**Olfactory attractants and parity affect prenatal androgens and territoriality of coyote
breeding pairs**

Christopher J. Schell^a, Julie K. Young^b, Elizabeth V. Lonsdorf^c, Jill M. Mateo^a, and Rachel M.
Santymire^{a,d}

^aCommittee on Evolutionary Biology, University of Chicago, 1025 E. 57th Street, Culver Hall
402, Chicago, IL 60637, United States

^bUSDA-WS-NWRC, Predator Research Facility, Department of Wildland Resources, Utah State
University, USU - BNR 163, Logan, UT 84322, United States

^cDepartment of Psychology, Franklin and Marshall College, P.O. BOX 3003, Lancaster, PA,
17603, United States

^dDepartment of Conservation and Science, Lincoln Park Zoo, 2001 N. Clark St., Chicago, IL
60614, United States

**Corresponding author:* Christopher J. Schell

Present Address: USDA/APHIS/WS/NWRC, 4101 LaPorte Avenue, Fort Collins, CO 80521

Phone: (626) 590-3595 / Email: cjschell@colostate.edu

Abstract

Hormones are fundamental mediators of personality traits intimately linked with reproductive success. Hence, alterations to endocrine factors may dramatically affect individual behavior that has subsequent fitness consequences. Yet it is unclear how hormonal or behavioral traits change with environmental stressors or over multiple reproductive opportunities, particularly for biparental fauna. To simulate an environmental stressor, we exposed captive coyote (*Canis latrans*) pairs to novel coyote odor attractants (i.e. commercial scent lures) mid-gestation to influence territorial behaviors, fecal glucocorticoid (FGMs) and fecal androgen metabolites (FAMs). In addition, we observed coyote pairs as first-time and experienced breeders to assess the influence of parity on our measures. Treatment pairs received the odors four times over a 20-day period, while control pairs received water. Odor-treated pairs scent-marked (e.g. urinated, ground scratched) and investigated odors more frequently than control pairs, and had higher FAMs when odors were provided. Pairs had higher FAMs as first-time versus experienced breeders, indicating that parity also affected androgen production during gestation. Moreover, repeatability in scent-marking behaviors corresponded with FGMs and FAMs, implying that coyote territoriality during gestation is underpinned by individually-specific hormone profiles. Our results suggest coyote androgens during gestation are sensitive to conspecific olfactory stimuli and prior breeding experience. Consequently, fluctuations in social or other environmental stimuli as well as increasing parity may acutely affect coyote traits essential to reproductive success.

Keywords: Androgens; coyotes; glucocorticoids; parity; personality; repeatability

1. Introduction

Organisms are constantly challenged by various external stimuli within their environment. Behavioral and morphological responses toward these environmental challenges are frequently initiated by neuroendocrine mechanisms [1–6]. For instance, glucocorticoids increase gluconeogenesis to activate energy stores necessary to respond toward environmental stressors [7,8]. Glucocorticoids are also associated with individual social status [3,9–11], mate preference and choice [12,13], and individually consistent behavioral differences (i.e. personality) [14–17]. Reproductive hormones such as androgens represent another pervasive suite of physiological factors that are intimately involved in reproduction and the social environment [7]. For example, increased androgens are often associated with sexually-selected ornamentation that constitute an honest signal of both fitness and social rank [18–21]. In many instances, increased androgens also augment territorial and aggressive behaviors that facilitate the acquisition and maintenance of increased social status [11,22–25]. Further, individual differences in foraging [26], exploration [27], and territorial marking behaviors [28,29] have demonstrated positive associations with androgens. Therefore, endocrine responses to environmentally-induced changes often have fitness consequences for the individual.

The role of endocrine factors in affecting personality may be particularly salient for social taxa. Social dynamics and structure are often regulated by the composition or diversity of personality types within a group [30–34]. For instance, increasingly aggressive individuals may secure more reproductive opportunities by maintaining higher social rank [33,35–37]. Bold or exploratory individuals may capitalize on rarely exploited food resources and subsequently affect overall group foraging success [31,38]. Further, the distribution of personalities within a group frequently dictates group success in competitive bouts against neighboring conspecifics for high-

quality resources [34,39]. Hence, in many instances personality is linked to life-history productivity (e.g. fecundity, longevity) [40]. Previous literature has suggested that the stability of personality traits is determined by underlying endocrine correlates and alterations to endocrine factors can induce behavioral plasticity [41]. Determining the proximate factors that affect endocrine traits may therefore help to predict accompanying changes to personality traits or vice versa.

For social mammals in particular, variation in density and environmental characteristics are prominent proximate causes affecting individual hormones [3]. Increases in the number of conspecifics within a landscape are often followed by the constriction of territorial boundaries, increasing the likelihood of conflict and competition for resources among neighboring conspecifics [42,43]. Mammals routinely utilize olfaction as a primary mode of communication, regularly urinating or defecating to demarcate home range boundaries and deter intrusion from conspecifics [44]. Greater abundances and densities are thus frequently accompanied by parallel increases in olfactory cues that are known to elicit behavioral responses (e.g. increased scent-marking, investigation) [45,46] potentially associated with glucocorticoid or androgen responses. Indeed, the mere perception of social competition generates individual hormonal changes [3,47]. However, changes to environmental cues (e.g. odors) are not constant nor do they persist over time; rather, such cues vary with time and likely induce plasticity in behavioral and hormonal traits [48].

Phenotypic consequences of environmental and temporal variation become considerably more far-reaching when considering breeding individuals and their overall influence on group or population dynamics. Breeding individuals undergo significant hormonal and behavioral changes in preparation for parenting [49,50] that can affect both breeders and even non-breeding

individuals in socially complex groups such as cooperative breeding systems [51–53]. Moreover, phenotypic changes during gestation may affect developing neonates epigenetically [54,55]. Gestation is therefore a critical period for study because density cues and temporal variation in such cues may likely have significant influences on personality and hormonal traits of an individual. Though several studies have quantified the effect of environmental stressors on gestating females, few studies to date have repeatedly measured individuals over successive breeding events to assess how temporal variation impacts endocrine profiles and behavior [56–58]. Even fewer have addressed the interplay among hormonal and personality correlates of individuals in biparental systems [59,60].

Coyotes (*Canis latrans*) are an excellent organism to examine the impact of environmental and temporal variation on gestational hormones and behavior. First, previous work has demonstrated increased serum testosterone and progesterone profiles over the mating season (December to February) that correspond with increased territorial behaviors such as urine-marking, ground scratching, and defecation [61–64]. Second, mated individuals frequently demarcate the boundaries of their territories via scent-marking and enforce home range limits using aggression against intruding conspecifics when necessary [65–67]. Social territorial incursions may likely represent a prominent stressor to breeding pairs. Third, previous work has demonstrated consistent individual differences in behavior of the species [68–70], providing empirical foundation for the current study. Associations among consistent individual differences in behavior and hormones may suggest that behavioral profiles of the species are hormonally mediated. Finally, previous work has provided methodological foundation to quantify individual variation in fecal glucocorticoid metabolites for coyotes [71].

We observed coyote breeding pairs exposed to commercial scent lures (i.e. coyote odor attractants) mid-gestation (February to March)[72] to determine whether such odors could affect fecal glucocorticoid (FGM) and fecal androgen metabolites (FAM), as well as behaviors. Coyote odor attractants such as scent lures have previously been identified to elicit strong marking and investigative behaviors, and are typically used to attract the attention of neighboring coyotes by simulating odors from foreign or novel conspecifics [46,73]. Here, we specifically predicted that increased marking and investigative behaviors would be correlated with FGM and FAM concentrations mid-gestation. In addition, we examined breeding pairs over successive breeding events in 2011 and 2013 to assess whether hormonal or behavioral measures exhibited temporal plasticity. We also examined whether coyotes demonstrated repeatability in marking and investigative behaviors (i.e. personality), as well as in FGM and FAM concentrations (i.e. hormone profiles).

2. Methods

2.1. Subjects

We observed eight breeding coyote pairs in 2011 at the United States Department of Agriculture National Wildlife Research Center (NWRC) Predator Research Facility in Millville, UT. At the beginning of the study all pairs had no prior breeding experience and were 1 or 2 years of age (1.4 ± 0.1 years [$X \pm SD$]). Prior to breeding, animals at the facility were housed in multiple enclosure types ranging from large outdoor pens (1000–6000 m²) to raised kennels (3.3 m²). In December 2011, coyotes were randomly selected from the NWRC population, then moved to 1000 m² outdoor “clover” pens optimized for long-term behavioral observations [69,74]. Clover pens were grouped into housing blocks of three pens per block, with each

individual pen containing a single breeding pair; hence, breeding pairs were adjacent to other study breeding pairs (Fig. 1). Pair relocation corresponded with the beginning of the breeding season [61,62,72]. To reduce potential effects of relocation stress on hormonal assays, each coyote pair was allowed one month to acclimate to their new pen before we collected fecal samples. From late December to January, each breeding pair was fed 1300g of commercial mink food (Fur Breeders Agricultural Cooperative, Logan, UT) daily and water was provided *ad libitum*. According to NWRC regulations, we doubled food rations in February to ensure that pregnant females were receiving adequate nutrition. We observed the same eight breeding pairs again in 2013 as experienced parents giving birth to their second litters.

Coyote parents were either wild-born and hand-reared (5 females, 5 males) or captive-born and coyote-reared (3 females, 3 males). Previous studies in multiple taxa have observed various differences in behavior and physiology attributed to hand-rearing (orange-winged Amazon parrots, *Amazona amazonica* [75]; red foxes, *Vulpes vulpes* [76]; gray wolves, *Canis lupus* [77]). Therefore, we considered rearing condition as a main effect in subsequent analyses (see 2.6. Statistical Analysis) to quantify the effect of early rearing experience on gestational hormones and scent-marking behaviors. Hand- or coyote-reared individuals were randomly assigned for pairing and were not exclusively paired with individuals of the same rearing condition.

