DOES PREGNANCY COLORATION REDUCE FEMALE CONSPECIFIC AGGRESSION IN
THE PRESENCE OF MATERNAL KIN?

Andrea Bailey, Lynn E. Eberly, and Craig Packer
University of Minnesota

Corresponding author:
Andrea Bailey
989 Van Buren Ave.
St. Paul, MN 55104
email: baile480@umn.edu
phone: 612-986-7996

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ABSTRACT

Color signals arise in a variety of sexual contexts, including advertising reproductive status. Despite potentially attracting negative attention from unrelated competitors, bright pregnancy coloration may communicate gestation to kin and potential fathers thereby garnering aid during agonistic encounters and reducing the overall amount of aggression received by pregnant females. To establish how rates of aggression might change after the appearance of this “pregnancy sign”, behavioral observations were conducted on, first, wild olive baboons (*Papio anubis*) in Gombe National Park, Tanzania, and, second, captive baboons at Southwest National Primate Center, where the pregnancy coloration was experimentally obscured. Playback experiments were also performed at Southwest to measure male responses to the distress calls of pregnant females. Free-ranging female baboons experience significantly less aggression from non-kin females after the onset of the pregnancy sign compared to pre-pregnancy sign, whereas captive pregnant females on which the pregnancy coloration was obscured with paint suffer significantly lower aggression rates compared to pre-painting from female conspecifics in the complete absence of maternal kin. Male aggression towards females does not differ in the presence or absence of the pregnancy sign in either wild or captive populations, though captive fathers pay significantly more attention to distress calls of pregnant rather than cycling cage-mates, suggesting a willingness to aid mothers that carry their unborn offspring.

KEYWORDS: communication, agonism, pregnancy sign

25 Conspicuous coloration serves as a reproductive signal in numerous species of fish
26 (Andersson 1994), birds (Baker and Parker 1979), reptiles (Cooper and Greenberg 1992),
27 and mammals (Caro 2005; Clutton-Brock and McAuliffe 2009). Such visual signals can
28 be easily localized, rapidly transmitted (Bradbury and Vehrencamp 2011; Rosenthal and
29 Ryan 2000), and sensitive to underlying hormonal profiles (Bagnara and Hadley 1973).
30 In particular, some females utilize color changes, such as bright plumage (Amundsen
31 2000), skin color (Weiss 2006; Dubuc *et al.* 2009), or swellings (Higham *et al.* 2008), to
32 signal sexual receptivity, and color displays by both sexes are known to attract mates
33 (Andersson 1994). However, in only a few cases do females employ color to
34 communicate other aspects of reproductive status such as gestation (e.g., some non-
35 human primates (Rowell and Chalmers 1970; Altmann 1973; Loy 1974; Dunbar 1977;
36 Setchell *et al.* 2006; Gerald *et al.* 2009)) or gravidity (e.g. some live-bearing fish (Farr
37 and Travis 1986; Deaton 2008) and lizards (Cooper and Greenburg 1992)).

38   Investigations into gravid signals in lizards suggest that females may use color to
39 signal the likelihood of aggressively responding to a male approach or courtship (Cooper
40 and Greenberg 1992; Watkins 1997). Specifically, female lizards in the genus
41 *Holbrookia* exhibit a bright “nuptial coloration” only at the onset of the breeding season,
42 but, once gravid, this coloration darkens and deters males from attempting further
43 copulations (Hager 2001). Yet, few studies have addressed the pregnancy coloration—
44 often referred to as the “pregnancy sign” (Altmann 1973)—found in many primates,
45 including gelada baboons (Dunbar 1977), savanna baboons (Altmann 1973), rhesus
46 macaques (Gerald *et al.* 2009), patas monkeys (Loy 1974), gray-cheeked mangabeys
47 (Rowell and Chalmers 1970), and mandrills (Setchell *et al.* 2006). This bright red or

magenta coloration typically manifests on exposed skin of the posterior in baboons and on the face in macaques, patas monkeys, and mandrills. Work with rhesus macaques suggests that the pregnancy sign may function as a “warning” to conspecifics, as males paid more visual attention and both males and females demonstrated higher amounts of appeasement behaviors to photographs of pregnant faces than to non-pregnant faces, though the nature of this warning is poorly understood (Gerald *et al.* 2009).

