Proximate and ultimate causes of personality in a non-aggressive, African ground squirrel

By

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Abstract

The study of consistent individual differences, or animal personality, has changed the way behavioral ecologists think about individual behavior. However, evidence regarding how personality traits are maintained, both proximately and ultimately, is not consistent and generally lacking in free-living populations. I examined 1) which extrinsic and intrinsic factors are related to personality traits and 2) if personality traits are maintained by differential survival and reproductive success in females from a highly social African ground squirrel, the Cape ground squirrel (*Xerus inauris*). I measured personality using three standard tests: open-field test for exploration, flight initiation distance tests for boldness, and handling indices for docility. I found that boldness, docility and two aspects of exploration, activity and a fecal index, were repeatable in Cape ground squirrels. Several extrinsic factors, such as time of day and date, affected the outcomes of personality tests. Further, I found no evidence that long-term cortisol isolated from hair and personality were related. Better body condition was correlated with higher activity and a lower fecal index. I found no relationship between personality and measures of reproductive success and survival. Overall, more research is needed to determine how personality traits arise and are maintained in a non-aggressive species like Cape ground squirrels.

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Chapter 1 — Thesis Introduction

In behavioral ecology, proximate causes attempt to explain how immediate mechanisms influence behavior whereas ultimate explanations address why behaviors exist in an evolutionary context (Alcock 2009). Understanding both levels of causation of behavior is key to painting a complete picture of how behaviors arise and are maintained. Only fairly recently have researchers recognized that consistent individual differences in behaviors across context and time can have influence on important life process such as habitat selection (Martin & Réale 2008a; Carrete & Tella 2010), dispersal (Fraser et al. 2001; Dingemanse et al. 2003; Krackow 2003), reproductive success (Dingemanse et al. 2004; Both et al. 2005; Cote et al. 2008; Smith & Blumstein 2008), and survival (Smith & Blumstein 2008). Research on animal personality (Sih et al. 2004a; Réale et al. 2007) has demonstrated that individuals do not behave optimally in every situation but instead are constrained, both evolutionarily and physiologically, in their responses (Dall et al. 2004; Dingemanse & Réale 2005).

Despite a myriad of recent work, the mechanisms that maintain personality are not fully understood. The Hypothalamus-Pituitary-Adrenal axis has been hypothesized as a proximate mechanism through which consistent individual differences in behavior are mediated (Koolhaas et al. 1999). Differential activation of stress pathways and release of hormones such as cortisol may cause individuals to be relatively more proactive, that is, bolder, more aggressive, and explore novel spaces faster than their reactive counterparts (Koolhaas et al. 1999). However, empirical research suggests that the cortisol response may be responsible for differences in some traits but not others

(Van Reenen et al. 2005; Koolhaas et al. 2010; Costantini et al. 2012; Clary et al. 2014). Additionally, few studies have examined how long-term cortisol levels, which are accumulated over weeks to months (Pragst & Balikova 2006), are linked to personality traits (but see Martin & Réale 2008b).

Alternatively, through feedback loops, personality traits may be maintained by an individual's state (i.e., anything that affects the costs and benefits of behavior; reviewed in Sih et al. 2015). Specifically, the state-dependent safety hypothesis postulates a positive feedback loop that predicts that individuals with more assets tend to be proactive and thus maintain their assets (McElreath et al. 2007; Sih & Bell 2008; Luttbeg & Sih 2010). However, recent work in Belding's ground squirrels (*Urocitellus beldingi*) has not supported this hypothesis but instead found support for a negative feedback loop between personality and condition (Dosmann et al. 2014). More work is needed in this field of study to determine how personality is influenced by such state variables.

Additionally, the life history trade-off hypothesis predicts that personality traits are ultimately preserved because they correlate with differences in life history strategies (Wolf et al. 2007). Proactive individuals are expected to sacrifice survival in favor of reproduction, whereas a reactive strategy favors survival (Koolhaas et al. 1999, 2010). When examined in free-living populations, however, support for this hypothesis has been mixed with studies finding relationships between personality and some life-history traits, but not others (Boon et al. 2007, 2008; Réale et al. 2009; Bijleveld et al. 2014; Hall et al. 2015; Haage et al. 2017).

In this thesis, I examined both proximate and ultimate causes of variation in personality in Cape ground squirrels (*Xerus inauris*). Cape ground squirrels are cooperative breeders that live on the open, arid plains of southern Africa (Waterman 1995). Ground squirrels, in general, make great candidates for studying behavior, as they are often diurnal and sedentary. Because of their small body size, they are also relatively easy to trap, handle and submit to personality tests. Cape ground squirrels are unique among traditionally studied sciurid species due to their highly social behavior (Waterman 1995; Pettitt et al. 2008). Furthermore, females demonstrate differential reproduction, with 43% of females producing no offspring in a year when 13% of individuals raised multiple litters (Waterman 1996). Despite this reproductive skew, female Cape ground squirrels exhibit very low levels of aggressive interactions and lack a measurable dominance hierarchy (Waterman 1995). Considering that research on personality traits has focused on species with established dominance hierarchies and aggression (Armitage 1991; Festa-Bianchet 1991; Slagsvold 1993), Cape ground squirrels offer a distinctive perspective in the study of how personality traits operate in what appears to be an egalitarian species.

I examined three commonly studied personality traits in Cape ground squirrels: exploration, defined as an animal's response to a novel situation (Réale et al. 2007) and measured using an open-field test (Walsh & Cummins 1976); boldness, defined as an animal's response to any non-novel potentially dangerous situation (Réale et al. 2007) and measured using a flight initiation distance test (Blumstein 2003); and docility, defined as a an animal's response to handling and measured using a handling index

(Réale et al. 2000). In chapter 2, I report on a study which tested the hypothesis that long-term cortisol and body condition were related to personality traits. I also examined which environmental factors affect the outcomes of personality tests. In chapter 3, I examined the life history trade-off hypothesis as it applies to animal personality. I quantified survival and reproductive success and compared it to personality traits.

Overall, my thesis examines the ultimate and proximate causes of personality in a population of adult female Cape ground squirrels. This research is important to understand how personality traits may be modulated through physiological mechanisms as well as how they are maintained through evolution.

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Chapter 2 — Extrinsic and intrinsic factors that affect personality in female Cape ground squirrels (*Xerus inauris*)

Abstract

Despite a growing body of work in the study of consistent individual differences, there is currently no consensus on which factors may be affecting the expression of personality, especially in free-living populations. The cortisol response is hypothesized to have a role in modulating behaviors, yet field studies have produced mixed results. Body condition may also play a role in the expression of traits as explained under the *state-dependent safety* hypothesis. Finally, extrinsic factors, such as weather conditions, may play a role in the expression of personality by influencing behavior. Here, we submitted adult female Cape ground squirrels to standard personality tests to examine extrinsic and intrinsic factors that affect boldness, exploration and docility. We compared these scores to measures of body condition and cortisol extracted from hair. We found that some environmental conditions impact the results of personality tests. Additionally, body condition was related to several traits. However, we did not detect a relationship between long-term cortisol and personality traits. This study highlights the role that extrinsic factors may play in influencing the outcomes of personality tests but found no support for long-term HPA-activity as measured by cortisol levels as an intrinsic mechanism that mediates the expression of traits.

Introduction

Personality traits, or consistent individual differences in behavior across time and/or context (*sensu* Sih et al. 2004), have been identified in an increasing number of species in a wide range of taxonomic groups (reviewed in Gosling 2001). The personality paradigm has changed the way ecologists examine behavior; differences among individuals are not simply regarded as non-adaptive variation around an ideal mean (Dall et al. 2004), but may instead be distinct tactics that are shaped by natural selection. Importantly, personality has been demonstrated to be at least partially heritable (Drent et al. 2003; Petelle et al. 2015) and to have important effects on factors such as habitat selection (Martin & Réale 2008a; Carrete & Tella 2010), dispersal (Fraser et al. 2001; Dingemanse et al. 2003; Krackow 2003), reproductive success (Dingemanse et al. 2004; Both et al. 2005; Cote et al. 2008; Smith & Blumstein 2008), and survival (Smith & Blumstein 2008). Despite a growing interest in this field, there is no current consensus on which extrinsic and intrinsic processes regulate the expression of personality traits.