2.2. Coyote odor attractants

We administered coyote attractant odors to four pairs, and a control (i.e. water) to the other four. For the treatment group, we provided four different commercial odors developed by Russ Carman® (Canine Call®, Pro's Choice®, and two versions of Magna Gland®, New Milford, PA). The odors were a blend of fermented glandular materials, urines, and other volatile

substances from other unrelated coyotes, known to increase marking and territorial behaviors in coyotes [46]. We manually administered odors to a treatment breeding pair territory (Fig. 1) every five days over a 20-day period (February 28th to March 15th) in 2011 (Table S1). We rotated the odor type provided to each breeding pair every five days to reduce potential presentation order effects. We provided control pairs with distilled water, and we provided all pairs with stimuli over the same timeframe. A single experimenter provided the stimuli and once administered, the tester and surrounding staff immediately vacated animal grounds to reduce species-typical caution to researcher presence [74]. The experimenter was inside each coyote pen for approximately 30 seconds. It took us approximately 55 minutes (54.88 ± 3.08 minutes [$X \pm SD$]) to provide all breeding pairs with their predetermined stimuli (i.e. either odor attractants or water). We video-recorded pairs beginning immediately before stimuli were provided and continued recording for approximately 140 minutes (137.75 ± 4.03 minutes [$X \pm SD$]) with one remote camera per pen (see Fig. 1). Cameras were stationed inside a closed environment in the center compound of three clover pens (Fig. 1). In sum, we recorded at least two hours of video after exiting the enclosure to ensure that any impact of human presence in the enclosure was diminished.

We repeated these methods in 2013 with the same male-female pairs but reversed the odor treatment: 2011 control pairs became 2013 odor pairs and vice versa. We changed pen location for each pair in the second breeding year to reduce potential habituation effects to familiar surroundings witnessed in the first year. In both years, we noted several marking and investigative behaviors following Gese and Ruff [67] and Kimball et al. [46] (Table 1). These are critical behaviors regularly used to assert hierarchical status, maintain social bonds, and demarcate territorial boundaries (Table 1) [67]. We began coding target behaviors at the

moment of stimulus deposition using all-occurrence methods [78] for a 70-minute period. Note that coyotes had immediate access to odor cues at the moment each stimulus was provided, and were not restricted by any physical barriers.

In 2012, coyotes were individually housed over the breeding season to prevent breeding, then repaired mid-spring. Pairs were also equally and periodically rotated through different pen types (excluding testing areas) to accommodate concurrent research projects on other captive coyotes and per NWRC regulations. Our study animals were not on any other NWRC related projects in the interim between the 2011 and 2013 breeding seasons.

2.3. Fecal sample collection

In both 2011 and 2013, we collected fresh fecal samples twice weekly from February to April. We fed animals multi-colored glitter particles according to previous methodology [79–81] to separate samples and determine their freshness. Specifically, we mixed glitter with surplus mink food, partitioned that food into small biscuits, froze them at -20°C , and then provided these mink food biscuits to each member of a breeding pair simultaneously the afternoon prior to sample collection. Glitter-marked samples retained their color once excreted the following morning. Each biscuit was mixed with a different color to identify sex within pairs. Certain individuals hesitated to approach mink food biscuits, allowing their mate enough time to eat both supplied biscuits. Individuals also tended to eat the biscuits while moving, which often resulted in crumbs spread for their mates to opportunistically eat. We therefore paired glitter biscuits (pre-excretion) with a previously established progesterone enzyme immunoassay (post-excretion:[82]). Females have significantly higher progesterone concentrations compared with

males during gestation [61]. We hypothesized that we could confirm the sex of fecal samples via the progesterone assay (see 4. Results).

We assessed freshness by appearance, odor, and stiffness in response to freezing temperatures. We restricted sample collection to feces excreted between 0600 and 1000 hours MST, as FGM content varies diurnally in coyotes [71]. Samples were stored at -20°C immediately to limit the amount of hormone metabolite degradation [83]. We collected multiple samples for each sampling period ($n=4$ per day, per pair) to ensure suitable fresh samples were collected for each individual in a breeding pair. Feces contaminated by urine ($n = 56$) were not collected, and all animals were sampled over the same time period for each collection event. All samples were shipped overnight on dry ice to the Lincoln Park Zoo Endocrinology Laboratory (Chicago, IL, U.S.A.) for hormonal analyses.

2.4. Fecal sample processing

Fecal samples (2011: $n = 588$; 2013: $n = 689$) were freeze-dried on a lyophilizer (Thermo Modulyo Freeze Dryer; Thermo Scientific, Waltham, MA, U.S.A.) for 3 days and crushed to a fine powder before extraction [71]. Briefly, sample powder was weighed (0.2 ± 0.02 g), combined with 5.0 ml of 90% ethanol (ethanol:distilled water), and agitated on a mixer (Glascol, Terre Haute, Indiana) for 30 min at setting 60. The samples were then centrifuged for 20 min at 1500 rpm and 10°C , and the supernatant was poured into clean glass tubes. The fecal pellets were re-suspended in 5.0 ml of 90% ethanol, vortexed for 30 sec, and re-centrifuged for 15 min at 1500 rpm. The supernatant was poured into the corresponding glass tubes and the combined supernatants were dried under air and a hot-water bath (60°C). Dry samples were then

reconstituted with 2.0 ml of phosphate-buffered saline (0.2 M NaH₂PO₄, 0.2 M Na₂HPO₄, NaCl), vortexed briefly, and sonicated for 20 min before analysis.

2.5. Enzyme Immunoassays

We used a previously validated cortisol enzyme immunoassay [71] to measure coyote fecal glucocorticoid metabolites. Polyclonal cortisol antiserum (R4866) and horseradish peroxidase were provided by C. Munro (University of California, Davis, CA, U.S.A.). Cortisol antiserum and cortisol horseradish peroxidase were used at dilutions of 1:8500 and 1:20,000, respectively [71,84]. Assay sensitivity was 1.95 pg/well and intra- and interassay coefficient of variation was <10%.

We also used a previously established testosterone enzyme immunoassay to measure coyote fecal androgen metabolites [85,86]. We biochemically validated the testosterone assay by (1) demonstrating parallelism between binding inhibition curves of fecal extract dilutions (1:2-1:8192) and hormonal standards (males: $R^2 = 0.990$; females: $R^2 = 0.993$) and (2) significant percent recovery (>90%) of exogenous testosterone (2.3-600 pg/well) added to pooled fecal extracts (1:3000; $\hat{y} = 0.8197x + 5.9562$; $R^2 = 0.9960$). Testosterone horseradish peroxidase and polyclonal antiserum were used at 1:30,000 and 1:10,000, respectively [84,85]. Assay sensitivity was 2.3 pg/well and intra- and interassay coefficient of variation was <10% for the testosterone enzyme immunoassay.

Finally, the progesterone assay used to differentiate samples by sex was biochemically validated by (1) demonstrating parallelism between binding inhibition curves of fecal extract dilutions (1:2-1:8192) and hormonal standards (males: $R^2 = 0.968$; females: $R^2 = 0.995$), and (2) significant percent recovery (>90%) of exogenous progesterone (0.78-200 pg/well) added to

pooled fecal extracts (1:3,000; $\hat{y} = 0.9999x + 1.4882$; $R^2 = 0.9945$). We also biologically validated fecal progesterone in the species by comparing samples collected during and after gestation. Progesterone horseradish peroxidase and polyclonal antiserum were used at 1:10,000 and 1:40,000, respectively. Assay sensitivity for fecal progesterone metabolites was 0.78 pg/well and intra- and interassay coefficient of variation was <10%. Cross-reactivities for all assays have been previously described [82].

2.6. Statistical Analyses

To assess the impact of our coyote odor attractants on scent-marking and investigative behaviors, we used generalized linear mixed models (GLMMs) with a Poisson error distribution for behavioral count data. All behavioral data are reported as per 70 minutes of recorded video. Here, we observed treatment group and breeding year as main effects in our model, as well as the interaction term between the two factors. Coyote identity and test number (see Table S1) were set as random effects in the model. We partitioned our data by sex to observe odor treatment and breeding year differences within each sex. In addition, we determined whether coyote investigative behaviors were in fact influenced by test number (see Table S1) by using post-hoc Tukey contrasts in pair-wise comparisons.

We used linear mixed models (LMMs) to determine how odor treatment and breeding year were associated with hormonal outcomes. Prior to using LMMs, we binned hormonal values by weeks until birth as there were unknown differences in date of conception for each pair. Weeks until birth were therefore projected according to each female's date of parturition and the typical length of coyote gestation (63 days; see [72]). To date, no data exist that have characterized fecal glucocorticoids and androgens in the species. We therefore used Tukey contrasts to assess

the general impact of weeks until birth on FGMs and FAMs. In subsequent LMMs, we set weeks until birth as a random effect to account for differences in the timing of conception that may have influenced FGM and FAM concentrations irrespective of their group identity (odor versus control).

We subsequently partitioned hormonal data into three descriptive categories: pre-test, test, and post-test. The test period specifically was the aforementioned 20-day period in which odors (treatment) and water (control) were provided (i.e. mid-gestation). The pre-test period (i.e. early gestation) comprised the 4 weeks of fecal collection before we provided odors, while the post-test period (i.e. late gestation) was the 3 weeks after. We set treatment group, breeding year, and test period as main effects for our LMMs. Mixed models were conducted separately for males and females, with coyote identity as the random effect term. We found a significant effect of period with LMMs focused within each period. Thus, within each separate test period we assessed the effect of odor treatment and breeding year as main effects. We tested hormonal data for normality using Shapiro-Wilk tests, and non-normally distributed data were log transformed [71].