Advertising gestational status may confer considerable risks from competing females and—in species with pronounced sexual dimorphism in body size—from incoming males. Agonistic encounters can provoke acute stress thereby causing decreased uterine blood flow and fetal hypoxemia in pregnant animals (Morishima *et al.* 1979; Shnider *et al.* 1979) and even lead to miscarriage (Clark *et al.* 1993; Field and Diego 2008). In social mammals, fetuses represent future competition for non-related group members and thus pregnant females may suffer from aggression by members of other matriline (Silk *et al.* 1981). Similarly, males with a low probability of paternity may attack gestating females to induce miscarriage, thereby causing the females to resume cycling (Berger 1983; Pereira 1983; Mori and Dunbar 1985; Agooromorthy *et al.* 1988; Storey and Snow 1990; van Schaik and Dunbar 1990; Roberts *et al.* 2012). Given these potential costs, the persistence of the pregnancy sign implies some over-riding evolutionary benefit.

In female philopatric species, females persistently associate with close kin. Genetic relatives can benefit by enhancing each other’s reproductive success (Hamilton 1964), including interventions that prevent stress or injury during pregnancy. Likewise, some vertebrate fathers defend their juvenile offspring, both from predators (Gross and

Shine 1981; Gubernick 1981; Montgomerie and Weatherhead 1988) and aggressive conspecifics (Itzkowitz and Nyby 1982; Grinnell *et al.* 1995; Watts 1997; Palombit 2000; Buchan *et al.* 2003; Ferreira *et al.* 2006). Juveniles often remain in close proximity to their mothers, thus males that respond to maternal distress calls protect their own genetic interests (Palombit *et al.* 1997). Fathers earn similar benefits from defending gestating females and are expected to intercede during conflicts with other group members (Kleiman and Malcolm 1981; Palombit *et al.* 1997; van Schaik and Kappeler 1997). Pregnancy coloration may be one mechanism by which females overtly advertise their reproductive status in order to garner such support from their mates as well as their maternal kin, thereby reducing the amount of aggression they receive during gestation.

Olive baboons are a highly gregarious primate species in which multiple females and males live in each group. Female baboons remain in their natal troop their entire lives (Packer 1979) and exhibit a deep reddening of the paracallosal skin approximately three weeks after conception (Altmann 1973). To test the “aggression-reduction” hypothesis, we collected observational data on wild baboons to establish whether pregnant females experienced reduced aggression after the appearance of pregnancy coloration and received greater support from mating partners and close maternal kin. We then experimentally obscured the pregnancy sign of captive female baboons and compared rates of aggression toward these females pre- vs. post-color alteration. Finally, we conducted playback experiments to test whether fathers were more attentive to the screams of pregnant females than of cycling females.

94 METHODS

95 Observational Study

96 Study area and population

97 All behavioral observations on wild baboons were collected at Gombe National
98 Park, Tanzania. Gombe is located on the eastern shore of Lake Tanganyika and is
99 characterized by steep valleys descending from a rift escarpment to the east (van Lawick-
100 Goodall 1968). Baboons at Gombe have been observed since 1967, with continuous
101 demographic data dating from 1972. All study animals are individually recognized by
102 natural markings. Data were collected during two distinct time periods when group size
103 averaged 22.0 (+/- 1.6 SE) adults. Group composition ranged from 4 - 11 adult males
104 (mean: 6.9 +/- 0.9 SE) and 12 – 19 adult females (mean: 15.1 +/- 1.2 SE).

105

106 Focal follows

107 Focal follows were conducted by two separate observers. First, focal follows
108 were conducted by CP on 23 adult male baboons between May and November, 1972 and
109 June 1974 – May 1975. Each focal follow lasted 2.5 hours and an average of 57.6 hours
110 (+/- 6.5 SE) was collected for each male. These observations included detailed data on
111 behavior during all interactions between the focal male and every other troop member,
112 including 14 pregnant females,. The second set of focal follows was conducted by AB on
113 six pregnant females between May and December, 2012. Each follow lasted 1.5 hours,
114 and each female was followed an average of 18.5 hours (+/- 0.3 SE) during the first 7
115 weeks of her pregnancy. These follows included all interactions with conspecifics,
116 including any aggressive encounters with either males or females and any incidences of

agonistic support, which was defined as any aggressive interaction involving the pregnant female when a third party directed aggression toward her opponent.