The Hypothalamus-Pituitary-Adrenal (HPA; Sherwood et al. 2013) axis is an important mechanism through which vertebrates respond to changes in their environment and has been identified as a candidate system through which behavioral differences may be modulated. When a stressor is encountered, the axis is stimulated, culminating in the release of glucocorticoids (cortisol and corticosterone) into the bloodstream, which facilitates the mobilization of energy stores and other physiological changes. Koolhaas et al. (1999) described the interaction between stress and personality by identifying two distinct suites of behavioral and physiological traits, or coping styles; ‘proactive’ individuals and ‘reactive’ individuals. Generally speaking, proactive individuals tend to have lower HPA activity and reactivity and are bolder, more aggressive, and explore novel spaces faster than their reactive counterparts (Koolhaas et al. 1999). Laboratory studies have further validated this hypothesis by demonstrating that lines selected for certain personality traits or behavioral syndromes differ in both baseline cortisol and stress reactivity (Carere et al. 2003; Veenema et al. 2003; Stöwe et al. 2010; van Oers et al. 2011).

However, recent empirical research reveals considerable variation in the role cortisol plays in personality differences, particularly in wild populations (Costantini et al. 2012). For instance, in Belding’s ground squirrels (*Urocitellus beldingi*), no evidence links cortisol to interindividual differences in activity, response to restraint or exploration. Indeed, some researchers have begun to suggest that the cortisol response may be responsible for differences in some traits but not others (Van Reenen et al. 2005; Koolhaas et al. 2010; Clary et al. 2014; Voellmy et al. 2014). For instance, Van Reenen et al. (2005) found that the cortisol response of Holstein calves (*Bos taurus*) was related to fearfulness but not locomotion in a novel object and open-field test.

Body condition is also hypothesized to be inextricably linked to personality. The “state-dependent safety hypothesis” postulates a positive feedback mechanism whereby individuals in a relatively better state (e.g. larger size, more energy reserves, or better condition) can be more proactive (aggressive, bold, or exploratory), thus maintaining their better condition (McElreath et al. 2007; Sih & Bell 2008; Luttbeg & Sih

2010). This state is reinforced if individuals in better body condition gain an advantage by being less susceptible to predation or by outcompeting lower state individuals. Alternatively, there are two hypothesized negative feedback mechanisms that relate body condition to personality; "asset protection" wherein individuals with assets are more reactive in order to protect said assets (Clark 1994) and "starvation avoidance" where a low enough state requires certain behaviors (i.e. proactivity) to avoid starving to death (Luttbeg & Sih 2010).

Extrinsic factors may also affect the expression of personality. By its very definition, (i.e. repeatable across context) personality is expected to be robust to environmental changes (Dingemanse & Wolf 2010). However, variables such as temperature, wind, time of day, precipitation and human activity are known to affect commonly measured behavioral traits such as vigilance and activity (Betts 1976; Caraco 1979; Hogstad 1988; McDonough & Loughry 1995; Pravosudov & Grubb, Jr. 1995, 1998; Dyck & Baydack 2004; Hayes & Huntly 2005; Mausbach et al. 2017). In field studies, where environmental conditions are not easily controlled and may vary widely among tests, these factors are considered important to include in analysis to control for behavioral plasticity as a result of changing conditions (Dingemanse et al. 2009; Pinter-Wollman et al. 2012). Indeed, Petelle et al. (2013) found a seasonal effect on docility in yellow-bellied marmots (*Marmota flaviventris*) and multiple studies on birds have shown that weather conditions can affect boldness (Hilton et al. 1999; Couchoux & Cresswell 2012).

We examined extrinsic and intrinsic factors that affect personality by performing a comprehensive study of consistent individual differences on a free-living population of Cape ground squirrels (*Xerus inauris*). Cape ground squirrels are highly social rodents found in arid savannah regions throughout southern Africa (Waterman 1995). Because they are fairly sedentary and diurnal, ground squirrels make an excellent candidate species for behavioral studies. Indeed, personality traits have been examined in a number of North American sciurids (Armitage 1986; Coss & Biardi 1997; Clary et al. 2014; Dosmann et al. 2014). Cape ground squirrels are unique among traditionally studied species because they are active year-round and are a sub-tropical species (Herzig-Straschil 1978). They are also highly social, living in matrilineal kin groups consisting of 1-6 adult females and up to 9 subadults of either sex (Waterman 1995; Pettitt et al. 2008), yet they display little aggression and have no detectable dominance hierarchy (Waterman 1995).

We first determined if personality traits existed in Cape ground squirrels by measuring three commonly identified personality traits: exploration, defined as an animal's response to a novel situation (Réale et al. 2007) and measured by an open-field test (Walsh & Cummins 1976); boldness, defined as an animal's response to any non-novel potentially dangerous situation (Réale et al. 2007) and measured by a flight initiation distance test (Blumstein 2003); and docility, defined as an animal's response to handling and measured by a handling index (Réale et al. 2000). We predicted that each trait would be repeatable across multiple trials and, due to the robustness of traits, environmental (extrinsic) variables would have little effect on the measured indices of personality. Finally, we examined whether body condition or hair cortisol concentration were related to personality traits. Following the state-dependent safety hypothesis, we predicted that individuals who were more proactive (i.e. more bold, more exploratory, less docile) would be in better body condition. We also predicted that proactive individuals would have lower levels of long-term cortisol isolated from their hair.

Methods

Study Site

We conducted our study at the SA Lombard Nature Reserve (27°35’S, 25°35’E) near Bloemhof, South Africa. The reserve is characterized by open *Cymbopogon*–*Themeda* grassland with small patches of bush (van Zyl 1965). All experiments were conducted during the austral winter (May-September 2014), which coincides with the dry season. Although squirrels reproduce year-round, the winter represents their peak breeding season (Herzig-Straschil et al. 1991; Waterman 1996). Squirrels at this site have been monitored since 2002. The study site consists of three distinct areas where female squirrels are primarily trapped: the house, the floodplain and the pan. Although not considered separate populations, these areas have similar vegetation (van Zyl 1965) but differ in levels of human contact; humans rarely go into the pan or floodplain areas but move regularly through the house area where there are permanent homes and other buildings (Unck et al. 2009). Predators also occur more frequently in the pan and floodplain and less frequently in the house area (Unck et al. 2009). Previous research has also found that squirrels in the floodplain area invest more time in vigilance behavior then those in the house area (Unck et al. 2009).

Trapping and Handling

We trapped squirrels using live traps (Tomahawk Live Trap Inc., Tomahawk, WI; 15 X 15 X 50 cm) baited with peanut butter and birdseed (Thokomon Foods SA LTD, Pretoria, SA; Kinko Birdfeed, D. Kingsbury, Johannesburg, SA). During handling, individuals were permanently marked using PIT tags inserted under the skin using a large gauge syringe (FECAVA, 125Khz, AVID Identification Systems Inc., Norco, CA). We also used freeze spray (Quick Freeze; Miller Stephenson Chemical Co., Danbury, CT; Rood & Nellis 1980) to brand individuals in the event of PIT tag failure. Additionally, we temporarily marked their dorsal region with black hair dye for identification during behavioral observations (Rodol D dye; Lowenstein and Sons, Inc., New York, NY; Melchior & Iwen 1965). Using trapping data, we determined the age of females either from the year of birth of marked individuals or by examining the state of the nipples; subadults have small, inconspicuous nipples, whereas adults have long, swollen nipples (Waterman 1995). Finally, we weighed individuals using a spring scale (Pesola AG, Baar, Switzerland) and took spine measurements from the base of the neck to the base of the tail using a measuring tape. We regressed body mass on spine length, and the residual for each individual was used as an estimate of body condition (Schulte-Hostedde et al. 2005).

