecological and
545 behavioral study. In: Rosenblum LA, editor. *Primate behavior: developments in field and*
546 *laboratory research.* New York: Academic Press; 1971, pp 2-106.
- 547 31. Wenyan Q, Yongzu Z, Manry D, Southwick CH. Rhesus monkeys (*Macaca mulatta*) in the
548 Taihang mountains, Jiyuan county, Henan, China. *Int J Primatol.* 1993;14(4):607-21.

- 549 32. Southwick C, Yongzu Z, Haisheng J, Zhenhe L, Wenyuan Q. Population ecology of rhesus
550 macaques in tropical and temperate habitats in China. In: Fa JE, Lindburg DG, editors. Evolution
551 and ecology of macaque societies. Cambridge: Cambridge University Press; 1996. pp 95-105.
- 552 33. Sade DS. A longitudinal study of social behavior of rhesus monkeys. The functional and
553 evolutionary biology of primates. In: Tuttle R, editor. Functional and evolutionary biology of
554 primates. Chicago: Aldine-Atherton, Chicago; 1972. pp 378-398.
- 555 34. Bernstein, I. Ehardt, C. Agonistic aiding: kinship, rank, age, and sex influences. *Am J*
556 *Primatol*; 1985; 8: 37-52.
- 557 35. Vessey SH. Dominance among rhesus monkeys. *Polit Psychol*. 1984:623-8.
- 558 36. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous
559 cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. 2006;147(3):255-
560 61.
- 561 37. Meyer JS, Novak MA. Minireview: hair cortisol: a novel biomarker of hypothalamic-
562 pituitary-adrenocortical activity. *Endocrinology*. 2012;153(9):4120-7.
- 563 38. Novak MA, Hamel AF, Kelly BJ, Dettmer AM, Meyer JS. Stress, the HPA axis, and
564 nonhuman primate well-being: a review. *App Anim Behav Sci*. 2013;143(2):135-49.
- 565 39. Schino G, Scucchi S, Maestriperi D, Turillazzi PG. Allogrooming as a tension-reduction
566 mechanism: a behavioral approach. *Am J Primatol*. 1988;16(1):43-50.
- 567 40. Judge P, Griffaton N, Fincke A. Conflict management by hamadryas baboons (*Papio*
568 *hamadryas hamadryas*) during crowding: a tension-reduction strategy. *Am J Primatol*.
569 2006;68(10):993-1006.

- 570 41. Dettmer A, Novak M, Meyer J, Suomi S. Population density-dependent hair cortisol
571 concentrations in rhesus monkeys (*Macaca mulatta*). *Psychoneuroendocrinology*. 2014;42:59-
572 67.
- 573 42. Dettmer AM, Woodward RA, Suomi SJ. Reproductive consequences of a matrilineal
574 overthrow in rhesus monkeys. *Am J Primatol*. 2015;77(3):346-52.
- 575 43. Novak M, Kinsey J, Jorgensen M, Hazen T. Effects of puzzle feeders on pathological
576 behavior in individually housed rhesus monkeys. *Am J Primatol*. 1998;46(3):213-27.
- 577 44. Altmann J. Observational study of behavior: sampling methods. *Behaviour*. 1974;49(3):227-
578 66.
- 579 45. Neumann C, Duboscq J, Dubuc C, Ginting A, Irwan AM, Agil M, et al. Assessing
580 dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating.
581 *Anim Behav*. 2011;82(4):911-21.
- 582 46. Albers PC, de Vries H. Elo-rating as a tool in the sequential estimation of dominance
583 strengths. *Anim Behav*. 2001;61(2):489-95.
- 584 47. Wooddell L, Kaburu SSK, Dettmer A, Suomi S. Elorating as a tool to measure rank changes
585 and dominance stability in semi-free ranging rhesus macaques. *Am J Primatol*. 2015; 77(S1), 80.
- 586 48. Elo AE. *The rating of chessplayers, past and present*: Arco Pub.; 1978.
- 587 49. Dettmer AM, Kaburu SS, Byers KL, Murphy AM, Soneson E, Wooddell LJ, et al. First-time
588 rhesus monkey mothers, and mothers of sons, preferentially engage in face-to-face interactions
589 with their infants. *Am J Primatol*. 2016; 78 (2): 238-246.
- 590 50. Beehner J, Bergman T, Cheney D, Seyfarth R, Whitten P. The effect of new alpha males on
591 female stress in free-ranging baboons. *Anima Behav*. 2005;69(5):1211-21.