Analysis of focal data

In the Gombe females, pregnancy coloration has a characteristic deep magenta hue that is distinct from the lighter pink of the sexual swelling and typically appears after the third week of pregnancy. Initial coloring was defined as any hint of red on the perineal skin after the onset of detumescence of the sexual swelling. Focal females at Gombe showed the first signs of pregnancy coloration 21.7 ($\pm$ 1.3 SE, n=6 females) days after initial detumescence. Based on this mean and low variance in the appearance of pregnancy coloration, the first three weeks of gestation were considered “pre-pregnancy sign”. However, if any female began to exhibit pregnancy coloration prior to the end of this three-week period, these data were excluded from the pre-pregnancy sign data analysis. Pregnancy coloration typically requires several days to become conspicuous, with overt coloration occurring on day 28.8 ($\pm$ 1.0 SE) post-detumescence for all focal females by which time the pregnancy was no longer in question to an experienced observer. Thus weeks 5-7 of gestation were defined as the “pregnancy-sign” period. Though females maintain bright coloration until parturition, we chose to compare two time spans in the early stages of pregnancy to limit any potential effects of other physical changes through later stages of gestation. Focal females were followed an average of 7.75 hours ($\pm$ 0.25 SE) pre-pregnancy sign and 8.25 hours ($\pm$ 0.34 SE) post-appearance of the pregnancy sign.

Male-female interactions

For both male and female focal follows at Gombe, we tallied all incidences of male aggression (combining physical attacks with visual/vocal threats) toward pregnant females in their pre-pregnancy sign and pregnancy sign periods. We also tallied female avoidance of males (defined as a female moving in the opposite direction of a male that approached to within 5 meters), minutes spent grooming and being groomed, incidents of support, and number of social interactions initiated by males and females, respectively. Because of the rarity of events, interaction rates were calculated for each male-female dyad during each reproductive phase by summing the total number of interactions of each type and dividing by the total observation time for focal individual. Each male-female dyad was then treated as an independent point and a linear mixed effects model with pregnancy-sign status, male rank, female rank, female age, and observer (CP or AB) as fixed effects and male and female identities as random effects. The least-squares means were compared across pregnancy sign status for analysis of each behavior and adjusted for the other fixed effects.

Female-female interactions

Female focal follows were analyzed for agonistic interactions between the focal pregnant female and other female group members, distinguishing kin from non-kin. Maternal kinship was well-established in the long-term data set at Gombe, and was restricted to mothers, maternal sisters, and daughters, as studies suggest the benefits of kin selection drop off significantly beyond immediate kin (Hamilton 1964) and DNA profiles were not available to determine paternal kin in this population. Analyses were performed in the

same way as for male-female interactions, and each female-female dyad was treated as an independent point. Kin and non-kin dyads were analyzed separately using linear mixed effects models with pregnancy sign status, focal female rank and age, and interacting female rank and age as fixed effects and the identities of pregnant and interacting females as random effects; least squares means were again compared across pregnancy sign status and adjusted for the other fixed effects.

Male Playback Experiments

Study Population

Female distress-call playback experiments were conducted on 17 captive adult male olive (*Papio anubis*) and olive-yellow hybrid (*Papio anubis/Papio cynocephalus*) baboons at the Southwest National Primate Research Center (SNPRC) in San Antonio, Texas. Each male was housed in a breeding enclosure measuring approximately 30 ft. x 30 ft. with 10-15 females. Groups consisted primarily of olive baboons, though a small number of olive-yellow, olive-hamadryas, and yellow-hamadryas hybrids were also present. Only the focal female and group male could be reliably recognized during data collection. All baboons were weaned by ~10 mos of age and reared in communal crèches by an unrelated male and female. Upon sexual maturity (typically ~4 yrs of age in this facility), females were introduced into breeding enclosures which were unlikely to include close kin (SNPRC, personal communication), but even if kin were present, kin recognition in baboons seems to depend heavily on socialization (Alberts 1999), thus females in enclosures were unlikely to know if they were housed with close relatives. Female rank is largely determined by the length of group tenure in captive

Cercopithecines (Tung 2012), but the dominance relationships were not known for the females in this study.