Personality Tests

All personality tests were performed on previously marked adult females. Open-field (OF) tests were performed immediately after approaching a trapped squirrel to measure exploration/activity (Walsh & Cummins 1976). Due to logistical constraints, we were unable to standardize the amount of time individuals spent in the trap prior to testing. Adult females who had not been handled in the previous 24 hours were identified by their dye mark and introduced into the arena using a door on the side. The trap containing the squirrel was placed flush against an opening in the arena and opened and squirrels were allowed to enter the arena of their own volition. Once the squirrel entered the arena, the trap was pulled away, the door was shut and locked and the trial commenced. The arena consisted of a 100 cm wide x 100 cm long x 60 cm high stained plywood box with a clear Plexiglas™ lid and a 4 x 4 grid marked on the bottom (Walsh & Cummins 1976). The box was finished with polyurethane and cleaned with a 50% vinegar solution after each use. A camera (Sony HandiCam, Sony, Tokyo, Japan) mounted on a tripod positioned immediately above the arena recorded the behavior of the subject for 5 minutes starting immediately after the subject entered the box. For each session, we recorded wind speed and temperature using a Kestrel 3000 weather meter (average over the first minute of the test; Nielsen-Kellerman Co., Boothwyn, PA) and estimated a percentage of cloud cover to examine any environmental effects. We also noted the occurrence of any vocalizations and counted the number of fecal pellets left behind after the test was completed. Defecation, in general, has been linked to stress through the activation of the sympathetic nervous system, and has been used as a measure of “emotionality” in the open-field (Archer 1973; Walsh & Cummins 1976; Hare 1998; Martin & Réale 2008b).

For all tests, we used JWatcher© to quantify behaviors (Blumstein et al. 2006). We used focal animal sampling (Martin & Bateson 1993) and quantified the total duration and all occurrences of sitting, walking, jumping, rearing up, biting at the box, visually scanning, grooming and vocalizing (Table 1). Finally, we recorded the proportion of time spent in each grid square of the box during all tests and used this information to quantify the total number of squares visited, total percent of squares visited and proportion of time spent in the center squares (centrality; Montiglio et al. 2012).

We also performed flight initiation distance tests on previously-marked females (Blumstein 2003). Two observers searched for adult female squirrels using binoculars; one from an elevated position (tower or hide) to allow for better viewing of the animals and one on the ground. The observers communicated using walkie-talkies (Motorola Talkabout, Chicago IL). Once an adult female was detected, both observers remained standing in the same place for five minutes to allow the subject to acclimate. During this time, we recorded the identity of the female, time of day, cloud cover, wind speed (on a scale from 0-4, with zero being the lowest), and group size within 10m in a notebook. Following the acclimation period, the observer on the ground walked directly toward the subject at a uniform, previously-practiced, pace of 1 m/s. We dropped markers at distances when the subject first alerted as the observer approached, as well as when the subject fled to her burrow. The elevated observer communicated to the walking observer when to drop the markers. We measured each of the distances as well as the squirrel’s initial distance to the burrow using pacing, which we later converted to meters (Runyan & Blumstein 2004). To avoid any effect of the identity or appearance of the walking human model, the same researcher (JLS) conducted each test wearing the same outfit.

We scored handling indices each time a squirrel was trapped and handled with the exception of first time captures. We did not score first captures because of longer handling times due to one-time procedures (i.e. permanent tagging of the squirrel and DNA collection), which may significantly alter the behavior of the squirrel. Based on Réale et al. (2000), we assigned points to an individual for the following behaviors: behavior of the squirrel when handlers approach the trap for the first time (0 = sits still, 1 = moderately agitated, alarm calling or hissing, 2 = highly agitated, alarm calling and/or hissing combined with thrashing around the trap); difficulty of transferring squirrel from trap to handling bag (0 = not difficult, runs right into handling bag, 1 = moderately difficult, takes 30 to 60s to run into handling bag, but does not require any additional measures other than banging on the trap, 2 = very difficult, requires additional measures, such as shaking the trap or opening the back to push the squirrel in); difficulty of controlling squirrel while in the handling bag (0 = calm, simply lays there, 1 = spends less than 50% of handling squirming and/or snorting, 2 = spends 50% or more of handling time squirming, snorting and alarm calling); and reaction upon release (0 = walks away from researchers, 1 = runs away from researchers). Finally, we added these scores up to give a total docility score which ranged from 0-7 with 0 being the most docile and 7 being the least docile.

Cortisol measurements

Part of the inconsistency in the results of personality and cortisol studies may be related to the difficulty of obtaining true baseline levels of cortisol from the blood. Especially in wild populations, trapping and handling methods can cause the release of cortisol into the blood typically within a period of minutes (Kenagy & Place 2000; Delehanty & Boonstra 2009; Wilkening et al. 2013; Hare et al. 2014) and may bias results. In fact, the mere presence of humans may cause elevated levels of cortisol in the blood and impact measurable circulating levels of cortisol (Creel et al. 2002; Barja et al. 2007; Martin & Réale 2008b). Methods have now been developed to isolate and measure cortisol from hair (Yang et al. 1999; Raul et al. 2004). This method is particularly advantageous because hair can be collected with minimal impact on the animal and stored with ease (Wennig 2000). Furthermore, cortisol accumulated in the shaft of the hair can be used to represent mean circulating levels during the time of growth, and thus is reflective of long-term (weeks to months) circulating levels of cortisol (Pragst & Balikova 2006). Consequently, unlike circulating levels in the blood, cortisol concentrations obtained from hair are not immediately affected by short-term trapping and handling methods (Yang et al. 1999; Macbeth et al. 2010) and may better represent actual long-term HPA axis activity and circulating levels of cortisol that individuals experience under natural condition in this species. For these reasons, we chose to isolate cortisol from hair

We obtained hair samples from the tails of adult females during handling. Cape ground squirrels at this site molt their tails between January and March (Herzig-Straschil 1978) during a time when they have minimal contact with humans (researchers are not present but a few people periodically move throughout the site). To properly prepare our samples for the radioimmunoassay, we followed the methods of Macbeth et al. (2010). To ensure that samples were clean and free from blood, dust or other contaminants, we washed each sample three times with 10mL of methanol per 100 milligrams of hair. Using a Retsch ball mill (Retsch MM 301 Mixer Mill, Retsch Inc., Newtown, PA, USA), we ground each sample for 0.03 min/mg at 30Hz until it was reduced to a fine powder. To extract the cortisol, we added 1mL of methanol to 50mg of hair powder and shook these samples for 24h at room temperature on a shaker table (Standard Analog Shaker Model 3500, VWR®, Radnor, PA, USA). Afterwards, we centrifuged the samples (15min, 4500rpm) and removed the supernatant. We then added another 1mL of methanol, vortexed the sample for 30s and centrifuged it again for 15 min at 4500rpm. This procedure was repeated for a total of three washes and approximately 3mL of supernatant was collected from each sample. A wash test confirmed that 93-100% (n = 5) of cortisol was extracted from the first three washes. We dried down the supernatant using a concentrator (Savant ISS110 SpeedVac Concentrator, Thermo Scientific, Waltham, MA, USA) and tubes were frozen at -18°C until use.

We performed radioimmunoassays following the methods of Ryan et al. (2012). We used tritium-labeled cortisol (diluted to 5000 disintegrations per minute; GE

Healthcare, Piscataway, NJ) and a cortisol-specific antibody (diluted to 1:10 000; cross-reactivity of 5.7% for 11-deoxycortisol, 3.3% for corticosterone, 36% for prednisolone and < 0.7% for cortisone; Fitzgerald Industries, North Acton, MA). Previously frozen samples were reconstituted at the time of the assay using 600 µL of RIA buffer (0.1 M phosphate buffer, 0.9% NaCl [w/v], and 0.5% bovine serum albumin [w/v]). We combined 100µL each of reconstituted sample (or known dilution of cortisol for standards) antibody, and labeled cortisol and allowed this mixture to incubate for 1h at room temperature and then for 12-24hrs at 4°C. After this time, we added 100 µL of separation buffer (0.5% dextran [w/v] and 5% charcoal [w/v] in RIA buffer) to stop the reaction and remove any unbound tritiated cortisol. These samples were then centrifuged (30min, 4500rpm, 4°C) and the supernatant was poured off into 6mL scintillation vials. Finally, we added 4mL of scintillation fluid (Ultima Gold, Perkin Elmer, Waltham, MA, USA) and measured the radioactivity for 5 minutes per sample using a scintillation counter (LS6500; Beckman Coulter, Brea, CA).