To quantify consistent individual differences in behavioral and hormonal measures, we used variance components extracted from previously described GLMMs to estimate repeatability (R) as the proportion of total variation attributable to among-individual variation versus between-individual variation [87]. Both the test number and test period were confounding factors partially explaining variance within our behavioral and hormonal results, respectively. We therefore calculated adjusted repeatabilities (R_{adj}) which estimate individual differences while controlling for confounding effects by including them in the model when calculating between-individual and residual variances. Adjusted repeatabilities were therefore calculated as:

297 $R_{adj} = \sigma_{\alpha}^2 / (\sigma_{\alpha}^2 + \sigma_e^2 + 0.25)$

298 in which σ_{α}^2 represents the between-individual variance, σ_e^2 represents the residual variance, and

299 (+0.25) is the distribution-specific variance of a Poisson model with a square root link function

300 and additive overdispersion [87]. Variances attributed to other random factors (test number and

301 test period) were included in repeatability calculations, allowing us to determine whether

302 consistent individual differences persisted despite confounding factors. We subsequently

303 determined whether repeatability estimates were significant by examining whether each

304 estimate's 95% confidence interval (CI) was pressed against zero, and if not we interpreted this

305 as evidence of a significant repeatability estimate [49,88]. Repeatabilities for behavioral count

306 and hormonal data were quantified using variance components extracted from GLMMs and

307 LMMs, respectively. Individual measures were compared between the 2011 and 2013 seasons

308 for our repeatability estimates. We then calculated best linear unbiased predictors (BLUPs) for

309 each individual, which is the value fitted for the individual based on the intercept of the model

310 and the standard deviation calculated for the individual random effect [89,90]. Hormonal

311 BLUPs were later correlated with our marking and investigative behaviors using Spearman rank

312 correlations. Finally, we examined Spearman correlations among maternal and paternal traits

313 because marking behaviors of paired individuals are expected to affect the marking traits of their

314 pairmate [67].

315 All analyses were conducted in R version 3.2.4 [91]. Linear mixed models were performed

316 using the lmer function from 'lme4' [92] and 'lmerTest' [93] packages. Mixed models with a

317 Poisson error distribution were performed using the glmer function from the 'nlme' package

318 [94]. We used restricted estimation maximum likelihood (REML) with a diagonal covariance

319 structure for all of our models, with Satterthwaite approximation for degrees of freedom.

Repeatabilities were either calculated using the rpt.remLMM.adj function from the ‘rpt.R’ package’ (Gaussian data) or from variance components of glmer model output (count data) [87]. Spearman correlations were performed using the corr function from the ‘corrplot’ package [95]. In all cases, we used two-tailed tests with alpha set to $P < 0.05$ and data reported as mean $\pm$ SE and for all data we used Shapiro-Wilk to test for normality before analyses. None of our hormonal or behavioral measures demonstrated an effect of rearing condition. Rearing effects were therefore not addressed further. All research and methods described above were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago (protocol no. 72184), the NWRC (protocol no. QA-1818), and the Lincoln Park Zoo Research Committee.

3. Results

3.1. Coyote Odor Attractants: Behaviors

We first observed overall differences by treatment group and breeding year in each sex separately (Fig. 2, Table S2). We found that both odor-treated females and males urinated, rubbed, sniffed, and visited the affected site more frequently than control individuals (Fig. 2A-B, Table S2). Odor-treated females in particular exhibited greater within-pair aggressive behaviors and ground scratching compared to their control counterparts (Table S2). Both odor-treated females and males spent more time at the odor site, but only females differed by treatment group in latency to visit the odor site (Table S2). Female aggression demonstrated an interaction between odor treatment and year, with 2011 odor-treated females behaving equally as aggressive in 2013 as control females (Table S2). Males demonstrated similar interaction effects for ground scratching, urination, rubs, and site visits (Table S2).

We also found that pairs scent-marked and investigated the scented areas more as experienced breeders (Fig. 2C-D, Table S2). Specifically, both experienced males and females urinated, rubbed, sniffed, and visited the affected site more frequently than first-time breeders despite the treatment group membership (Fig. 2C-D, Table S2). Experienced breeders also spent more time at the affected area. However, only females differed in their latency to visit the site, in which experienced breeders approached the stimulus site quicker than first-time breeders.

To assess whether individuals adjusted their site time or latency to visit the stimulus site with each successive test (see Table S1), we used Tukey contrasts to compare coyotes within treatment groups in each of the four odor provisioning events (Fig. 3A-B). Again, we found that odor-treated males and females spent more time at the stimulus site within each test date (Fig. 3A-B). However, over each successive trial odor-treated females spent less time at the stimulus site ($F_{3,27} = 4.237$, $P = 0.014$) compared with control females ($F_{3,27} = 0.646$, $P = 0.592$). Males did not exhibit a significant decrease in time spent at the odor site for males as a function of treatment group (control: $F_{3,27} = 0.334$, $P = 0.801$; treatment: $F_{3,27} = 1.293$, $P = 0.297$; Fig. 3).

Finally, we determined whether coyotes demonstrated repeatability in our behavioral measures. Both females and males demonstrated repeatability in ground scratching, urination, and site visits across years (Table 2). We found that females were specifically repeatable in their aggression, whereas males demonstrated repeatability in their latency to visit the odor- or water-provisioned site (Table 2).

3.2. Coyote Odor Attractants: Hormones

First, our progesterone assays were able to distinguish previously unidentified fecal samples by sex: females had consistently higher dilution rates compared to their male partners (females:

1:1500 to 1:15,000; males: 1:300), indicating higher progesterone concentrations for female samples. We therefore were able to successfully identify a total of 560 fecal samples for our 8 breeding pairs across the 2011 and 2013 seasons. Both FGMs and FAMs decreased toward parturition for both sexes (FGMs: females – $F_{10,257} = 2.945$, $P = 0.002$; males – $F_{10,254} = 1.986$, $P = 0.035$; FAMs: females – $F_{10,257} = 15.96$, $P < 0.001$; males – $F_{10,254} = 7.739$, $P < 0.001$). Post-hoc Tukey tests comparing gestational weeks demonstrated lower FGMs and FAMs in the latter half of gestation compared to early gestation (Fig. 4).

When hormonal data were partitioned by testing period (i.e. pre-test, test, and post-test), we found that odor-treated females – but not odor-treated males – had lower FGM concentrations during the test period (females – $F_{1,67.2} = 10.77$, $P = 0.002$; males – $F_{1,69.3} = 0.84$, $P = 0.36$) compared with control pairs (Fig. 5A). Odor-treated females also had lower FGM concentrations during the pre-test period ($F_{1,115.1} = 4.19$, $P = 0.043$), but did not differ from controls in the post-test period ($F_{1,57.3} = 1.61$, $P = 0.21$), indicating that differences in FGMs may have existed prior to receiving odors (Fig. 5A). Odor-treated females generally had lower FGMs than their control counterparts (Table S2). Odor-treated males did not differ from controls in FGMs over the pre-test ($F_{1,110.4} = 0.59$, $P = 0.45$) or post-test periods ($F_{1,58.2} = 2.77$, $P = 0.10$), suggesting odor attractants did not influence male fecal glucocorticoids (Fig. 5B). We did not find any significant FGM differences as a function of breeding experience in either males or females (Table S2).

We did find higher FAM concentrations during the test period for odor-treated pairs versus control pairs (females – $F_{1,68.6} = 6.11$, $P = 0.012$; males – $F_{1,72.0} = 6.18$, $P = 0.015$; Fig. 5C-D). Fecal androgen metabolites during the pre-test (females: $F_{1,117.5} = 0.62$, $P = 0.43$; males: $F_{1,112.8} = 1.29$, $P = 0.26$) and post-test periods (females: $F_{1,63.0} = 2.35$, $P = 0.13$; males: $F_{1,60.0} = 3.64$, $P =$

0.061) did not differ as a function of odor treatment for either sex (Fig. 5C-D). Within both sexes, we found that FAMs over the entirety of gestation were greater for first-time breeders versus experienced breeders (females: $F_{1,251.4} = 9.33$, $P = 0.003$; males: $F_{1,250.1} = 6.14$, $P = 0.014$). In addition, males had significantly greater FAMs during gestation compared with females ($F_{1,7} = 84.8$, $P < 0.001$).

Last, both female and male coyotes demonstrated repeatability in their FGMs across years (Table 2). Only males demonstrated consistency in their FAMs with successive breeding years (Table 2).

3.3. Correlations among Behaviors and Hormones

We found that both female ($r_s = 0.61$, $N = 16$, $P = 0.012$) and male ($r_s = 0.58$, $N = 16$, $P = 0.019$) androgen best linear unbiased predictors (BLUPs) were positively correlated with latency to visit the stimulus site (Table S3), indicating that individuals with higher androgen BLUPs took longer to investigate the odor or water-affected site. Male androgen BLUPs in particular were negatively associated with time at the odor site ($r_s = -0.51$, $N = 16$, $P = 0.044$), and number of site visits ($r_s = -0.69$, $N = 16$, $P = 0.003$), indicating that males with higher androgen BLUPs spent less time performing investigative behaviors (Table S3). Male androgen BLUPs were also negatively associated with the mean number of urinations ($r_s = -0.67$, $N = 16$, $P = 0.005$). Further, urination, body rubs, site sniffs, site visits, and time at the site all positively covaried (Table S4). Finally, we found positive correlations between identical male and female behaviors, in which high-marking and investigatory males were matched with high-marking and investigatory females (Table 3). Additional correlational data among BLUPs for glucocorticoid

metabolites, androgen metabolites, and behaviors, as well as behavior-behavior relationships are in the supplementary materials (Tables 3, S3, and S4).