Playback Methodology

Female screams were collected opportunistically from individuals throughout the facility using a Marantz PMD620 handheld digital recorder and a Sennheiser ME66 shotgun microphone with a K6 power module. The identity, cage number, and reproductive status of each caller were recorded. Focal males were then exposed to six different call types over the subsequent three months: screams from 1) pregnant females in their home cage (probability of paternity = 100%); 2) pregnant females in a neighboring cage (males can interact with these females through the wire mesh but likelihood of paternity = 0); 3) pregnant females from enclosures far from the focal male with whom he has had no opportunity to interact); 4) cycling females in their home cage; 5) cycling females in a neighboring cage; 6) “distant” cycling females. Cycling females had sexual swellings both when their calls were recorded and when their calls were played to males, and pregnant females exhibited pregnancy coloration during both call collection and playback to control for any potential differences deriving from changes in reproductive status. 16 of the 17 males were exposed to at least one of each call type, with some males hearing multiple samples of one or more call types, for a total of 199 playbacks. However, males only heard each individual female on a single occasion. Mean scream length was 4.74 +/- 0.063 SE seconds (n=57??) and calls were introduced in a random order. To avoid habituation, no male experienced more than one call in a 72-

hour period, and several “dummy” playbacks were conducted on each male, where equipment was set up but no call played.

During the playback procedure, a Sony RDPX200iP speaker connected to a MacBook Pro laptop was set up at a distance of 3-5 meters from the focal male. A JVC Everio digital camcorder mounted on a tripod was placed behind the speaker to record reactions to the playback vocalization. The equipment was set up 10 minutes before each playback to allow individuals to acclimate. After the waiting period, the camcorder was started to record at least 20 seconds of male behavior prior to the playback, during which time the experimenter remained out of view. The call was played, and the video recorded at least an additional 20 seconds. Calls were only played if the calling female was neither near the target male nor in his line of sight, and if no female from the home or neighboring cage had given a distress call for a minimum of 10 minutes.

The length of time each male spent gazing at the speaker pre- vs. post-call was noted, as well as any behaviors directed toward the speaker, including approaches (defined as any movement toward the camera), and appeasement (such as tongue protrusion or grunting) or agonistic behaviors. Neither of the two student judges who scored these behaviors was involved in the playback procedures or knew the identities and call types in each test. Net gaze length post-call was calculated by subtracting the length of male gaze toward the camera in the 20 seconds before the playback from the length of gaze in the 20 seconds after the playback was begun. For playbacks with disagreement between scorers (13% of calls), a third individual viewed the video to adjudicate the judges’ interpretations.

A logistic regression was performed to determine if males were more likely to respond to calls from pregnant or cycling females from the home or the neighboring cage. For calls to which males reacted, linear mixed effects models were then used to compare the net time males spent gazing at the speaker after each call type. The least squares means were compared across call types for each of these analyses using a Tukey-Kramer adjustment for multiple-comparisons. Calls from unknown cycling and pregnant females were compared separately to assess if males could differentiate between the calls of pregnant and cycling females.

Color Manipulation Experiments

Painting methodology

Color manipulation experiments were conducted on 18 parous pregnant female olive baboons at the Southwest National Primate Research Center. Each female was at least in her second trimester of pregnancy and therefore had been exhibiting pregnancy coloration for a minimum of five weeks. Each resided in the cage with the father of her fetus, and each was randomly assigned a painting treatment, either grey/purple or clear paint. The grey-purple paint was mixed to visually match photographs of the rump skin color of a flat, cycling female, with the same paint mixture used on each test subject so that each experienced the same color change, while the clear paint was used as a control. Test females (n=9) were anesthetized and the paracallosal and sexual skin of each individual cleaned. A thin coat of non-toxic grey/purple paint (Createx™ Airbrush Colors, opaque purple, red, black, and white) was applied over the entire rump area to obscure all visible pregnancy coloration. After ~15 minutes, a coat of the non-toxic clear

glossy paint (Createx™ Airbrush Colors, gloss top coat) was applied. Test females were then transferred to a recovery area prior to reintroduction to their group. An identical procedure was completed for control females, using the same clear glossy paint without the color application. The paint typically persisted for only 4-5 days (maximum ~14 days) thereby eliminating the need to re-anesthetize the animals for paint removal. Each cage only included one painted female at a time, with at least a two-week gap between the final observation of the first female and the first observation of the second.