- 592 51. Sapolsky RM. Cortisol concentrations and the social significance of rank instability among
593 wild baboons. *Psychoneuroendocrinology*. 1992;17(6):701-9.
- 594 52. Sapolsky RM. Endocrine aspects of social instability in the olive baboon (*Papio anubis*).
595 *Am J Primatol*. 1983;5(4):365-79.
- 596 53. Sapolsky RM. Endocrinology alfresco: psychoendocrine studies of wild baboons. *Recent*
597 *Prog Horm Res*. 1993;48:437.
- 598 54. Bergman T, Beehner J, Cheney D, Seyfarth R, Whitten P. Correlates of stress in free-
599 ranging male chacma baboons, *Papio hamadryas ursinus*. *Anim Behav*. 2005;70(3):703-13.
- 600 55. Creel S, Creel NM, Mills MG, Monfort SL. Rank and reproduction in cooperatively
601 breeding African wild dogs: behavioral and endocrine correlates. *Behav Ecol*. 1997;8(3):298-
602 306.
- 603 56. Czoty PW, Gould RW, Nader MA. Relationship between social rank and cortisol and
604 testosterone concentrations in male cynomolgus monkeys (*Macaca fascicularis*). *J*
605 *neuroendocrinol*. 2009;21(1):68-76.
- 606 57. Fox HE, White SA, Kao MH, Fernald RD. Stress and dominance in a social fish. *J Neurosci*.
607 1997;17(16):6463-9.
- 608 58. Boccia ML, Reite M, Laudenslager M. On the physiology of grooming in a pigtail macaque.
609 *Physiol Behav*. 1989;45(3):667-70.
- 610 59. Gust DA, Gordon TP, Hambright MK, Wilson ME. Relationship between social factors and
611 pituitary-adrenocortical activity in female rhesus monkeys (*Macaca mulatta*). *Horm Behav*.
612 1993;27(3):318-31.
- 613 60. Aureli F, Preston SD, de Waal F. Heart rate responses to social interactions in free-moving
614 rhesus macaques (*Macaca mulatta*): a pilot study. *J Comp Psychol*. 1999;113(1):59.

615 61. Shutt K, MacLarnon A, Heistermann M, Semple S. Grooming in Barbary macaques: better
616 to give than to receive? Biol Lett. 2007;3(3):231-3.

617 62. Engh AL, Beehner JC, Bergman TJ, Whitten PL, Hoffmeier RR, Seyfarth RM, et al.
618 Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas*
619 *ursinus*). Proc R Soc B. 2006;273(1587):707-12.

620 63. Koren L, Mokady O, Geffen E. Social status and cortisol levels in singing rock hyraxes.
621 Horm Behav. 2008;54(1):212-6.

622 64. Beisner, B. McCowan, B. Signaling context modulates social function of silent bared-teeth
623 displays in rhesus macaques (*Macaca mulatta*). Am J Primatol. 2014; 76(2): 111-121.

624 65. Beisner, B. Hannibal, D. Finn, K. Fushing, H. McCowan, B. Social power, conflict policing,
625 and the role of subordination signals in rhesus macaque society. Am J Phys Anthropol. 2016;
626 160 (1): 102-112.

627 66. Watts, D. A preliminary study of selective visual attention in female mountain gorillas
628 (*Gorilla gorilla beringei*). Primates; 1998; 39, 71-78.

629 67. Pannoza, P. Phillips, K. Haas, M. Mintz, E. Social monitoring reflects dominance
630 relationships in a small captive group of brown capuchin monkeys (*Cebus apella*). Ethology.
631 2007; 113(9): 881-888.

632 68. Mallow GK. Aggressive behavior during the breeding season of adult female rhesus
633 monkeys (*Macaca mulatta*). Aggressive Behav. 1980;6(3):217-32.

634 69. Ruehlmann TE, Bernstein IS, Gordon TP, Balcaen P. Wounding patterns in three species of
635 captive macaques. Am J Primatol. 1988;14(2):125-34.

636