4. Discussion

We have demonstrated here that coyote odor attractants were both effective at eliciting strong territorial responses, as well as increasing FAMs of both sexes. In addition, coyote pairs had increased FAMs as first-time breeders, indicating that either parity or increasing age played a partial role in affecting FAM concentrations. We observed steady declines in both FGMs and FAMs toward parturition for both sexes, elucidating the temporal component of these hormones during gestation. Individual coyotes demonstrated repeatability for several territorial behaviors and hormones despite altering treatment conditions, highlighting the robustness of coyote personality and hormonal traits to novel disturbances. Moreover, pairs were consistent in marking behaviors and glucocorticoids suggesting that individual coyote responses to the odor attractants were partially influenced by the behavior of their pairmate. Finally, though relatively minimal and sex-specific, we found that-androgen BLUPs were correlated with a subset of observed investigative behaviors, suggesting that specific androgen profiles may be partially associated with coyote personality.

4.1. Odor attractants, parity, and behavioral responses

Scent-marking and investigative behaviors greatly increased for individuals that received novel odors. This is consistent with other odor studies in coyotes [46,73], as well as with other studies in Canidae (African wild dogs, *Lycaon pictus* [45]; Ethiopian wolves, *Canis simensis* [96]), highlighting the importance of olfactory cues in stimulating parallel territorial behaviors

across the clade. What is unique to our study is that these behaviors exhibited plasticity as a function of odor presentation and either parity or increasing age (Table S2). From 2011 to 2013, both sexes increased the number of visits they made to the experimental site, despite treatment group. There was also an overall increase in the number of site sniffs and urine-marking events for all coyotes. Increased marking with age suggests that older individuals become more involved in demarcating territorial boundaries. In fact, older coyotes mark more frequently than yearlings or early-aged adults [67]. Our study also demonstrated that pair latency to visit the site dramatically decreased from 2011 to 2013, in which individuals in 2013 generally approached the affected site faster than they did in 2011. These results suggest several possibilities: (1) coyotes' prior experience with the mere process of stimulus application (i.e. an observer entering and placing a foreign substance in their pen) influenced their resultant visit latencies or (2) older coyotes are typically more investigative and therefore individuals were more likely to visit the site quickly in 2013 versus 2011. However, the covariance between coyote age and prior experience in our study does not allow us to definitively determine which factor is a better explanatory variable for visit latencies.

Multiple scent-marking and investigative behaviors were highly correlated with one another irrespective of the odor treatment (Table S4). For instance, ground scratching, urination, body rubs, and site sniffs all covaried. The multiple associations among these marking behaviors likely accentuate individual territoriality characteristic within this species [67,97]. Specifically, only particular individuals ascend to alpha pair status, and those individuals demarcate territorial boundaries more frequently than betas or transients [67]. Alpha individuals also maintain status via successful territorial defense from neighboring conspecifics and suppression of insurgency within a pack [65]. Because being an alpha coyote increases breeding opportunities for that

individual [65,98], consistent individual differences in territoriality represent a tangible set of characteristics that can directly influence reproductive fitness. This interplay between rank and consistent individual differences may not be restricted to coyotes, but also found in African wild dogs [24], Ethiopian wolves [99], and gray wolves [28]. We temper our predictions as to how relevant these findings are to social dynamics of coyotes, however, because our design solely observed single pairs. However, our results provide a foundation for future research to closely examine pack systems and how individual differences in behavior shape the development of pack dynamics in Canidae.

Other studies have used similar manufactured and commercial odors that have elicited near identical behavioral responses from coyotes to varying degrees [46,73]. The use of coyote attractants that have trace odors from novel conspecifics suggest that our coyote attractants were a proxy for social cues. This is similar to Dantzer et al. [47], which presented conspecific audio playbacks toward pregnant red squirrel mothers as a proxy for social challenge and increased conspecific density. Because coyotes similarly respond to odors such as wolf urine (C. Schell, unpublished) it is not conclusive whether the behavioral responses observed were socially-motivated or if coyotes regularly follow a set marking protocol whenever the intensity of a scent is highly volatile. Nevertheless, it is known that coyotes are able to differentiate between subtle differences in volatile odors and respond accordingly [46]. Indeed, individual coyotes have been shown to delineate among varying volatile odors by altering the degree of marking, biting, pulling, and rubbing behaviors they perform specific to the odor received. Water was therefore a suitable control in our study to compare against receiving coyote odor attractants, particularly given the profound behavioral differences we observed among our treatment and control breeding pairs. Further, the potential for indirect olfactory contact was negligible: though

control and odor pairs were adjacent to each other, the stark behavioral differences between treatment groups indicate that having stimuli inside your pen was more salient than indirect detection of odors outside of your home pen. Our results support data from coyotes in the wild, as resident individuals are more responsive to foreign substances provided within their territories [67].

4.2. Odor attractants, parity, and hormonal responses

This study is the first to physiologically quantify gestational FGMs and FAMs in coyotes of both sexes, as previous studies were restricted to plasma samples and did not measure both hormones in each sex [61,64,100]. Our initial analyses to characterize FGM and FAM patterns demonstrated steady declines for both sexes as gestation progressed regardless of odor treatment. The observed hormonal patterns are in accord with previous findings on female progesterone [61] and male testosterone [64,100] in coyotes, and may be explained by several factors. First, glucocorticoids and androgens peak early during the breeding season, which corresponds with a peak in scent-marking behaviors of previous studies [63,72,101]. It is likely that hormonal physiology accompanies the onset and regression of marking behaviors, similar to urine-marking and testosterone in gray wolves [28]. Second, constant territorial maintenance over mating may require expectant pairs to have elevated glucocorticoids and androgens to cope with the stress of territorial intrusions. As competition wanes, however, it may be unnecessary to maintain elevated stress and reproductive hormone concentrations, especially as chronic activation of glucocorticoids can compromise maternal health and developing offspring [4]. Third, hormonal declines may also be evolutionarily conserved: related Canidae mothers demonstrate similar decreases in stress and reproductive hormones closer to parturition (domestic dog, *Canis lupus*

503 *familiaris* [102]; Ethiopian wolves [99]). More distantly related mammals show the opposite
504 trend (yellow baboons, *Papio cynocephalus* [103]; pygmy rabbits, *Brachylagus idahoensis*
505 [104]), suggesting that decreases in reproductive and stress hormones over pregnancy are
506 specific to Canidae.

507 Hormonal patterns of expectant coyote fathers closely followed maternal patterns over the
508 entirety of gestation, suggesting that males are sensitive to maternal cues over pregnancy.
509 Similarly, expectant cotton-top tamarin (*Saguinus oedipus*) fathers track glucocorticoid
510 responses of paired pregnant partners [51]. The authors suggest that responsiveness of fathers is
511 primarily explained by female deposition of periovulatory scents rather than increased rates of
512 behavioral communication, as there were no observed interaction changes between mates.
513 Expectant common marmoset (*Callithrix jacchus*) fathers show similar responsiveness, as males
514 exhibited increased testosterone shortly after sniffing periovulatory scents of pregnant females
515 [105]. Periovulatory scents may signal that the female is receptive to solicit copulation with the
516 male and mate guarding from neighboring males, both of which are often accompanied by
517 increased testosterone. Periovulatory scents may also signal maternal health status to expectant
518 fathers, which can alter glucocorticoids and paternal behavior to assist the mother during
519 gestation. Coincidentally, coyotes, cotton-top tamarins, and common marmosets are all socially
520 and reproductively monogamous [51,72,98,105], which suggests that in monogamous biparental
521 systems it is beneficial for fathers to be highly responsive toward maternal cues over pregnancy.
522 Periovulatory scents may signal that the female is receptive to solicit copulation with the male
523 and mate guarding from neighboring males, both of which are often accompanied by increased
524 FAMs. Periovulatory scents may also signal maternal health status to expectant fathers, which
525 can alter glucocorticoids and paternal behavior to assist the mother during gestation. This study

provides further evidence to suggest that hormonal patterns of expectant fathers are highly responsive to female stimuli.

Both sexes had increased FAMs when provided odor attractants, suggesting that novel odors were effective at soliciting a physiological response. Paired with increased scent-marking and investigatory behavior, it is likely that the coyote odor attractants were effective proxies for territorial incursion. These results support the challenge hypothesis previously described by Wingfield et al. [106] and revisited by Goymann et al. [107], in which individuals (specifically males) that are challenged for their social rank during the mating season exhibit increased androgens and aggression in response. Golden lion tamarin males (*Leontopithecus rosalia*) exhibit this trend, as dominant breeding males exhibit higher androgens during the mating season [22]. Similarly, in male chacma baboons (*Papio hamadryas ursinus*) testosterone concentrations and changes in rank are positively correlated, in which males rising in rank have higher testosterone than males falling in rank [23]. Here, our data suggest that the challenge hypothesis applies to both males and females, which may be due to the biparental nature of the coyote system. Future work should address the challenge hypothesis in socially monogamous systems to examine how androgens of both sexes are affected by artificial (i.e. odors) or actual challenges to social rank.

In addition to effects of the coyote attractants, we observed an effect of breeding experience on male and female androgens, in which pairs as first-time breeders had higher FAM concentrations. This is in contrast to cotton-top tamarins, in which FAM changes pre-partum are independent of breeding experience [58]. For coyotes, it is possible that as a young breeding pair, securing a territory and guarding against territorial intrusions may present a greater challenge than maintaining a territory is for experienced breeders. Consequently, increased

FAMs during gestation may accompany increased territorial defense and maintenance. It is also possible that increased familiarity between individuals within a pair is related to decreased FAMs over time. Specifically, reduction of intra-pair aggression over time may result in decreased FAMs. An alternative explanation may be that unfamiliar physical changes such as pair relocation and first breeding event may have placed physical stress on the body that manifested as increased FAMs. In addition, this unfamiliarity may stem from novel experiences of young animals to captive conditions, and FAMs of experienced pairs merely reflect a perceived comfort or predictability of housing conditions. More data are necessary to examine these hypotheses on how age of pairs and familiarity within pairs impact FAMs.