Behavioral Observations

Prior to the painting treatment, each test subject was observed for a total of ten hours over the course of three days (3 hours and 20 minutes per day). All interactions between the focal female and male and female conspecifics were recorded, including all aggressive (including threat and attack, displacement and avoidance) and affiliative (mutual greetings and grooming) behaviors, as well as any incidents of third-party support during an agonistic encounter. Greetings were defined as any behavior in which either the focal female or her conspecific approached and either touched or grunted at the other and in which neither individual exhibited a subordinate behavior (e.g., subordinate vocalization, fear grin, or avoidance). After the initial observations, females were painted and returned to their home cage the same morning and allowed to re-acclimate to their group until the following day. Over the next three days, females were observed for an additional ten hours at similar times of day as the pre-painting observations. The percent of paint coverage was noted, as paint deteriorated at different rates for different females.

Analysis

All incidents of aggression and support were compared between pre-painting and post-painting treatments for gray-painted females using a 2-tailed Wilcoxon signed rank test. The amount of aggression and support received by clear-painted females pre- vs. post-painting were also compared as a means to test for any effects of the painting treatment.

Number of approaches by and toward the focal female, total negative interactions (aggression, displacement, and avoidance), total number of positive interactions (mutual greetings and grooming events), and minutes spent grooming and being groomed were also compared. Separate analyses were conducted for pregnant female interactions with the group male and other females. Observations on females with paint coverage below 65% were not used and pre-painting observations were reduced at random so that pre- vs. post-painting comparisons included the same number of observation hours. This procedure was necessary for 6 of the 9 females, with 1 female observed 6 hrs, 40 minutes, before and after painting, 4 females for 3 hrs, 20 minutes, before and after, and 1 female's data was not used at all as her paint wore away to a level of 55% before the first post-painting observation period. Paint wear was fairly uniform, typically beginning in the urogenital region and around the edges of the rump; the primary aspect of the rump remained colored longest, giving an overall impression of color coverage despite wear.

Ethical Note: Capture for females for application of color did not differ significantly from routine movement of individuals for daily cleaning and is a familiar process to all animals

at SNPRC. All females recovered from the anesthetic without event and did not suffer any injuries as a result of the experimental protocol.

All analyses were carried out in R version 3.1.1. All reported p-values are two-sided, with $p < 0.05$ considered statistically significant.

RESULTS

Observational Study

Aggression towards pregnant focal female baboons by female maternal kin was rare at Gombe (7 distinct instances in 96 focal hours), and levels did not differ before or after the appearance of the pregnancy sign ($t(22.12) = -0.69$, $p = 0.50$; note: all tests performed on data from the observational study are summarized in Table S1 of the supplementary materials). However, pregnant focal females received significantly less aggression from non-kin females while exhibiting pregnancy coloration than pre-pregnancy sign ($t(48.8) = -2.585$, $p = 0.013$ Fig. 1). Avoidance rates by focal females of non-kin female conspecifics did not differ significantly ($t(46.03) = -1.276$, $p = 0.21$), nor did grooming rates on ($t(21.84) = 0.397$, $p = 0.70$) or by ($t(24.28) = -0.747$, $p = 0.46$) focal females, pre- vs. post-pregnancy sign. Interaction rates between the focal female and other non-kin female group members also remained unchanged ($t(105.08) = -0.781$, $p = 0.44$), and there was no effect of rank or age in any of the analyses.

Male rates of aggression toward pregnant Gombe females did not change after the appearance of pregnancy coloration ($t(53.72) = -0.268$, $p = 0.79$; Fig. 2). However, pregnant females avoided males less after the coloration appeared ($t(59.92) = -2.634$, $p =$