We determined absolute values of cortisol by interpolating values to our standard curve and adjusting for a 96.6% extraction efficiency. Inter and intra-assay variation confirmed that our technique was consistent (12.8% and 8.6% coefficient of variation, respectively) and samples demonstrated good parallelism (linear model; $p < 0.05$, $r^2 = 0.94$). All components of this assay have previously been validated using Richardson's ground squirrels (*Urocitellus richardsonii;* Hare et al. 2014)

Statistical Analysis

To reduce the number of correlated variables on the measured behaviors from the open-field test, we performed principal components analysis using the “psych” package (Revelle 2010) in R (R v3.2.1, R Development Core Team 2015). Because they occurred at very low frequencies (< 10% of tests), we removed biting the box, grooming and vocalizations from our analysis. Each trial was then assigned a score on each of the three components.

Using the package “lme4” (Bates et al. 2012) we then constructed linear mixed-effects models (LMMs) with an identity link function for each personality assay to test for effects of environmental factors. All continuous variables were standardized and the residuals of each model were inspected for normality. In each model, we used individual identity as a random effect. We used the component scores extracted from the principal components analysis for each open-field test as the dependent variables in three separate models, while Julian date, time of day, trial number, area within the study site (house, pan or floodplain), presence of clouds, wind speed, and temperature were fixed effects. For FIDs, the first flight distance was the dependent variable while Julian date, time of day, area within the study site, presence of clouds, wind, number of squirrels within 20m, posture (on 2 feet or 4), initial behavior (feed, sit or other), start distance and distance to burrow were fixed effects. For handling indices, we used total score as the dependent variable with Julian date, hour, area, trial number and body condition as fixed effects. We then used backwards elimination until all remaining factors were significant (Boon et al. 2008). We also determined repeatability of each measured trait by using the "LMM-based repeatability estimates" as described by Nakagawa & Schielzeth (2015).

From each model, we extracted the best linear unbiased predictors, or BLUPs (also known as random effect predictions) for each individual identity. This analysis allowed us to have a single score per individual for each trait that better estimates an individual "behavior profile" than the mean alone. BLUPs are commonly used in animal personality analysis (Kruuk 2004; Boon et al. 2007; Martin & Réale 2008b).

Finally, we used Pearson's correlations to compare BLUP scores to determine if behavioral syndromes exist. We also used Pearson's correlations to compare BLUP scores to body condition and hair cortisol concentration. We set significance to $\alpha = 0.05$ and all residuals were checked for normality.

Results

In total, we performed 98 open-field tests on 42 adult females (one test, n = 9; two tests, n = 10; three tests, n = 23). Principal components analysis yielded three components with eigenvalues greater than one (PC1: 2.12, PC2: 3.32, PC3: 1.06; Table 2). Together, these three components explained 72% of the variance of the total data set (PC1: 35%, PC2: 25%, PC3: 12%; Table 2). The first component was considered the "exploration/activity component," in that its variation along that axis was characterized by time spent walking, total number of squares visited and the percentage of squares visited. Exploration/activity had a repeatability of $r = 0.11$ (Table 4). Component 2, or the "vigilance component", was characterized by the amount of time vertical as opposed to time sitting, and had a repeatability of *r* = 0.02 (Table 4). The third component was characterized by the number of scats left in the arena at the end of an individual's open field test and had a repeatability of *r* = 0.18 (Table 4). We considered PC3 the "fecal index component."

Mixed models revealed that trial number was significantly related to the exploration component, with activity decreasing with each subsequent trial. Interestingly, squirrels tested late in the season also tended to have higher activity than those tested earlier. Vigilance within the open-field increased as date increased. For the fecal index component, we found no significant effects of any recorded variables (Table 3).

A total of 74 FIDs were obtained for 37 individuals. Boldness was repeatable (*r* = 0.27, Table 4). Flight distance increased significantly as start distance increased and when squirrels were farther from their burrow. Squirrels also had a shorter flight distance as the time of day increased (Table 3).

We scored 86 docility indices on 36 individuals. Combined docility scores were not repeatable (*r* = 0.02). However, when using only difficulty of transfer score, repeatability increased to *r* = 0.21 (Table 4). Because the transfer score seems to accurately reflect the way squirrels interacted with handlers, we hereafter use the transfer score as measure of docility. Mixed models revealed that docility was significantly lower (higher scores) in the house area compared with the floodplain (Table 3).

Corrected cortisol concentrations ranged from 1.31-11.69 ng cortisol/g hair with an average of 5.36ng/g. We found no relationship between cortisol and body condition (t_{48} = 0.42, *p* = 0.67) and no significant correlations between personality assays and levels of cortisol (Appendix 1, Pearson's product moment correlation; activity, t_{38} = 0.53, *p* = 0.61; vigilance, t_{38} = 1.37, *p* = 0.18; fecal index, t_{38} = 0.59, *p* = 0.56; docility, t_{31} = 0.28, *p* = 0.78; boldness, t_{32} = 0.69, *p* = 0.50). We found a significant negative relationship between body condition and fecal index (PC3, t_{37} = -2.45, *p* = 0.02 Figure 8) and better body condition was associated with higher activity/exploration (PC1, t_{37} = -2.00, *p* = 0.05, Figure 7). Condition was not significantly related to any other personality scores (Appendix 1, Pearson's product moment correlation; vigilance, t_{37} = 0.71, *p* = 0.48; docility, t_{30} = 1.21, *p* = 0.24; boldness, t_{32} = -1.21, *p* = 0.23).

Discussion

We found support for several common repeatable personality traits in Cape ground squirrels (Martin & Réale 2008a). Our findings suggest that environmental conditions have some impact on the results of personality tests. Additionally, because body condition was related to fecal index and activity/exploration, we found some support for the state-dependent safety hypothesis but we did not detect a relationship between long-term cortisol and personality traits.

This study is the first to comprehensively examine consistent individual differences via standardized procedures in Cape ground squirrels. Our results suggest that activity/exploration, boldness, docility and fecal index were repeatable and although they are at the low end of the spectrum, are comparable to repeatability values found in similar studies (Dingemanse 2002; Petelle et al. 2013; Baugh et al. 2014). In a meta-analysis, Bell et al. (2009) found an average behavioral repeatability of 0.37 across a variety of taxa with activity and antipredator behavior showing relatively low repeatability compared to other behaviors. Estimating repeatability is important to show that inter-individual variation is higher than intra-individual variation and thus establishing that consistent individual differences exist in the studied population (Boake 1989; Stamps & Groothuis 2010).

We found no evidence that personality was related to long-term cortisol measurements. Similar studies performed on wild populations have reported varying results (Costantini et al. 2012; Montiglio et al. 2012; Ferrari et al. 2013; Clary et al. 2014; Dosmann et al. 2014). For example, in Belding's ground squirrels (*Urocitellus beldingi*), Dosmann et al. (2014) found that cortisol concentrations did not correlate with restraint or open-field behaviors either within or among individuals. We may not have detected a relationship because cortisol levels may not be as tightly correlated to personality traits as once thought. Koolhaas et al. (2010) suggested a revised two-tier model of personality whereby stress reactivity (measured by cortisol levels) is independent from coping style (i.e. personality traits). The two-tiered model has been demonstrated using exploratory PCA by showing that cortisol and other stress reaction behaviors constitute one axis whereas personality traits or coping styles make up another (Van Reenen & Blokhuis 2004; Van Reenen et al. 2005). In the same way, Clary et al. (2014) found that cortisol was independent from exploration but not boldness. Our data suggest that such a two-tier model may be operative in Cape ground squirrels.