4.3. Repeatability of behaviors and hormones

Both females and males demonstrated repeatability in urination and ground scratching behaviors across years despite differences in odor treatment condition (Table 2). Previous work has indicated that between-individual differences in wild coyote marking rates are primarily attributed to social organization (i.e. resident versus transient), social class (i.e. alpha versus beta), and season (i.e. breeding versus non-breeding), in which resident alpha adults have the highest rates of marking behaviors [67]. Our captive coyote pairs were individually housed, eliminating the potential for social hierarchies to develop among coyote packs. Our results therefore suggest marking rates are not merely determined by social organization or class but also by inherent differences in individual personalities. Consequently, personality type may be a credible predictor in determining which individual coyotes will establish home ranges and outcompete conspecifics to ascend in social rank.

Our results also demonstrated strong positive correlations between identical behaviors in male-female pairs except for aggression and ground scratching (see Table 3). Because most of these traits demonstrated repeatability, these data suggest female personality may have affected male behaviors and vice versa. These data support findings from wild coyotes, in which one partner of a resident pair will urinate and the other partner subsequently responds by sniffing and urine-marking the same affected area [67]. Individuals within a breeding pair therefore have the potential to augment the expression of personality traits of their partner, indicating that pairmates are a potential source of plasticity in personality traits. Coalescing personalities between pairmates may provide a fitness benefit, as parents with relatively similar personality types may have greater reproductive success [108,109]. For coyotes, highly territorial pairs tend to be residents that can outcompete neighboring conspecifics and secure high-quality resources [66,67]. If individuals with strong personalities in scent-marking and investigatory behaviors can augment the personality traits of their partner, this may positively affect their ability to secure and defend source habitats in which to rear offspring. Moreover, the significant correlation between male and female androgen BLUPs may indicate that individuals also partially influence the reproductive hormone profiles of their pairmate (Table 2). Hence, influences from the father during pregnancy may likely affect maternal androgen profiles that eventually affect developing offspring epigenetically, shaping pup developmental trajectories [54,55]. Our results therefore highlight the importance of pairmates in affecting both phenotypic outcomes for their partner and for subsequent offspring as well.

Both sexes demonstrated repeatability in their FGM concentrations over time, suggesting that coyotes either have stable stress physiology or distinct stress profiles. These stress profiles are reminiscent of the Hawk-Dove hypothesis proposed by Korte et al. [4], where individuals

employ different behavioral strategies that are facilitated by underlying physiology. Korte et al. [4] originally delineated Hawk-Dove differences by levels of aggression and its association with the biological stress response of an individual, though the conceptual framework can be expanded to different behaviors and hormones. In contrast, only males demonstrated repeatability in FAMs (Table 2). Given that testosterone is generally higher in male mammals [110], it is likely that repeatability in coyote male FAMs reflect sex-linked traits important for reproduction.

Individually-consistent differences in hormones (i.e. BLUPs) were correlated with multiple scent-marking and investigative behaviors (Table S3). This is similar to male great tits (*Parus major*), in which testosterone levels were both repeatable over time and correlated with exploratory behaviors [27]. Likewise, individual white-eared hummingbirds (*Hylocharis leucotis*) show consistent individual differences in testosterone over time and those with higher testosterone concentrations are more risk-prone foragers (quantified by frequent visits to variable flowers)[26]. For this study, increased androgen BLUPs were positively associated with latency to visit the affected site for both sexes (Table S3). For male coyotes specifically, androgen BLUPs were also correlated with the number of visits made and the time spent at the affected site (Table S3), suggesting male coyote androgens are particularly salient for individually-specific investigative behaviors. Our results therefore suggest that individual differences in territoriality are partially mediated by androgens. However, the paradoxical negative association between androgen BLUPs and urine-marking may also suggest other environmental factors like pairmate behavior may mitigate behavioral expression of males. Indeed, we have provided evidence to suggest that coyote female marking behaviors may be met with matching behaviors

from males (Table 3); hence, the potential of pairmates to influence behavioral profiles may be understated.

We have demonstrated that both conspecific odor attractants and parity affect behavior and hormones of breeding coyotes. Moreover, coyotes demonstrated personality for multiple territorial behaviors, and distinct hormone profiles for both glucocorticoid metabolites and androgen metabolites. Given the pervasive effects that parental influences may have pre-partum, it will be important to consider how individual differences in coyote traits and plasticity of those traits affect both parents and offspring. Indeed, changes to parental glucocorticoid and androgen concentrations directly interact with offspring during gestation and greatly dictate offspring development in the process [47,54,55,111]. Hence, a deeper understanding of the myriad effects environmental stressors and temporal variation have on pregnant parents and subsequent developmental trajectories of neonates will enrich our understanding of epigenetic processes [112–114]. This is particularly relevant for coyotes in the context of rapid adaptation to nonnative and urban habitats, which may suggest that environmental experiences of parents play some role in shaping offspring phenotypic traits integral for colonization and survival of novel environments.

Acknowledgments

We would like to thank B. Patterson and T. Price for extensive and valuable feedback on this manuscript, as well as members of the Mateo and Santymire laboratories. This study would not have been possible without the input, time, and animal expertise of S. Brummer, J. Schultz, M. Davis, E. Stevenson, and other staff at the Wildlife Services National Wildlife Research Center's Predator Research Facility in Millville, UT, USA. Special thanks to D. Schell, B. Flack, and

639 other volunteers for providing data collection assistance, behavioral observations, and laboratory
640 analyses. Additional thanks to the University of Chicago Hinds Fund and GAANN Fellowship,
641 the United Negro College Fund (UNCF)-Merck Fellowship, the National Science Foundation
642 (NSF), and United States Department of Agriculture–Wildlife Services–NWRC as funding
643 sources for this endeavor. This work was performed with the approval of the Institutional
644 Animal Care and Use Committees (IACUC) at the University of Chicago (protocol no. 72184),
645 the NWRC (protocol no. QA-1818), and the Lincoln Park Zoo Research Committee. Mention of
646 companies or commercial products does not imply recommendation or endorsement by USDA
647 over others not mentioned. USDA neither guarantees nor warrants the standard of any product
648 mentioned. Product names are mentioned solely to report factually on available data and to
649 provide specific information.

REFERENCES

- [1] Bijlsma R, Loeschcke V. Environmental stress, adaptation and evolution: An overview. *J Evol Biol* 2005;18:744–9. doi:10.1111/j.1420-9101.2005.00962.x.
- [2] Boonstra R. The ecology of stress: a marriage of disciplines. *Funct Ecol* 2013;27:7–10. doi:10.1111/1365-2435.12048.
- [3] Creel S, Dantzer B, Goymann W, Rubenstein DR. The ecology of stress: effects of the social environment. *Funct Ecol* 2013;27:66–80. doi:10.1111/j.1365-2435.2012.02029.x.
- [4] Korte SM, Koolhaas JM, Wingfield JC, McEwen BS. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neurosci Biobehav Rev* 2005;29:3–38. doi:10.1016/j.neubiorev.2004.08.009.
- [5] McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. *Horm Behav* 2003;43:2–15. doi:10.1016/S0018-506X(02)00024-7.
- [6] Wingfield JC. The concept of allostasis: coping with a capricious environment. *J Mammal* 2005;86:248–54. doi:10.1644/BHE-004.1.
- [7] Möstl E, Palme R. Hormones as indicators of stress. *Domest Anim Endocrinol* 2002;23:67–74. doi:10.1016/S0739-7240(02)00146-7.
- [8] Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. *Ann N Y Acad Sci* 2005;1046:54–74. doi:10.1196/annals.1343.006.
- [9] Creel S. Social dominance and stress hormones. *Trends Ecol Evol* 2001;16:491–7. doi:10.1016/S0169-5347(01)02227-3.
- [10] Creel S. Dominance, Aggression, and Glucocorticoid Levels in Social Carnivores. *J Mammal* 2005;86:255–64. doi:10.1644/BHE-002.1.

- [11] Goymann W, Wingfield JC. Allostatic load, social status and stress hormones: the costs of social status matter. *Anim Behav* 2004;67:591–602. doi:10.1016/j.anbehav.2003.08.007.
- [12] Husak JF, Moore IT. Stress hormones and mate choice. *Trends Ecol Evol* (Personal Ed 2008;23:532–4. doi:10.1016/j.tree.2008.06.007.
- [13] Miles DB, Sinervo B, Hazard LC, Svensson EI, Costa D. Relating endocrinology, physiology and behaviour using species with alternative mating strategies. *Funct Ecol* 2007;21:653–65. doi:10.1111/j.1365-2435.2007.01304.x.
- [14] Atwell JW, Cardoso GC, Whittaker DJ, Campbell-Nelson S, Robertson KW, Ketterson ED. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behav Ecol* 2012;23:960–9. doi:10.1093/beheco/ars059.
- [15] Carere C, Caramaschi D, Fawcett TW. Covariation between personalities and individual differences in coping with stress : Converging evidence and hypotheses. *Curr Zool* 2010;56:728–41.
- [16] Dosmann AJ, Brooks KC, Mateo JM. Within-Individual Correlations Reveal Link Between a Behavioral Syndrome, Condition, and Cortisol in Free-Ranging Belding's Ground Squirrels. *Ethology* 2014;120:1–10. doi:10.1111/eth.12320.
- [17] Dosmann AJ, Brooks KC, Mateo JM. Evidence for a mechanism of phenotypic integration of behaviour and innate immunity in a wild rodent : implications for animal personality and ecological immunology. *Anim Behav* 2015;101:179–89. doi:10.1016/j.anbehav.2014.12.026.
- [18] Buchanan KL, Evans MR, Goldsmith AR, Bryant DM, Rowe LV. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc R*

696 Soc B Biol Sci 2001;268:1337–44. doi:10.1098/rspb.2001.1669.

697 [19] Drea CM, Place NJ, Weldele ML, Coscia EM, Licht P, Glickman SE. Exposure to
698 naturally circulating androgens during foetal life incurs direct reproductive costs in female
699 spotted hyenas, but is prerequisite for male mating. *Hungarian Q* 2008;49:1981–7.
700 doi:10.1098/rspb.2002.2109.