0.011). Females with the pregnancy sign also interacted less with males ($t(188.53) = -2.089$, $p = 0.038$; Fig. 3), with males initiating fewer of these interactions than with the same females pre-pregnancy sign ($t(199.87) = -1.979$, $p = 0.049$). Pregnant females initiated interactions with males at similar rates before and after the appearance of pregnancy coloration ($t(194.35) = -1.294$, $p = 0.20$). The proportion of interactions involving avoidance behavior did not differ significantly with pregnancy sign ($t(58.4) = -0.524$, $p = 0.60$), suggesting the decreased avoidance behavior after the appearance of the pregnancy sign was a by-product of decreasing rates of interaction. Similarly, the proportion of interactions involving threats or attacks did not differ significantly with pregnancy status ($t(55.65) = 0.078$, $p = 0.94$). Pregnant females groomed males and were groomed by males at similar rates regardless of pregnancy coloration (grooming of males: $t(54.37) = 0.479$, $p = 0.63$; grooming of females: $t(51.41) = -1.055$, $p = 0.30$). Neither male nor female rank affected the significance of these results.

Third-party support was rarely witnessed during focal follows, but of 8 incidents, 4 occurred prior to the onset of pregnancy coloration, 2 during the 4th week when the pregnancy sign was developing, and 2 during the first three weeks after the appearance of the pregnancy sign. Seven cases involved matrilineal kin; in the eighth case, an adult male supported a female with the pregnancy sign.

Figure 1. Least squares mean rates of wild non-kin female aggression (attacks/threats per hour $\pm$ SE) received by wild female baboons in the observational study before and after the appearance of pregnancy coloration, averaged across female-female dyads ($t(45.8) = -2.585$, $p = 0.013$).

Figure 2. Least squares mean rates of wild male aggression (attacks/threats per hour $\pm$ SE) received by wild pregnant female baboons in the observational study before and after the appearance of pregnancy coloration, averaged across male-female dyads ($t(53.72) = -0.268$, $p = 0.79$).

Figure 3. Least squares mean rates of interaction per hour($\pm$ SE) between wild males and pregnant females pre- vs. post-appearance of the pregnancy sign in the observational study, averaged across male-female dyad ($t(188.53) = -2.089$, $p = 0.0380$). Males initiated significantly fewer interactions post-sign ($t(199.87) = -1.979$, $p = 0.049$), while there was no difference in rate of initiation by pregnant females ($t(194.35) = -1.294$, $p = 0.20$).

Male Playback Experiments

Males at SNPRC gazed longer at the speaker when played screams from known pregnant females than screams from known cycling females ($t(45.35) = -2.64$, $p = 0.011$; Fig. 4). In particular, males gazed longer at calls from pregnant females from their home cage than those from either cycling females from their home ($t(40.03) = -2.77$, $p = 0.040$) or neighboring cages ($t(42.71) = -2.86$, $p = 0.032$; Fig 4). Males did not appear to differentiate between calls from the home cage and neighboring cages ($t(44.81) = 1.281$, $p = 0.21$; Fig. 4), nor calls from pregnant females from the home cage vs. a neighboring cage ($t(40.25) = 1.77$, $p = 0.30$; Fig. 4). Males did not differentiate between the calls of unknown pregnant and cycling females ($t(21.82) = -1.23$, $p = 0.23$), nor were they more

likely to react to any one call type ($X^2(3) = 1.8$ $p = 0.61$). Note that males only approached the speaker 5 times in response to 154 calls and that males never directed threat gestures toward the speaker.

Figure 4. Mean captive male net gaze length (in seconds $\pm$ SE) at distress calls from six female types: 1) cycling females from the home cage; 2) cycling females from a neighboring cage; 3) cycling females from a distant cage that were unknown to the male; 4) pregnant females from the home cage; 5) pregnant females from a neighboring cage; 6) pregnant females from a distant cage that were unknown to the male. Males gazed significantly longer at the calls of pregnant females from their home cage vs. calls from cycling females from their home ($t(40.03) = -2.77$, $p = 0.040$) or neighboring ($t(42.71) = -2.86$, $p = 0.032$) cages.

Color Manipulation Experiment

Pregnant captive females received significantly less aggression from other females when the pregnancy sign was obscured with gray paint ($W(7) = 21$, $p = 0.031$, Fig. 5). There was no significant change in the amount of aggression received by females in the clear-painted control group ($W(8) = 18.5$, $p = 0.49$, Fig. 5). Pregnant females

received similar amounts of aggression from the group male pre- vs. post-painting in both gray ($W(7) = 0$, $p = 1.00$) and clear paint groups ($W(8) = 9$, $p = 0.77$); Fig. 6).

