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Effects of population density on corticosterone levels of prairie voles in the field

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ABSTRACT

High population density is often associated with increased levels of stress-related hormones, such as corticosterone (CORT). Prairie voles (*Microtus ochrogaster*) are a socially monogamous species known for their large population density fluctuations in the wild. Although CORT influences the social behavior of prairie voles in the lab, the effect of population density on CORT has not previously been quantified in this species in the field. We validated a non-invasive hormone assay for measuring CORT metabolites in prairie vole feces. We then used semi-natural enclosures to experimentally manipulate population density, and measured density effects on male space use and fecal CORT levels. Our enclosures generated patterns of space use and social interaction that were consistent with previous prairie vole field studies. Contrary to the positive relationship between CORT and density typical of other taxa, we found that lower population densities (80 animals/ha) produced higher fecal CORT than higher densities (240/ha). Combined with prior work in the lab and field, the data suggest that high prairie vole population densities indicate favorable environments, perhaps through reduced predation risk. Lastly, we found that field animals had lower fecal CORT levels than laboratory-living animals. The data emphasize the usefulness of prairie voles as models for integrating ecological, evolutionary, and mechanistic questions in social behavior.

1. Introduction

Glucocorticoids function to mobilize resources and channel them to meet environmental demands, ranging from daily activity rhythms to responses to threats and stressors (Sapolsky, 2002). Many natural stressors are profoundly influenced by social factors: conspecifics may compete for territories, commit infanticide, or fuel the growth of predator populations; they may also facilitate food finding, share parental care, or satiate predators with their abundance (Adkins-Regan, 2005; Becker et al., 2002; Solomon, 1949; Wolff and Sherman, 2007). Hence, the social environment has dynamic and complex consequences for individual fitness. It seems fitting that much current attention is focused on the intersection of stress steroids, environmental variability, and social behavior (Creel et al., 2013). In the current study, we test the hypothesis that high population densities promote increases in agonistic encounters and elevate glucocorticoids in the socially monogamous prairie vole, *Microtus ochrogaster*.

Population density cycles are a well-investigated dimension of environmental variation. With respect to glucocorticoids, historical perspectives emphasize how density-associated predation or resource depletion can promote stress and inhibit reproduction, leading to a reduction in population growth (Christian, 1950; Wolff and Sherman, 2007). More recently, researchers have examined how predator abundance or other density-related stressors influence the developing phenotypes of young, causing cycles in stress reactivity that can alter ecological processes as offspring become adults (Breuner, 2008; Love et al., 2013). While population cycles have been examined extensively, their interactions with stress and social behavior are still not fully understood (Creel et al., 2013), and the subject remains a remarkably fruitful area of work.

Because glucocorticoids mobilize resources to deal with unfavorable conditions and limit reproduction, the effects of population density on glucocorticoids often mirror how density contributes to population growth (Christian, 1950; Creel et al., 2013). In many

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taxa, high population density depletes resources, drives agonistic interactions, and/or promotes predation, a set of stressors that also promote glucocorticoid secretion and impair reproduction (Boonstra and Boag, 1992; Christian, 1950; Creel et al., 2013). Perhaps not surprisingly, high density is often associated with high glucocorticoid secretion (Boonstra and Boag, 1992; Christian, 1961; Creel et al., 2013; Wang et al., 2009). Alternatively, environments may actually improve with density (Allee, 1931). For example, higher densities can lower the per-capita risk of predation (Parrish and Edelstein-Keshet, 1999), and facilitate mate-finding (Crowley et al., 1991; Kokko and Rankin, 2006). When the social system includes complex social groups with dominance hierarchies, the relationship between glucocorticoids and population density becomes still more complicated (Creel et al., 2013; Sapolsky, 2005; Wingfield and Sapolsky, 2003), with glucocorticoids affecting individuals unequally depending on dominance status and the stability of social hierarchies.

To investigate the relationship between population density and glucocorticoids, we study the socially monogamous prairie vole. In the wild, most male and female prairie voles form enduring pair-bonds and participate in the rearing of young (Getz and Carter, 1996). Studies spanning decades reveal that their population density undergoes wide annual fluctuations (Getz et al., 1993, 2006, 1990), with heavy spring-summer mortality and low densities (as low as 11 animals/hectare; Getz et al., 1993), contrasted with autumn–winter population spikes (as high as 600 animals/hectare; Getz et al., 1993). The time from conception to adulthood is approximately 9 weeks (Mateo et al., 1994; Stehn and Richmond, 1975); depending on the time of year young are born, offspring may grow to live in similar densities or wildly different densities, providing an interesting challenge to the development of the young. The combination of this well-characterized natural history and the extensive use of prairie voles as laboratory subjects in social neuroscience (Carter et al., 1995; Winslow et al., 1993; Young et al., 1999; Young and Wang, 2004) make them an especially valuable model for the integrative study of stress, sociality, and ecology.