701 [20] Gonzalez G, Sorci G, Smith LC. Testosterone and sexual signalling in male house
702 sparrows (*Passer domesticus*). *Behav Ecol Sociobiol* 2001;50:557–62.
703 doi:10.1007/s002650100399.

704 [21] Setchell JM, Smith T, Wickings EJ, Knapp LA. Social correlates of testosterone and
705 ornamentation in male mandrills. *Horm Behav* 2008;54:365–72.
706 doi:10.1016/j.yhbeh.2008.05.004.

707 [22] Bales KL, French JA, McWilliams J, Lake RA, Dietz JM. Effects of social status, age, and
708 season on androgen and cortisol levels in wild male golden lion tamarins (*Leontopithecus*
709 *rosalia*). *Horm Behav* 2006;49:88–95. doi:10.1016/j.yhbeh.2005.05.006.

710 [23] Beehner JC, Bergman TJ, Cheney DL, Seyfarth RM, Whitten PL. Testosterone predicts
711 future dominance rank and mating activity among male chacma baboons. *Behav Ecol*
712 *Sociobiol* 2006;59:469–79. doi:10.1007/s00265-005-0071-2.

713 [24] Creel S, Creel NM, Mills MGL, Monfort SL. Rank and reproduction in cooperatively
714 breeding African wild dogs: behavioral and endocrine correlates. *Behav Ecol* 1997;8:298–
715 306. doi:10.1093/beheco/8.3.298.

716 [25] Koren L, Mokady O, Geffen E. Elevated testosterone levels and social ranks in female
717 rock hyrax. *Horm Behav* 2006;49:470–7. doi:10.1016/j.yhbeh.2005.10.004.

718 [26] Chávez-Zichinelli CA, Gómez L, Ortiz-Pulido R, Lara C, Valdéz R, Romano MC.

719 Testosterone levels in feces predict risk-sensitive foraging in hummingbirds. *J Avian Biol*
720 2014;45:501–6. doi:10.1111/jav.00387.

721 [27] van Oers K, Buchanan KL, Thomas TE, Drent PJ. Correlated response to selection of
722 testosterone levels and immunocompetence in lines selected for avian personality. *Anim*
723 *Behav* 2011;81:1055–61. doi:10.1016/j.anbehav.2011.02.014.

724 [28] Asa CS, Mech LD, Seal US, Plotka ED. The influence of social and endocrine factors on
725 urine-marking by captive wolves (*Canis lupus*). *Horm Behav* 1990;24:497–509.
726 doi:10.1016/0018-506X(90)90038-Y.

727 [29] Fuxjager MJ, Knaebe B, Marler CA. A single testosterone pulse rapidly reduces urinary
728 marking behaviour in subordinate, but not dominant, white-footed mice. *Anim Behav*
729 2015;100:8–14. doi:10.1016/j.anbehav.2014.11.006.

730 [30] Sih A, Watters J V. The mix matters: behavioural types and group dynamics in water
731 striders. *Behaviour* 2005;142:1417–31. doi:10.1163/156853905774539454.

732 [31] Sih A. Behavioral Syndromes: A Behavioral Ecologist’s View on the Evolutionary and
733 Ecological Implications of Animal Personalities. In: Weiss A, King JE, Murray L, editors.
734 Personal. Temperament Nonhum. Primates, New York, NY: Springer New York; 2011, p.
735 313–36. doi:10.1007/978-1-4614-0176-6.

736 [32] Cote J, Fogarty S, Brodin T, Weinersmith K, Sih A. Personality-dependent dispersal in the
737 invasive mosquitofish: group composition matters. *Proc R Soc B Biol Sci* 2011;278:1670–
738 8. doi:10.1098/rspb.2010.1892.

739 [33] David M, Auclair Y, Cézilly F. Personality predicts social dominance in female zebra
740 finches, *Taeniopygia guttata*, in a feeding context. *Anim Behav* 2011;81:219–24.
741 doi:10.1016/j.anbehav.2010.10.008.

- 742 [34] English S, Nakagawa S, Clutton-Brock TH. Consistent individual differences in
743 cooperative behaviour in meerkats (*Suricata suricatta*). *J Evol Biol* 2010;23:1597–604.
744 doi:10.1111/j.1420-9101.2010.02025.x.
- 745 [35] Sands J, Creel S. Social dominance, aggression and faecal glucocorticoid levels in a wild
746 population of wolves, *Canis lupus*. *Anim Behav* 2004;67:387–96.
747 doi:10.1016/j.anbehav.2003.03.019.
- 748 [36] Smith BR, Blumstein DT. Fitness consequences of personality: a meta-analysis. *Behav*
749 *Ecol* 2007;19:448–55. doi:10.1093/beheco/arm144.
- 750 [37] Vucetich JA, Creel S. Ecological Interactions, Social Organization, and Extinction Risk in
751 African Wild Dogs. *Conserv Biol* 1999;13:1172–82. doi:10.1046/j.1523-
752 1739.1999.98366.x.
- 753 [38] Sih A, Kats LB, Maurer EF. Behavioural correlations across situations and the evolution
754 of antipredator behaviour in a sunfish–salamander system. *Anim Behav* 2003;65:29–44.
755 doi:10.1006/anbe.2002.2025.
- 756 [39] Wolf M, Weissing FJ. Animal personalities: consequences for ecology and evolution.
757 *Trends Ecol Evol* 2012;27:452–61. doi:10.1016/j.tree.2012.05.001.
- 758 [40] Biro PA, Stamps JA. Are animal personality traits linked to life-history productivity?
759 *Trends Ecol Evol* 2008;23:361–8. doi:10.1016/j.tree.2008.04.003.
- 760 [41] Duckworth RA. Neuroendocrine mechanisms underlying behavioral stability: implications
761 for the evolutionary origin of personality. *Ann N Y Acad Sci* 2015:1–21.
762 doi:10.1111/nyas.12797.
- 763 [42] Cubaynes S, MacNulty DR, Stahler DR, Quimby KA, Smith DW, Coulson T. Density-
764 dependent intraspecific aggression regulates survival in northern Yellowstone wolves

765 (Canis lupus). J Anim Ecol 2014;83:1344–56. doi:10.1111/1365-2656.12238.

766 [43] Fryxell JM, Falls JB, Falls EA, Brooks RJ, Dix L, Strickland MA. Density dependence,
 767 prey dependence, and population dynamics of martens in Ontario. Ecology 1999;80:1311–
 768 21. doi:10.1890/0012-9658(1999)080[1311:DDPDAP]2.0.CO;2.

769 [44] Gorman ML, Trowbridge BJ. The Role of Odor in the Social Lives of Carnivores. In:
 770 Gittleman JL, editor. Carniv. Behav. Ecol. Evol., Boston, MA: Springer US; 1989, p. 57–
 771 88. doi:10.1007/978-1-4757-4716-4_3.

772 [45] Rafacz ML, Santymire RM. Using odor cues to elicit a behavioral and hormonal response
 773 in zoo-housed African wild dogs. Zoo Biol 2014;33:144–9. doi:10.1002/zoo.21107.

774 [46] Kimball BA, Mason JR, Blom FS, Depot PS, Street ED, Johnston JJ, et al. Development
 775 and Testing of Seven New Synthetic Coyote Attractants. J Agric Food Chem
 776 2000;2000:1892–7.

777 [47] Dantzer B, Newman AEM, Boonstra R, Palme R, Boutin S, Humphries MM, et al.
 778 Density triggers maternal hormones that increase adaptive offspring growth in a wild
 779 mammal. Science (80-) 2013;340:1215–7. doi:10.1126/science.1235765.

780 [48] Sih A, Cote J, Evans M, Fogarty S, Pruitt J. Ecological implications of behavioural
 781 syndromes. Ecol Lett 2012;15:278–89. doi:10.1111/j.1461-0248.2011.01731.x.

782 [49] Stein LR, Trapp RM, Bell AM. Do reproduction and parenting influence personality
 783 traits? Insights from threespine stickleback. Anim Behav 2016;112:247–54.
 784 doi:10.1016/j.anbehav.2015.12.002.

785 [50] Leuner B, Glasper ER, Gould E. Parenting and plasticity. Trends Neurosci 2010;33:465–
 786 73. doi:10.1016/j.tins.2010.07.003.

787 [51] Ziegler TE, Washabaugh KF, Snowdon CT. Responsiveness of expectant male cotton-top

788 tamarins, *Saguinus oedipus*, to mate's pregnancy. *Horm Behav* 2004;45:84–92.
789 doi:10.1016/j.yhbeh.2003.09.003.

790 [52] van Kesteren F, Paris M, Macdonald DW, Millar R, Argaw K, Johnson PJ, et al. The
791 physiology of cooperative breeding in a rare social canid; sex, suppression and
792 pseudopregnancy in female Ethiopian wolves. *Physiol Behav* 2013;122:39–45.
793 doi:10.1016/j.physbeh.2013.08.016.

794 [53] Clutton-Brock TH, Thavarajah NK, Fenkes M. The determinants of dominance
795 relationships among subordinate females in the cooperatively breeding meerkat.
796 *Behaviour* 2014;151:89–102. doi:10.1163/1568539X-00003124.

797 [54] Meylan S, Miles DB, Clobert J. Hormonally mediated maternal effects, individual strategy
798 and global change. *Philos Trans R Soc Lond B Biol Sci* 2012;367:1647–64.
799 doi:10.1098/rstb.2012.0020.