Males supported pregnant females in agonistic encounters with other females a total of six times and each incident occurred while females had pregnancy coloration (5 incidents prior to painting and 1 after being treated with clear paint). One pregnant female was supported twice by another female: once while exhibiting pregnancy coloration and once after the coloration had been obscured.

The total amount of time a pregnant female was groomed by other females did not differ pre- vs. post-painting with gray ($W(7) = 8$, $p = 0.20$) or clear paint ($W(8) = 12$, $p = 0.24$), nor did pregnant females groom other females at different rates with gray ($W(7) = 12.5$, $p = 0.48$) or clear ($W(8) = 16.5$, $p = 0.89$) treatments. Pregnant females were not approached by other females at different levels in gray ($W(7) = 6$, $p = 0.79$) or clear treatments ($W(8) = 23.5$, $p = 0.95$), nor did they approach those females at different levels (gray: $W(7) = 16.5$, $p = 0.89$; clear: $W(8) = 35$, $p = 0.16$). Pregnant females also did not change their levels of aggression toward other females in gray ($W(7) = 10$, $p = 0.58$) or clear treatments ($W(8) = 25.5$, $p = 0.06$). There was no significant difference in the rates of male interactions with pregnant females pre- vs. post-painting, including approaches toward pregnant females (gray: $W(7) = 11$, $p = 0.36$; clear: $W(8) = 31.5$, $p = 0.31$), approaches by pregnant females (gray: $W(7) = 27$; $p = 0.23$; clear: $W(8) = 34.5$, $p = 0.17$), grooming on pregnant females (gray: $W(7) = 0$; $p = 1$; clear: $W(8) = 3$, $p = 0.35$), or grooming by pregnant females (gray: $W(7) = 3$, $p = 0.37$; clear: $W(8) = 10$, $p = 0.10$).

Figure 5. Mean number of captive female attacks (+/- SE) on pregnant females pre- vs. post-painting of pregnancy coloration with gray (n=8) ($W(7) = 21$, $p = 0.031$) or clear (n=9) ($W(8) = 18.5$, $p = 0.49$) paint. Of the females treated with gray paint, 7 of the 8 showed a decline in received aggression, while 1 of the 8 experienced similar levels of aggression.

Figure 6. Mean number of captive male attacks (+/- SE) on pregnant females pre- vs. post-painting of pregnancy coloration with gray (n=8) ($W(7) = 0$, $p = 1.00$) or clear (n=9) paint ($W(8) = 9$, $p = 0.77$).

DISCUSSION

The pregnancy signal elicited female aggression in captivity, where individuals were completely surrounded by non-kin. However, pregnant females received less aggression from other females after the appearance of the pregnancy sign at Gombe, where families were available to support pregnant kin. These results are consistent with the hypothesis that the pregnancy sign not only reduces aggression but that it elicits protective responses from family members, thereby inhibiting attacks from members of rival matriline. The bright coloration may make a female easier to identify and locate, and communicate a warning to non-kin that family members are willing to intercede on her behalf.

If the risk of intercession by family members reduces aggression directed at pregnant females, rates of agonistic support should have increased after the appearance of pregnancy coloration, but we only rarely observed third-party support. However, these intercessions may not be necessary if the signal successfully deters harassers through an elevated *risk* of retaliation. Thus, it is notable that coloration increased the amount of aggression received in captivity, where pregnant females did not live among protective kin. Also, warning coloration across species deters interaction because it communicates a cost to receivers (Mappes *et al.* 2005) through the use of colors that contrast well with the surrounding environment (Arenas *et al.* 2014),

Likely, the nature of captivity influenced the rate of aggression at SNPRC. In this setting, competitors are not only in constant proximity to their aggressors, but they have no means to escape repeated attacks. Thus, the rates of aggression among captive baboons are likely higher than those in the wild. Our results suggest that pregnancy coloration makes captive individuals a target for unrelated females, and, as such, these

females may be especially vulnerable to repeated attacks. However, these elevated rates of aggression in captivity do not fully explain the contrasting patterns between the captive and wild populations. Though females at Gombe may be able to avoid aggressors more easily, we should still expect to see increases in aggression with the appearance of pregnancy coloration, since rates of aggression toward pregnant females at SNPRC were higher prior to paint treatment than when pregnancy coloration was obscured. Yet, the opposite pattern emerged, suggesting the existence of an agonism-reduction mechanism in populations with intact kinship ties.