Furthermore, hair may provide too coarse of a measurement to detect the differences in cortisol that we might expect to find in relation to personality. For example, there would be no measurable difference between an individual that had mounted HPA-axis responses to several moderate stressors versus an individual that had experienced one intense and/or prolonged stressor. Individuals who have higher tissue cortisol concentrations may also be behaviorally mediating their stress response, and thus overall cortisol levels by actively avoiding high stress situations (Martin & Réale 2008b; Costantini et al. 2012). Eastern chipmunks (*Tamias striatus*) that were more exploratory and docile were likely to be found in areas with high levels of human activity yet did not have higher stress levels (Martin & Réale 2008b). Individuals that do not pay a high cost in terms of cortisol response may be dispersing to these areas to gain other benefits associated with humans (better territory, decreased predation, supplemental food, etc.). In the present study, although we found no differences in cortisol among squirrels exposed to the varying levels of human activity, we also found no differences in behavior. Female Cape ground squirrels do not disperse; therefore, we would not expect to see similar sorting of individual home ranges as found in eastern chipmunks. However, behavioral changes attributable to stress responses may be happening in a similar fashion at a lower level (e.g. changes in space-use within a colony, changes in placement within a social group).

We found that better body condition was associated with a lower fecal index and higher activity (lower score). This finding is in keeping with the state-dependent safety hypothesis for animal personality, which predicts that individuals in relatively better condition can afford to be more active and less emotional. Recent research, however, has found the opposite relationship in a variety of vertebrates (Monclús & Rödel 2009; Kurvers et al. 2010; Dosmann et al. 2014; Crino et al. 2016) which supports the asset protection and/or starvation avoidance hypotheses. We did not find any correlation between three of five personality traits and body condition, suggesting that state dependent safety may exert some influence but that other mechanisms, such as starvation avoidance also may be in play when it comes to the maintenance of consistent animal personality traits (Luttbeg & Sih 2010).

Only three environmental variables significantly affected personality tests. Squirrels were bolder later in the day. This shift may be due to changes in temperature (Petelle et al. 2013). In fish, even small increases in temperature can have a significant impact on personality traits such as boldness and exploration, which may be mediated through an increase in metabolic activity (Biro et al. 2010; Forsatkar et al. 2016). However, such a relationship is likely to be less pronounced in endothermic organisms. Furthermore, we measured temperature directly during open-field tests and we found no correlation between temperature and activity, exploration or fecal index. More research is needed in this area, particularly in mammals, to determine why personality is affected by time of day.

Date significantly influenced both activity/exploration and vigilance; later in the trapping season squirrels had lower activity/exploration and higher vigilance. Cape ground squirrels spend more time vigilant when predation risk is higher, suggesting that predator activity may increase over the course of the trapping season (Unck et al. 2009). An increase in predators overall might also explain why activity/exploration decreases through the season, as squirrels may be less willing to explore space away from their burrow cluster owing to fear of predators. Previous research has shown that risk of predation can alter foraging patterns significantly (Holmes 1991; Lagos et al. 1995; Brown et al. 1999; van der Merwe & Brown 2008). Additionally, predation has been demonstrated to affect personality (Bell 2005; Dingemanse et al. 2007).

Finally, docility was significantly lower (higher scores) in the house area compared with the pan and floodplain areas, which may be related to the relatively lower amount of human contact in the floodplain/pan compared with the house area. Previous work has shown that human presence can influence personality traits as animals become more habituated (Li et al. 2011; Gravolin et al. 2014). Martin and Réale (2008b) found that docility actually decreased with both frequency of human contact and cortisol levels. Interestingly, Cape ground squirrels react oppositely, tending to be more reticent to transfer from the trap to the handling bag during intense trapping seasons regardless of area within the field site (JM Waterman, personal observation) although the reason for this reticence is unclear.

Because squirrels decreased their activity/exploration with each subsequent trial, our data provide evidence that habituation occurred in the open-field (Groves &

Thompson 1970). This result is not surprising, as many studies have reported habituation to personality tests (Archer 1973; Dingemanse 2002; Martin & Réale 2008b). Interestingly, we did not find evidence of habituation in most of the traits examined. Previous studies have regarded habituation as a measure of learning (Dukas 1998; File 2001; Elliott & Grunberg 2005), and squirrels at this site seem to adapt rather quickly to the presence of researchers. For instance, individuals behave differently between the first capture of the season and subsequent trapping events (JM Waterman, personal observation). We took steps to avoid the effects that novelty might have on the docility test by not scoring the first capture and we did not start collecting FIDs until the population was fully marked and we had been on site for several weeks. This history of trapping could be why we did not find evidence of habituation to docility or boldness tests.

In general, Cape ground squirrels are sensitive to weather conditions; they avoid temperature extremes by emerging later on cold days and reduce overall activity during rainy and overcast days (Herzig-Straschil 1978, 1979; Scantlebury et al. 2012). In total, however, we did not detect many effects of environmental conditions such as wind speed, temperature or cloud cover on personality traits. The general lack of influential environmental variables indicates that Cape ground squirrel personality traits are robust and that they perform similarly in personality assays regardless of conditions. Couchoux and Cresswell (2012) suggest that changing weather conditions lead to changes in predation risk which in turn affect behavior. It may be that predation risk in Cape ground squirrels is not significantly affected by weather conditions. Certainly, snakes mediate their activity based on temperature, but the most active predators during the austral winter (i.e. black-backed jackals, *Canis mesomalas*, and raptors like the pale chanting goshawk, *Melierax canorus*, and black-shouldered kite, *Elanus axillaris*) might not be affected by weather conditions (Unck et al. 2009). Although these results are promising for the field of animal personality, we suggest that future studies be mindful of the robustness of some traits and justify which variables to include in models of animal personality.

In summary, we found some support that both intrinsic and extrinsic factors were associated with personality traits in Cape ground squirrels. We found no relationship between measures of long-term cortisol, suggesting that other mechanisms may be modulating the expression of differing personality traits. However, we cannot rule out the possibility that varying HPA-activity modulates personality traits but was not detected in the analysis of cortisol in hair. Although personality traits are expected to be consistent over time and across context, our findings suggest that differing external factors affect the expression of a variety of behaviors under testing conditions. This often-overlooked aspect of personality tests should be carefully considered and, if possible, controlled, when working with free-ranging populations. Additionally, intrinsic factors like body condition are also related to several personality traits and provide support for the state-dependent safety hypothesis. Further research will be necessary to determine if such factors directly affect personality or vice versa.

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Figures and Tables

Table 2.1. Ethogram for behaviors observed during the open-field test.

Behavior	Description
Sitting	Proportion of time immobile either on four legs or two legs, crouched low
Walking	Proportion of time spent in motion, walking or running
Jumping	Frequency of four feet leaving the ground at the same time
Rearing	Proportion of time on two feet, stretched vertically
Biting	Frequency of biting or chewing the box
Scanning	Head moving more than 30° while body was still, frequency only
Grooming	Frequency of grooming behavior such as itching and tail fluffing
Vocalizing	Frequency of alarm calls emitted

Table 2.2. Summary of principal components analysis for open-field tests performed on Cape ground squirrels. Components with eigenvalue greater than 1 were retained for further analysis. Important coefficients (> 0.4) for retained components are bolded.