The principal glucocorticoid secreted by prairie voles is corticosterone (CORT). In the lab, acute stressors and exogenous CORT facilitate social bonding in males (Blondel Thesis 2013; DeVries et al., 1996), but does not influence bonding in females (DeVries et al., 1996). Interestingly, stressed males also exhibit increased levels of paternal care (Bales et al., 2006). In several species, parental care or developmental CORT shapes the stress reactivity and social behavior of offspring as adults (Meaney, 2001). Among prairie voles, developmental CORT exposure can alter subsequent parental care and affiliation (Roberts et al., 1996).

CORT levels in the lab are traditionally quantified by invasive procedures such as retro-orbital bleeding (DeVries et al., 1995; Taymans et al., 1997), which are less appropriate for measuring CORT in the field than non-invasive fecal hormone assays (Good et al., 2003). Fecal hormone glucocorticoid assays have been validated successfully in many other mammalian taxa (Crino et al., 2010; Goymann et al., 1999; Harper and Austad, 2000; Mateo and Cavigelli, 2005; Monfort et al., 1997; Wasser et al., 1995), and are currently considered the most reliable, the most practical, and the least invasive method of measuring chronic stress (Dantzer et al., 2014; Sheriff et al., 2011). The fecal hormone assay method has considerable advantages over traditional bleeding methods (all reviewed in Dantzer et al., 2014; Goymann, 2005; Harper and Austad, 2000; Palme et al., 2005; Sheriff et al., 2011): beyond its non-invasive nature and more straightforward collection procedure, each sample also contains an averaged hormone level covering the previous several hours. Hence, measures are more representative of an individual's general hormone exposure than the point sampling of bleeding methods.

In the current study, we begin by validating a fecal hormone assay for CORT in prairie voles, demonstrating that our assay is precise and replicable, and that it can detect acute rises in CORT induced by an arbitrary stressor. We next manipulate population density in semi-natural enclosures, to ask whether density causes changes in social interaction and CORT titers in the field. We focus on males because CORT levels are known to influence both social bonding and parental care of males in the lab, and because limited resources prevented us from looking at both sexes. By using semi-natural enclosures, we allowed social interactions while controlling for factors such as predation, food and water resources (Creel et al., 2013).

2. Materials and methods

2.1. Experimental design summary

We first validated a fecal CORT metabolite hormone assay using a test for assay linearity, followed by a swim challenge to confirm that the assay can detect fecal CORT responses to a standardized acute stressor. Having validated our fecal CORT assay, we conducted a field experiment using a different set of animals. We measured fecal CORT metabolites for these animals in the lab, then placed them in semi-natural enclosures in the field for 19–24 days at one of two densities: low-density (LD) = 80 animals/ha, $n = 12$ males and 12 females per trial, and high-density (HD) = 240 animals/ha, $n = 12$ males and 12 females per trial. In these enclosures we measured individual space use, male–female pairing patterns, and CORT metabolite levels over two separate replicate trials, a "summer trial" and a "fall trial". Animals were briefly trapped and feces were collected during the trial and again at the end of the trial; thus, we collected feces at three time points: pre-field (lab), midway (days 8–13), and at the end of the field trial (days 19–24).

2.2. Subject animals

Study animals were descendants of wild-caught voles from Illinois and Tennessee. Subjects ranged from F6 to F10 generations from the original wild-caught voles. All animals were lab born and raised, and were weaned at 21 days. Upon weaning, voles were placed in same-sex sibling groups. Housing conditions have been known to affect CORT levels, with solo-housed prairie voles exhibiting higher levels of CORT (Ruscio et al., 2007); therefore, all animals used in this study were socially housed in same-sex sib groups of 2–5. Prairie voles reach sexual maturity at 45 days (Mateo et al., 1994); only animals 45 days or older were used as subjects. All procedures were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) in accordance with local, state, and federal regulations to minimize pain and discomfort. Our IACUC protocol number was D289.

2.3. Fecal CORT metabolite extraction and radioimmunoassays

Our sample collection, extraction, and validation protocols included methods established by Harper and Austad (2000), Mateo and Cavigelli (2005) and Crino et al. (2010). For all fecal collection, we collected up to a maximum of five pellets per animal produced during a 15-min defecation period; any feces contaminated with urine were excluded (after Cavigelli et al., 2005). Pellets were stored in a −20 °C freezer.

Feces were freeze-dried and weighed. To minimize variation in weight prior to extraction and radioimmunoassay (RIA), we combined dry pellets, collected at the same time and from the same individual, to a weight of 15–20 mg. If all combined pellets from a given defecation period were less than 15 mg, we still used them;