It is also possible that the Gombe females could have changed their behavior as their pregnancies progressed or undergone physiological changes that were obvious to other female group members but not to human observers. Mammals experience behavioral and hormonal changes throughout pregnancy (R.M. Marchant-Forde and J.M. Marchant Forde 2004; Bazer and Spencer 2011; Kinsley and Armory-Meyer 2011) and conspecifics may be sensitive to these changes. As in the captive population, however, the number and nature of most interactions with other females did not alter pre- vs. post-appearance of the pregnancy sign, suggesting that the changes in aggression likely stemmed from the pregnant female's changing reproductive status *per se*. However, it is uncertain whether the wild females relied on the visual color signal or responded to some other cue, such as olfaction.

Males at Gombe initiated fewer interactions with pregnant females after the onset of bright coloration, perhaps because these females were now clearly unavailable as potential reproductive partners. These males, too, may have reacted to other cues besides the pregnancy sign, and further investigation is merited. Alternatively, aposematic

coloration is a common deterrent across the animal kingdom (Ruxton *et al.* 2004) and pregnant females may be employing a similar tactic to warn males of the danger of a heightened familial response to male harassment (Gerald *et al.* 2009). It should also be noted that only recent immigrants in group-living mammals truly gain from harassing pregnant females to the point of miscarriage (Lukas and Huchard 2014), and only 3 out of 20 pregnant Gombe females were exposed to such males during their first seven weeks of pregnancy.

Males do, however, stand to benefit from protecting mothers carrying their unborn offspring. Although adult males only rarely supported pregnant females — one observation in the wild population vs. six in captivity — all seven cases occurred when females exhibited pregnancy coloration (sign test, $p = 0.016$). In addition, captive males spent significantly longer gazing in the direction of distress calls from pregnant females than of cycling females. Curiously, these males paid similar attention to the distress calls of females carrying their offspring—of which they had high paternity certainty, being the only male in the group—and those from neighboring females, with which they could interact through a barrier but of whom their paternity certainty was quite low. Though selection is likely to favor males in polygamous species to correctly assess their probability of paternity (Westneat and Sherman 1993; Neff 2003), males may use a rule of thumb, assuming some probability of paternity if they were present at the time of conception. Male baboons have been shown to increase care toward offspring for whom they have high paternity estimates based upon mating effort with the mother (Moscovice *et al.* 2010) and, though males with low estimates are unlikely to invest heavily, there may be selection on males to assume some probability of paternity based on residency

patterns to avoid injuring or killing their own offspring. In many infanticidal rodent species, males limit infanticidal behavior toward females with whom they have mated, regardless of actual paternity (vom Saal and Howard 1982; Gubernick *et al.* 1994). Thus, male baboons may show care toward any female that became pregnant during their residency. Also, despite a chain-link barrier, males may have engaged in some limited mating contact with neighboring females during their conceptive cycle, causing a modest possibility of paternity. Attention, though, is not direct support, and it is likely that paternity certainty plays a substantial role in male willingness to actively intercede on behalf of a pregnant female (Trivers 1972; Maynard Smith 1977; Moscovice *et al.* 2010). The lone example of male support in the wild baboons was by the top-ranking male in the group. Since rank is highly correlated with paternity in baboons (Cowlshaw and Dunbar 1991; Bulger 1993; but see also Alberts *et al.* 2003), this male was a likely father. Further investigation will be necessary to elucidate the role of paternity certainty in these male-female interactions.

Though pregnancy status and, specifically, coloration apparently alter the behavior of both male and female group members, further exploration is required to determine the importance of male and kin protection to pregnant females. In particular, a focus on family structure, with comparisons of aggression toward pregnant females with and without numerous close female kin may clarify the role of familial support on inhibiting aggression. Further, the existence of pregnancy signals in baboons and analogous signals in lizards (Cooper and Greenburg 1992; Hager 2001) and fish (Farr and Travis 1986; Deaton 2008) suggests that similar mechanisms may exist throughout the

- 522 animal kingdom and may even extend beyond visual cues, opening new avenues for
523 understanding the challenges faced by pregnant/gravid females.
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