	Principal Component								
Variable	1[1]	2[2]	3[3]	4	5	6	7	8	9
Jumps	-0.390	-0.219	-0.215	-0.187	0.418	-0.614	-0.234	-0.331	
Scans	-0.251	0.255	0.360	-0.514	0.443	0.467	-0.247		
Rear (proportion of time)	-0.193	**0.599**		0.314	0.115	-0.122			-0.680
Sit (proportion of time)	0.305	**-0.526**		-0.322					-0.716
Walk (proportion of time)	**-0.447**	-0.129		0.162	-0.487	0.353	-0.303	-0.529	-0.154
Total squares visited	**-0.499**	-0.211	-0.102		-0.193		-0.240	0.772	
Centrality (proportion of time)		0.378		-0.630	-0.579	-0.353			
Feces		-0.138	**0.901**	0.198		-0.350			
Percent of squares	**-0.450**	-0.166		-0.183			0.851		
Standard deviation	1.822	1.456	1.030	0.974	0.896	0.628	0.568	0.159	0.039
Proportion of variance	0.369	0.236	0.118	0.105	0.089	0.044	0.036	0.003	0.000
Cumulative proportion	0.369	0.605	0.723	0.828	0.917	0.961	0.997	1.000	1.000
Eigenvalues	3.321	2.121	1.061	0.949	0.803	0.395	0.323	0.025	0.002

1. Activity/exploration component
2. Vigilance component
3. Fecal index component

Table 2.3. Estimates of fixed effects of linear mixed-effects models after backwards elimination. Cape ground squirrel ID was used as a random effect.

	Docility			
	Estimate (±SD)	t-value	*p*-value	
Intercept	1.09 ± 0.24	4.62	0.00	***
Colony (floodplain)	-0.57 ± 0.27	-2.11	0.04	*
Colony (pan)	-0.97 ± 0.30	-3.23	0.00	**
	Boldness			
(Intercept)	-0.19 ± 0.13	-1.52	0.13	
Time	-0.12 ± 0.06	-1.96	0.05	*
Trial	0.10 ± 0.06	1.66	0.10	
Initial Observer Distance	0.86 ± 0.06	13.64	0.00	***
Distance to Burrow	0.17 ± 0.06	2.82	0.01	**
	Activity/exploration (PC1)			
Intercept	-1.44 ± 0.24	-6.13	0.00	***
Date	-0.22 ± 0.10	-2.13	0.00	***
Trial	0.80 ± 0.12	6.59	0.03	*
	Vigilance (PC2)			
Intercept	0.00 ± 0.11	-0.02	0.98	
Date	0.33 ± 0.10	3.40	0.00	***
	Fecal Index (PC3)			
Intercept	0.02 ± 0.16	0.12	0.90	
Colony (house)	0.56 ± 0.34	1.67	0.09	
Colony (pan)	-0.38 ± 0.27	-1.39	0.17	
Temperature	-0.15 ± 0.09	-1.60	0.11	

Table 2.4. Estimates of random effects of Cape ground squirrel ID in linear mixed-models after backwards elimination and repeatability (*r*) estimates.

Activity/exploration (PC1)				**Docility**			
	Variance (±SD)				Variance (±SD)		
ID	0.19	±	0.43	ID	0.17	±	0.42
Residual	0.51	±	0.72	Residual	0.34	±	0.58
	r = 0.12				*r* = 0.19		
Vigilance (PC2)				**Boldness**			
ID	0.13	±	0.36	ID	0.09	±	0.30
Residual	0.77	±	0.88	Residual	0.15	±	0.39
	r = 0.03				*r* = 0.27		
Fecal Index (PC3)							
ID	0.29	±	0.54				
Residual	0.66	±	0.81				
	r = 0.16						

Figure 2.1. Activity/exploration score per trial in the open-field tests of Cape ground squirrels. Increasing activity/exploration score is characterized by decreasing proportion of time walking, total squares visited and percent of squares visited. Mixed-effects models found that activity score increased significantly as trial increased. Each line represents a single individual.

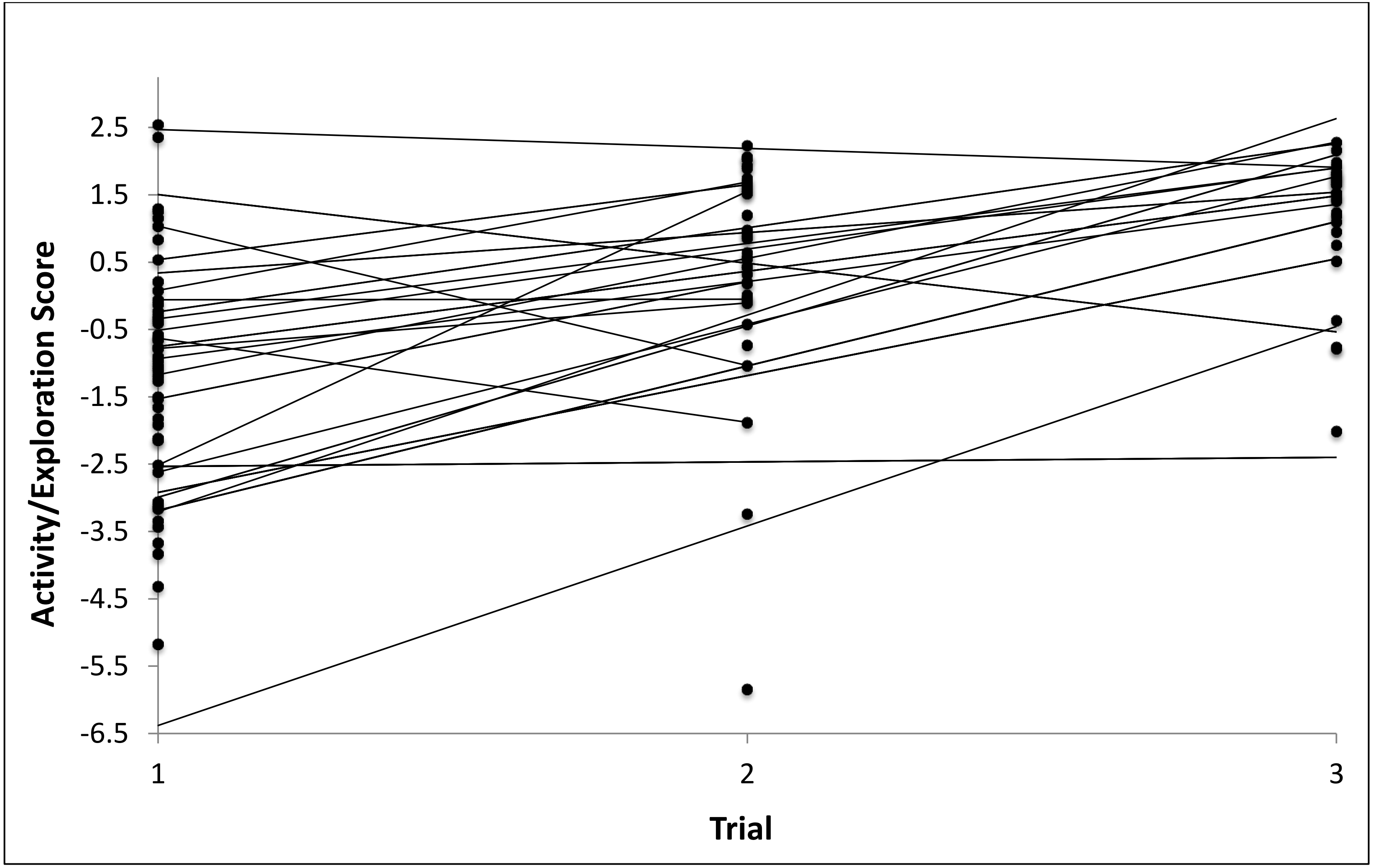


Figure 2.2. Activity/exploration score over increasing date in the open-field tests of Cape ground squirrels. Increasing activity/exploration score is characterized by decreasing proportion of time walking, total squares visited and percent of squares visited. Mixed-models found that activity score decreased as date increased.