800 [55] Reynolds RM, Labad J, Buss C, Ghaemmaghami P, Räikkönen K. Transmitting biological
801 effects of stress in utero: Implications for mother and offspring.
802 *Psychoneuroendocrinology* 2013;38:1843–9. doi:10.1016/j.psyneuen.2013.05.018.

803 [56] Almond RE, Ziegler TE, Snowdon CT. Changes in prolactin and glucocorticoid levels in
804 cotton-top tamarin fathers during their mate's pregnancy: the effect of infants and paternal
805 experience. *Am J Primatol* 2008;70:560–5. doi:10.1002/ajp.20529.

806 [57] Sheriff MJ, Krebs CJ, Boonstra R. The ghosts of predators past: Population cycles and the
807 role of maternal programming under fluctuating predation risk. *Ecology* 2010;91:2983–
808 94. doi:10.1890/09-1108.1.

809 [58] Ziegler TE, Snowdon CT. Preparental hormone levels and parenting experience in male
810 cotton-top tamarins, *Saguinus oedipus*. *Horm Behav* 2000;38:159–67.

doi:10.1006/hbeh.2000.1617.

[59] Charpentier MJE, Van Horn RC, Altmann J, Alberts SC. Paternal effects on offspring fitness in a multimale primate society. *Proc Natl Acad Sci* 2008;105:1988–92. doi:10.1073/pnas.0711219105.

[60] Schweitzer C, Schwabl H, Baran NM, Adkins-Regan E. Pair disruption in female zebra finches: Consequences for offspring phenotype and sensitivity to a social stressor. *Anim Behav* 2014;90:195–204. doi:10.1016/j.anbehav.2014.01.022.

[61] Carlson DA, Gese EM. Reproductive biology of the coyote (*Canis latrans*): integration of mating behavior, reproductive hormones, and vaginal cytology. *J Mammal* 2008;89:654–64. doi:10.1644/06-MAMM-A-436R1.1.

[62] Carlson DA, Gese EM. Influence of exogenous gonadotropin-releasing hormone on seasonal reproductive behavior of the coyote (*Canis latrans*). *Theriogenology* 2009;72:773–83. doi:10.1016/j.theriogenology.2009.05.012.

[63] Carlson DA, Gese EM. Integrity of mating behaviors and seasonal reproduction in coyotes (*Canis latrans*) following treatment with estradiol benzoate. *Anim Reprod Sci* 2010;117:322–30. doi:10.1016/j.anireprosci.2009.05.008.

[64] Minter LJ, DeLiberto TJ. Seasonal variation in serum testosterone, testicular volume, and semen characteristics in the coyote (*Canis latrans*). *Theriogenology* 2008;69:946–52. doi:10.1016/j.theriogenology.2008.01.010.

[65] Gese EM. Response of neighboring coyotes (*Canis latrans*) to social disruption in an adjacent pack. *Can J Zool* 1998;76:1960–3. doi:10.1139/cjz-76-10-1960.

[66] Gese EM. Territorial defense by coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming: who, how, where, when, and why. *Can J Zool* 2001;79:980–7. doi:10.1139/cjz-

834 79-6-980.

835 [67] Gese EM, Ruff RL. Scent-marking by coyotes, *Canis latrans*: the influence of social and
836 ecological factors. *Anim Behav* 1997;54:1155–66. doi:10.1006/anbe.1997.0561.

837 [68] Darrow PA, Shivik JA. Bold, shy, and persistent: Variable coyote response to light and
838 sound stimuli. *Appl Anim Behav Sci* 2009;116:82–7.
839 doi:10.1016/j.applanim.2008.06.013.

840 [69] Mettler AE, Shivik JA. Dominance and neophobia in coyote (*Canis latrans*) breeding
841 pairs. *Appl Anim Behav Sci* 2007;102:85–94. doi:10.1016/j.applanim.2006.03.012.

842 [70] Young JK, Mahe M, Breck S. Evaluating behavioral syndromes in coyotes (*Canis latrans*).
843 *J Ethol* 2015;33:137–44. doi:10.1007/s10164-015-0422-z.

844 [71] Schell CJ, Young JK, Lonsdorf E V., Santymire RM. Anthropogenic and physiologically
845 induced stress responses in captive coyotes. *J Mammal* 2013;94:1131–40. doi:10.1644/13-
846 MAMM-A-001.1.

847 [72] Bekoff M, Wells MC. Behavioral Ecology of Coyotes: Social Organization, Rearing
848 Patterns, Space Use, and Resource Defense. *Z Tierpsychol* 1982;60:281–305.
849 doi:10.1111/j.1439-0310.1982.tb01087.x.

850 [73] Shivik JA, Wilson RR, Gilbert-Norton L. Will an artificial scent boundary prevent coyote
851 intrusion? *Wildl Soc Bull* 2011;35:494–7. doi:10.1002/wsb.68.

852 [74] Gilbert-Norton LB, Leaver LA, Shivik JA. The effect of randomly altering the time and
853 location of feeding on the behaviour of captive coyotes (*Canis latrans*). *Appl Anim Behav*
854 *Sci* 2009;120:179–85. doi:10.1016/j.applanim.2009.06.007.

855 [75] Fox RA, Millam JR. The effect of early environment on neophobia in orange-winged
856 Amazon parrots (*Amazona amazonica*). *Appl Anim Behav Sci* 2004;89:117–29.

doi:10.1016/j.applanim.2004.05.002.

[76] Pedersen V, Jeppesen LL. Effects of early handling on later behaviour and stress responses in the silver fox (*Vulpes vulpes*). *Appl Anim Behav Sci* 1990;26:383–93. doi:10.1016/0168-1591(90)90037-E.

[77] Gácsi M, Gyori B, Miklósi Á, Virányi Z, Kubinyi E, Topál J, et al. Species-specific differences and similarities in the behavior of hand-raised dog and wolf pups in social situations with humans. *Dev Psychobiol* 2005;47:111–22. doi:10.1002/dev.20082.

[78] Altmann J. *Observational Study of Behavior: Sampling Methods*. Behaviour 1974;49:227–66. doi:10.1163/156853974X00534.

[79] Guertin DA, Harestad AS, Ben-David M, Drouillard KG, Elliott JE. Fecal genotyping and contaminant analyses reveal variation in individual river otter exposure to localized persistent contaminants. *Environ Toxicol Chem* 2010;29:275–84. doi:10.1002/etc.53.

[80] Fuller G, Margulis SW, Santymire RM. The effectiveness of indigestible markers for identifying individual animal feces and their prevalence of use in North American zoos. *Zoo Biol* 2011;30:379–98. doi:10.1002/zoo.20339.

[81] Young JK, Glasscock SN, Shivik JA. Does Spatial Structure Persist Despite Resource and Population Changes? Effects of Experimental Manipulations on Coyotes. *J Mammal* 2008;89:1094–104. doi:10.1644/07-MAMM-A-198.1.

[82] Loeding E, Thomas J, Bernier D, Santymire RM. Using fecal hormonal and behavioral analyses to evaluate the introduction of two sable antelope at Lincoln Park Zoo. *J Appl Anim Welf Sci* 2011;14:220–46. doi:10.1080/10888705.2011.576968.

[83] Goymann W. On the use of non-invasive hormone research in uncontrolled, natural environments: The problem with sex, diet, metabolic rate and the individual. *Methods*

880 Ecol Evol 2012;3:757–65. doi:10.1111/j.2041-210X.2012.00203.x.

881 [84] Santymire RM, Armstrong DM. Development of a field-friendly technique for fecal
882 steroid extraction and storage using the African wild dog (*Lycaon pictus*). Zoo Biol
883 2010;29:289–302. doi:10.1002/zoo.20248.

884 [85] Armstrong DM, Santymire RM. Hormonal and Behavioral Variation in Pied Tamarins
885 Housed in Different Management Conditions. Zoo Biol 2013;32:299–306.
886 doi:10.1002/zoo.21023.

887 [86] Rafacz ML, Margulis S, Santymire RM. Hormonal Correlates of Paternal Care
888 Differences in the Hylobatidae. Am J Primatol 2011;74:247–60. doi:10.1002/ajp.21994.

889 [87] Nakagawa S, Schielzeth H. Repeatability for Gaussian and non-Gaussian data: A practical
890 guide for biologists. Biol Rev 2010;85:935–56. doi:10.1111/j.1469-185X.2010.00141.x.

891 [88] Stein LR, Bell AM. Consistent individual differences in paternal behavior: a field study of
892 threespined stickleback. Behav Ecol Sociobiol 2015;58:45–52. doi:10.1007/s00265-014-
893 1835-3.

894 [89] Betini GS, Norris DR. The relationship between personality and plasticity in tree swallow
895 aggression and the consequences for reproductive success. Anim Behav 2012;83:137–43.
896 doi:10.1016/j.anbehav.2011.10.018.

897 [90] Carter A, Goldizen A, Heinsohn R. Personality and plasticity: temporal behavioural
898 reaction norms in a lizard, the Namibian rock agama. Anim Behav 2012;84:471–7.
899 doi:10.1016/j.anbehav.2012.06.001.

900 [91] R Core Team. R: A language and environment for statistical computing. 2016.

901 [92] Bates D, Maechler M, Bolker B. lme4: Linear mixed-effects models using S4 classes
902 2012:R package version 0.999999–0.