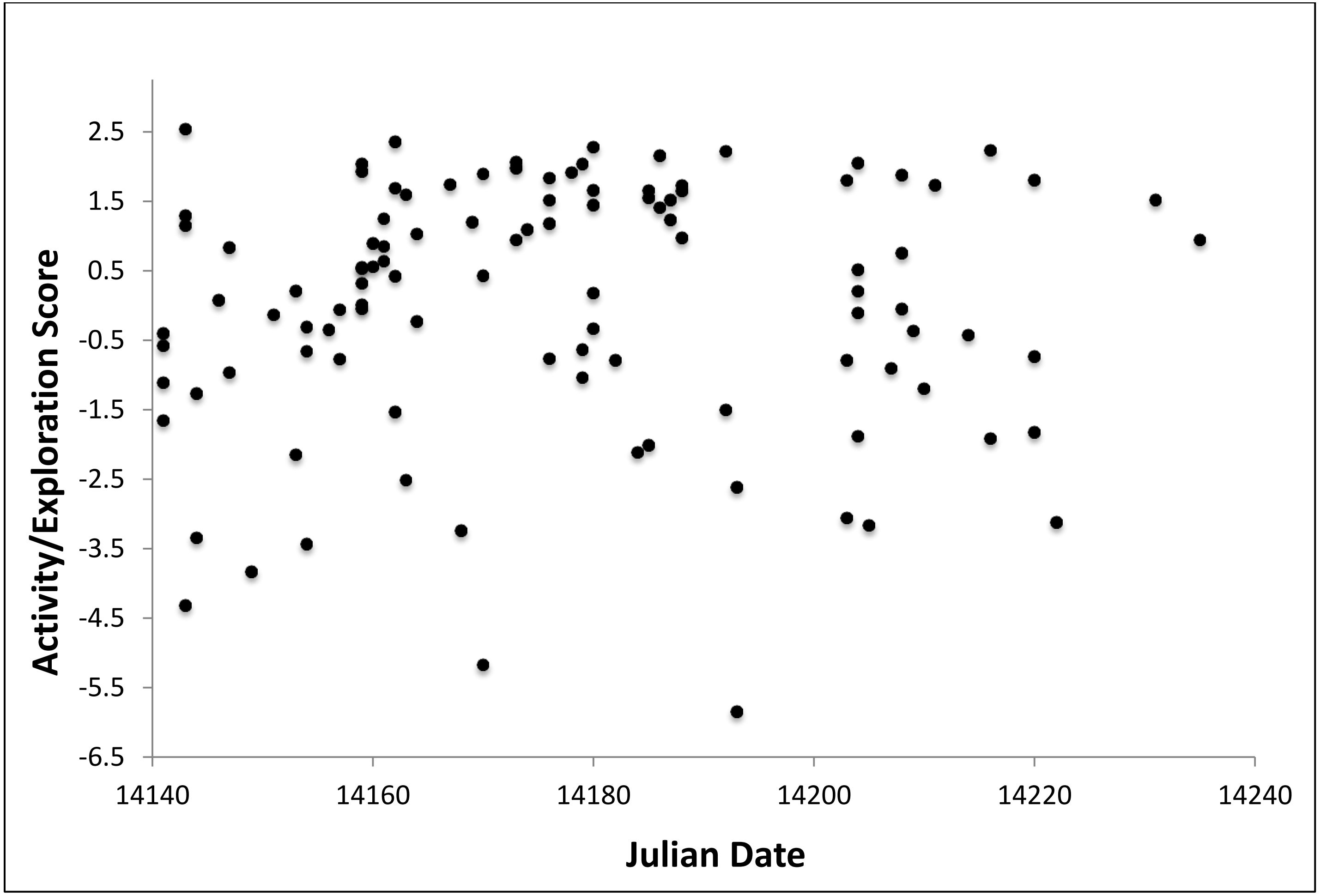


Figure 2.3. Vigilance score over increasing date in the open-field tests of Cape ground squirrels. Increasing vigilance score is characterized by more time spent in high vigilance (rearing up) and less time spent walking. Mixed models found that as date increases, vigilance score increases significantly.

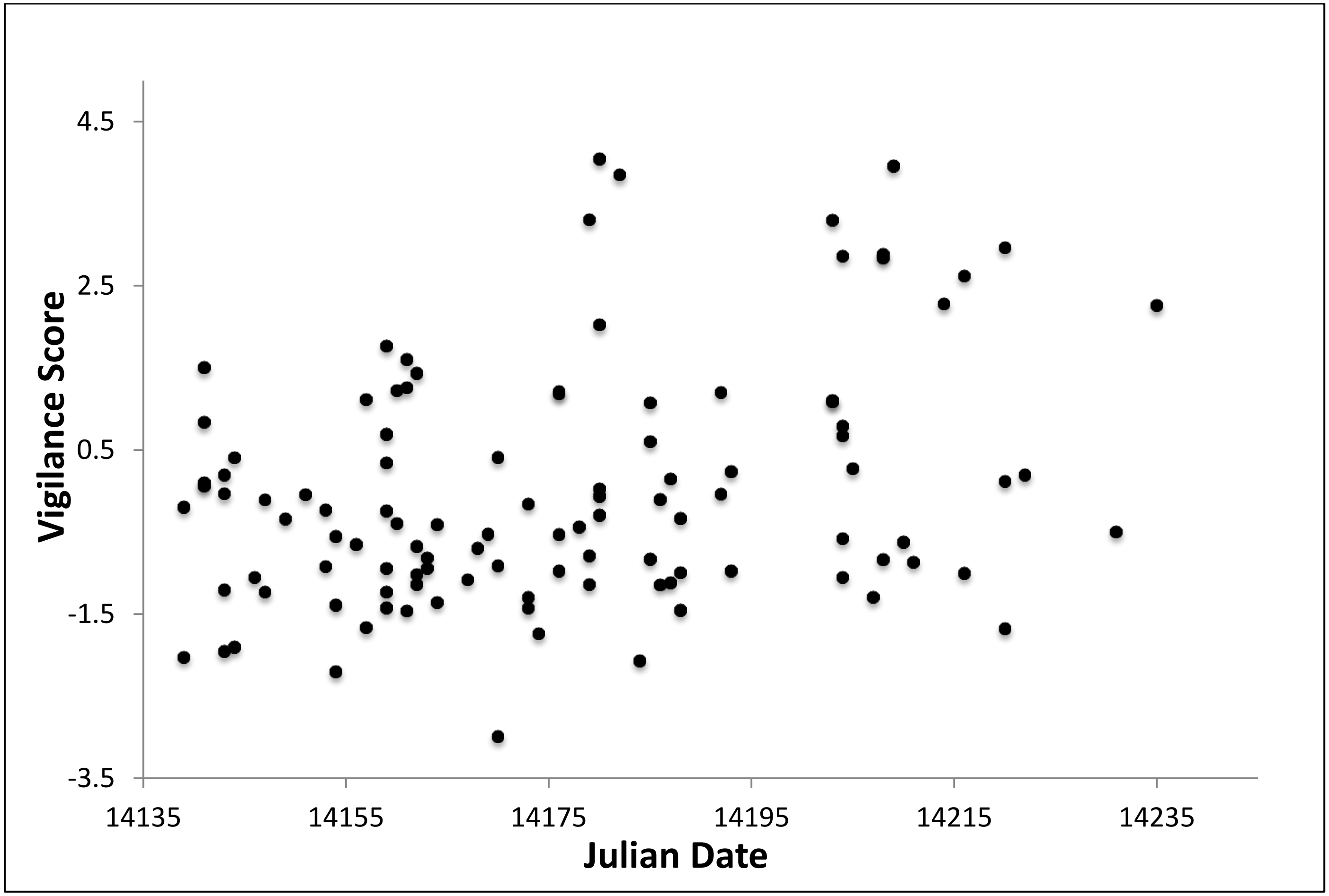


Figure 2.4. Flight distance over varying start distances in flight initiation tests of Cape ground squirrels. Increasing flight distance is associated with decreasing boldness. Mixed-effects models found that as start distance increases, flight distance increases significantly.