- 903 [93] Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest: Test for random and fixed
904 effects for linear mixed models (lmer objects of lme4 package) 2013:R package version
905 1.2–0.
- 906 [94] Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. nlme: Linear and Nonlinear
907 Mixed Effects Models 2016:R package version 3.1–126.
- 908 [95] Wei T. corrplot: Visualization of a correlation matrix 2013:R package version 0.73.
- 909 [96] Sillero-Zubiri C, Macdonald DW. Scent-marking and territorial behaviour of Ethiopian
910 wolves *Canis simensis*. *J Zool* 1998;245:351–61. doi:10.1017/S0952836998007134.
- 911 [97] Gese EM, Ruff RL. Howling by coyotes (*Canis latrans*): variation among social classes,
912 seasons, and pack sizes. *Can J Zool* 1998;76:1037–43. doi:10.1139/cjz-76-6-1037.
- 913 [98] Gehrt SD, Riley SPD. Coyotes (*Canis latrans*). In: Gehrt SD, Riley SPD, Cypher BL,
914 editors. *Urban Carniv. Ecol. conflict, Conserv.*, Baltimore: JHU Press; 2010, p. 79–95.
- 915 [99] Van Kesteren F, Sillero-Zubiri C, Millar R, Argaw K, Macdonald DW, Paris M. Sex,
916 stress and social status: Patterns in fecal testosterone and glucocorticoid metabolites in
917 male Ethiopian wolves. *Gen Comp Endocrinol* 2012;179:30–7.
918 doi:10.1016/j.ygcen.2012.07.016.
- 919 [100] Amoss Jr. MS, Hodges CM. Selected Parameters of the Reproductive Physiology and
920 Endocrinology of Coyotes. *Coyotes Southwest A Compend. Our Knowl.*, Lincoln:
921 University of Nebraska; 1995, p. 12–6.
- 922 [101] Messier F, Barrette C. The social system of the coyote (*Canis latrans*) in a forested habitat.
923 *Can J Zool* 1982;60:1743–53. doi:10.1139/z82-227.
- 924 [102] Concannon PW. Reproductive cycles of the domestic bitch. *Anim Reprod Sci*
925 2011;124:200–10. doi:10.1016/j.anireprosci.2010.08.028.

- 926 [103] Nguyen N, Gesquiere LR, Wango EO, Alberts SC, Altmann J. Late pregnancy
927 glucocorticoid levels predict responsiveness in wild baboon mothers (*Papio*
928 *cynocephalus*). *Anim Behav* 2008;75:1747–56. doi:10.1016/j.anbehav.2007.09.035.
- 929 [104] Scarlata CD, Elias BA, Godwin JR, Powell RA, Shepherdson D, Shipley LA, et al.
930 Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits
931 (*Brachylagus idahoensis*). *Gen Comp Endocrinol* 2011;171:373–80.
932 doi:10.1016/j.ygcen.2011.03.002.
- 933 [105] Ziegler TE, Prudom SL, Zahed SR. Variations in male parenting behavior and physiology
934 in the common marmoset. *Am J Hum Biol* 2009;21:739–44. doi:10.1002/ajhb.20920.
- 935 [106] Wingfield JC, Hegner RE, Duffy AM, Ball GF. The “Challenge Hypothesis”: Theoretical
936 Implications for Patterns of Testosterone Secretion, Mating Systems, and Breeding
937 Strategies. *Am Nat* 1990;829–46. doi:10.2307/2678832.
- 938 [107] Goymann W, Landys MM, Wingfield JC. Distinguishing seasonal androgen responses
939 from male-male androgen responsiveness-Revisiting the Challenge Hypothesis. *Horm*
940 *Behav* 2007;51:463–76. doi:10.1016/j.yhbeh.2007.01.007.
- 941 [108] Both C, Dingemanse NJ, Drent PJ, Tinbergen JM. Pairs of extreme avian personalities
942 have highest reproductive success. *J Anim Ecol* 2005;74:667–74. doi:10.1111/j.1365-
943 2656.2005.00962.x.
- 944 [109] Schuett W, Dall SRX, Royle NJ. Pairs of zebra finches with similar “personalities” make
945 better parents. *Anim Behav* 2011;81:609–18. doi:10.1016/j.anbehav.2010.12.006.
- 946 [110] Wynne-Edwards KE. Hormonal changes in mammalian fathers. *Horm Behav*
947 2001;40:139–45. doi:10.1006/hbeh.2001.1699.
- 948 [111] Dloniak SM, French JA, Holekamp KE. Rank-related maternal effects of androgens on

949 behaviour in wild spotted hyaenas. *Nature* 2006;440:1190–3. doi:10.1038/nature04540.

950 [112] Räsänen K, Kruuk LEB. Maternal effects and evolution at ecological time-scales. *Funct*

951 *Ecol* 2007;21:408–21. doi:10.1111/j.1365-2435.2007.01246.x.

952 [113] Marshall DJ, Uller T. When is a maternal effect adaptive? *Oikos* 2007;116:1957–63.

953 doi:10.1111/j.2007.0030-1299.16203.x.

954 [114] Uller T. Developmental plasticity and the evolution of parental effects. *Trends Ecol Evol*

955 2008;23:432–8. doi:10.1016/j.tree.2008.04.005.

956

Tables – Schell et al. 2016

Ms. Ref. No: PHB-D-16-00139R2

Title: Olfactory attractants and parity affect prenatal androgens and territoriality of coyote breeding pairs

Table 1

Behavioral ethogram used during coyote odor attractants testing.

Behavior	Description
Aggression	Teeth baring, growling, and/or physical confrontation directed toward pairmate
Ground scratching	Digs and kicks down and backward; often follows urination
Urination	Discharges urine
Rubs	Descends head-first toward the ground and rakes, undulates body across the floor
Site sniffs	Individual directly placed its nose toward the stimulus site for ≥ 3 seconds
Site visits	Individual gets within ≤ 1 meter from stimulus site
Site time (sec)	Total time spent at the stimulus site
Latency to visit (sec)	Length of time before individual gets ≤ 1 meter from the stimulus site

Table 2

Adjusted repeatabilities (R_{adj}) for marking and investigative behaviors across the odor attractant tests, as well as fecal glucocorticoid (FGMs) and androgen metabolites (FAMs) during gestation for females and males. Confidence intervals not pressed against zero (bolded) are considered statistically significant.

Behavior/Hormone	R_{adj}	95% CI
Females		
Aggression ^a	0.289	0.166–0.820
Ground scratching ^a	0.296	0.282–1.161
Urination ^a	0.320	0.238–0.929
Rubs ^a	0.130	0.000–1.291
Site sniffs ^a	0.088	0.000–1.026
Site visits ^a	0.357	0.374–1.320
Site time (sec) ^b	0.140	0.000–0.162
Latency to visit (sec) ^b	0.144	0.007–0.349
FGMs ^b	0.561	0.434–0.720
FAMs ^b	0.004	0.000–0.039
Males		
Aggression ^a	0.193	0.000–0.461
Ground scratching ^a	0.544	0.539–1.668
Urination ^a	0.173	0.056–0.690
Rubs ^a	0.049	0.000–0.865
Site sniffs ^a	0.000	0.000–0.577
Site visits ^a	0.261	0.245–1.097
Site time (sec) ^b	0.027	0.000–0.179
Latency to visit (sec) ^b	0.224	0.198–0.434
FGMs ^b	0.300	0.043–0.524
FAMs ^b	0.204	0.060–0.468

Repeatabilities for count data were quantified by comparing between-individual variance (σ^2_{α}) and residual variance components (σ^2_{ϵ}) of generalized linear mixed models (GLMMs) with a Poisson error distribution and square root link function (a); Repeatabilities for Gaussian data were quantified using the rpt.remLMM.adj function from the 'rpt.R' package (b). Test number and test order were controlled for in estimating repeatabilities, and all behavioral data are reported as per 70 minutes of recorded video.

Table 3

Spearman rank correlations among identical male and female behaviors and hormonal best linear unbiased predictors (BLUPs) during odor cue provisioning (i.e. mid-gestation; $N = 16$). Bolded values indicate statistical significance.

Trait	r_s	P
Aggression	0.39	0.135
Ground scratching	0.35	0.184
Urination	0.79	<0.001
Body rubs	0.85	<0.001
Site sniffs	0.84	<0.001
Visits	0.76	<0.001
Site Time	0.88	<0.001
Latency to visit	0.88	<0.001
BLUP Cortisol	-0.01	0.971
BLUP Testosterone	0.63	0.009

All behavioral data are reported as per 70 minutes of recorded video.

Fig. 1. Schematic depicting three of the 0.1 ha clover pens within a single housing block at the National Wildlife Research Center, Predator Research Facility (Millville, UT, USA), including the location of remote cameras during testing, and placement of the stimuli (odor versus water). Each clover pen contained a single breeding pair.

Fig. 2. Differences in marking and investigative behaviors (mean $\pm$ SE) among odor and control females and males (A and B), and first-time (2011) versus experienced (2013) breeders (C and D). Asterisks indicate statistical differences (* P <0.05, ** P <0.01, *** P <0.001). All behavioral count data are reported as per 70 minutes of recorded video.

Marking and Investigative Behaviors

Fig. 3. Mean ($\pm$ SE) time spent ≤ 1 meter within the odor- (treatment) or water-treated (control) test site. Asterisks indicate differences between treatment groups within each test date (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Contrasts in subscript letters indicate statistical differences within treatment groups across test dates. Data are pooled among the 2011 and 2013 seasons. All behavioral data are reported as per 70 minutes of recorded video.

Fig. 4. Fecal glucocorticoid and androgen metabolite concentrations during gestation before parturition. Uppercase and lowercase superscripts correspond to within-maternal and within-paternal metabolites, respectively. Contrasts between uppercase letters and between lowercase letters indicate a statistical difference within each sex. Figures pool data from both treatment and control animals.

Fig. 5. Fecal glucocorticoid (A, B) and androgen metabolite concentrations (C, D) before (pre-test), during (test), and after (post-test) female (A, C) and male coyotes (B, D) received odor attractants or water as stimuli. Data represent means $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and bars denote differences across experimental (i.e. testing) periods.