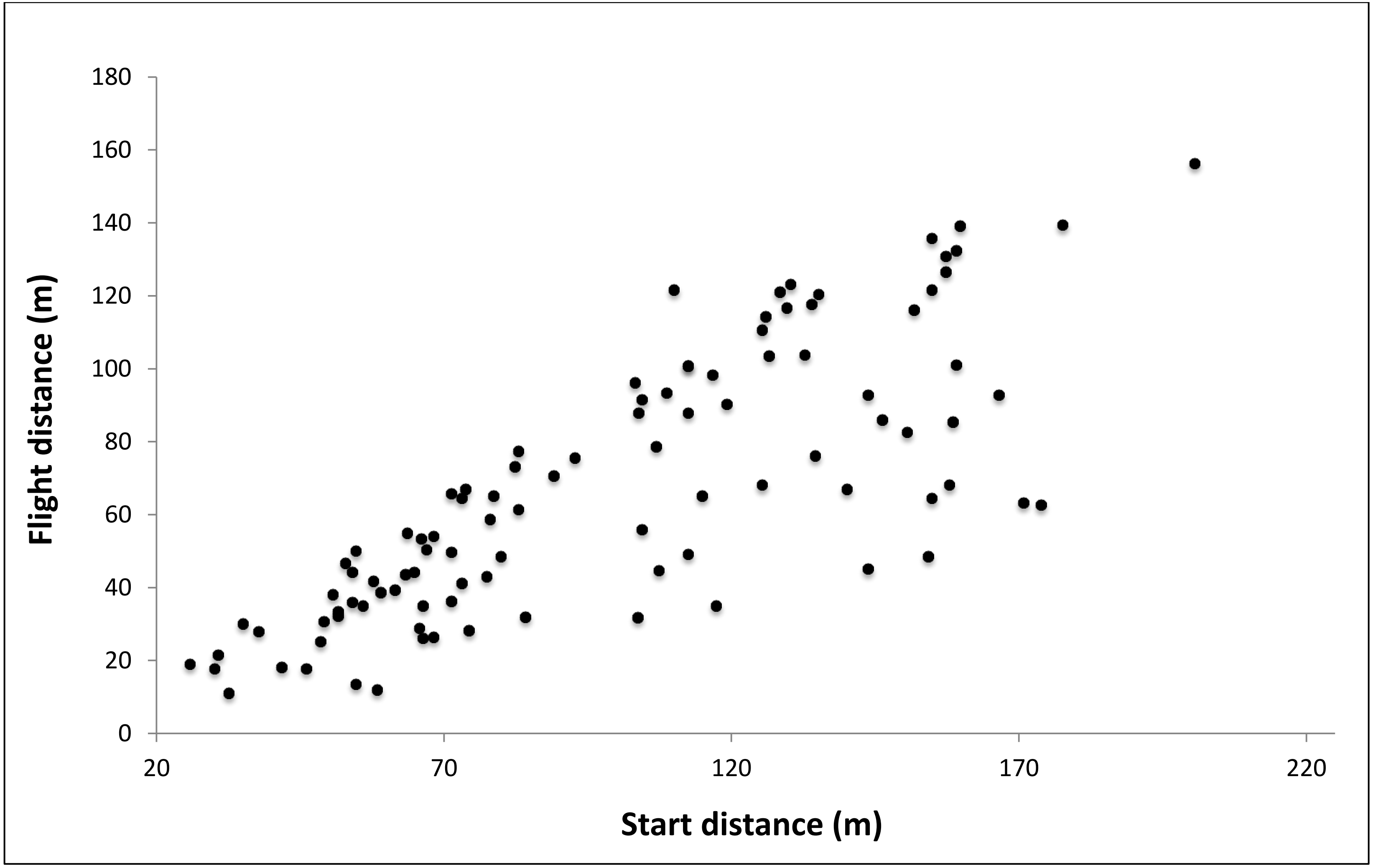


Figure 2.5. Flight distance over varying distances to burrow in flight initiation tests of Cape ground squirrels. Increasing flight distance is associated with decreasing boldness. Mixed-effects models found that as distance to burrow increases, flight distance increases.

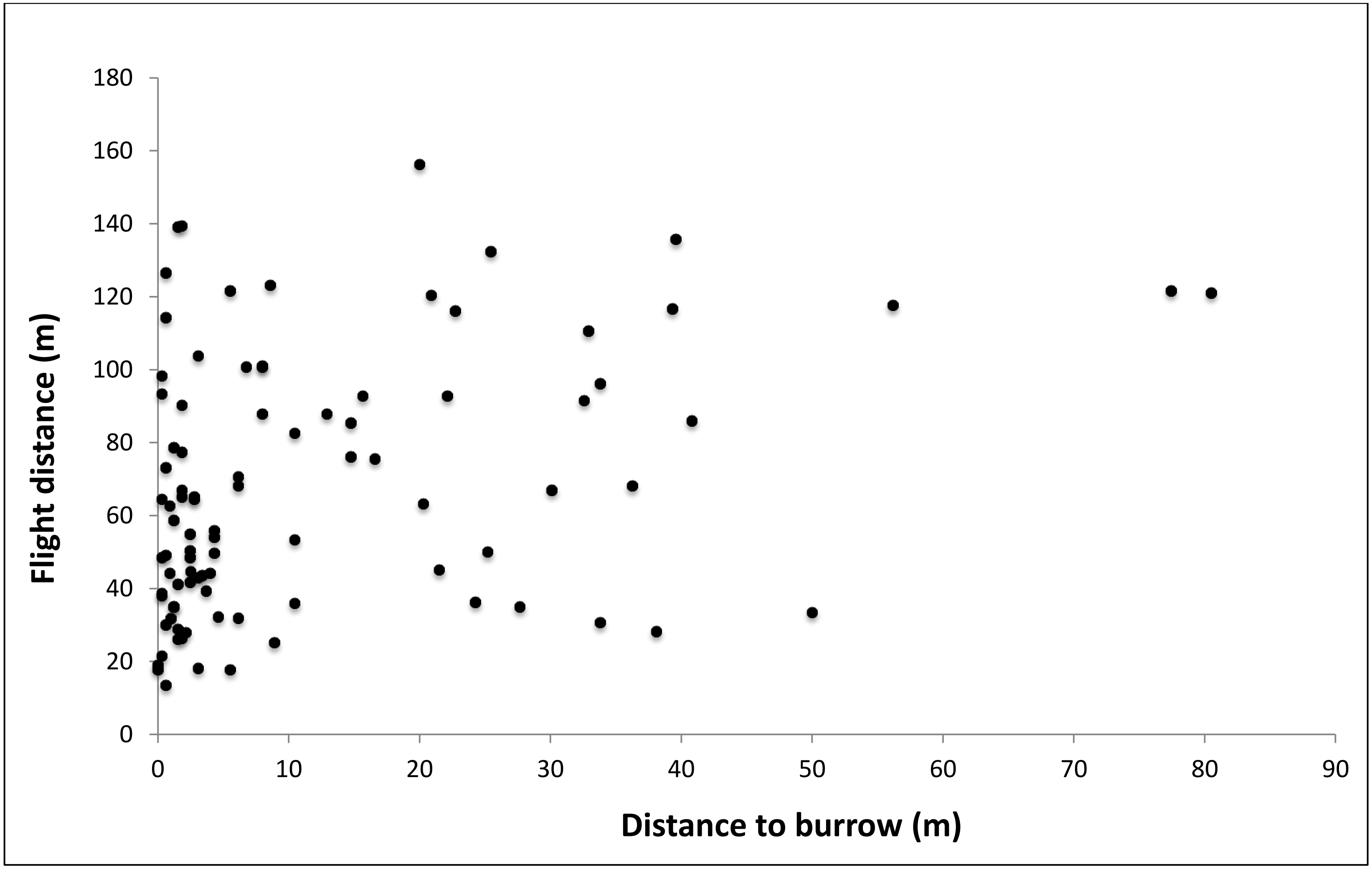


Figure 2.6. Docility scores over three areas in the study site: house, pan and floodplain. Increased docility score is associated with increased difficulty of handling. Mixed-effects models revealed that Cape ground squirrel docility scores were significantly higher in the house than in the pan and the floodplain.

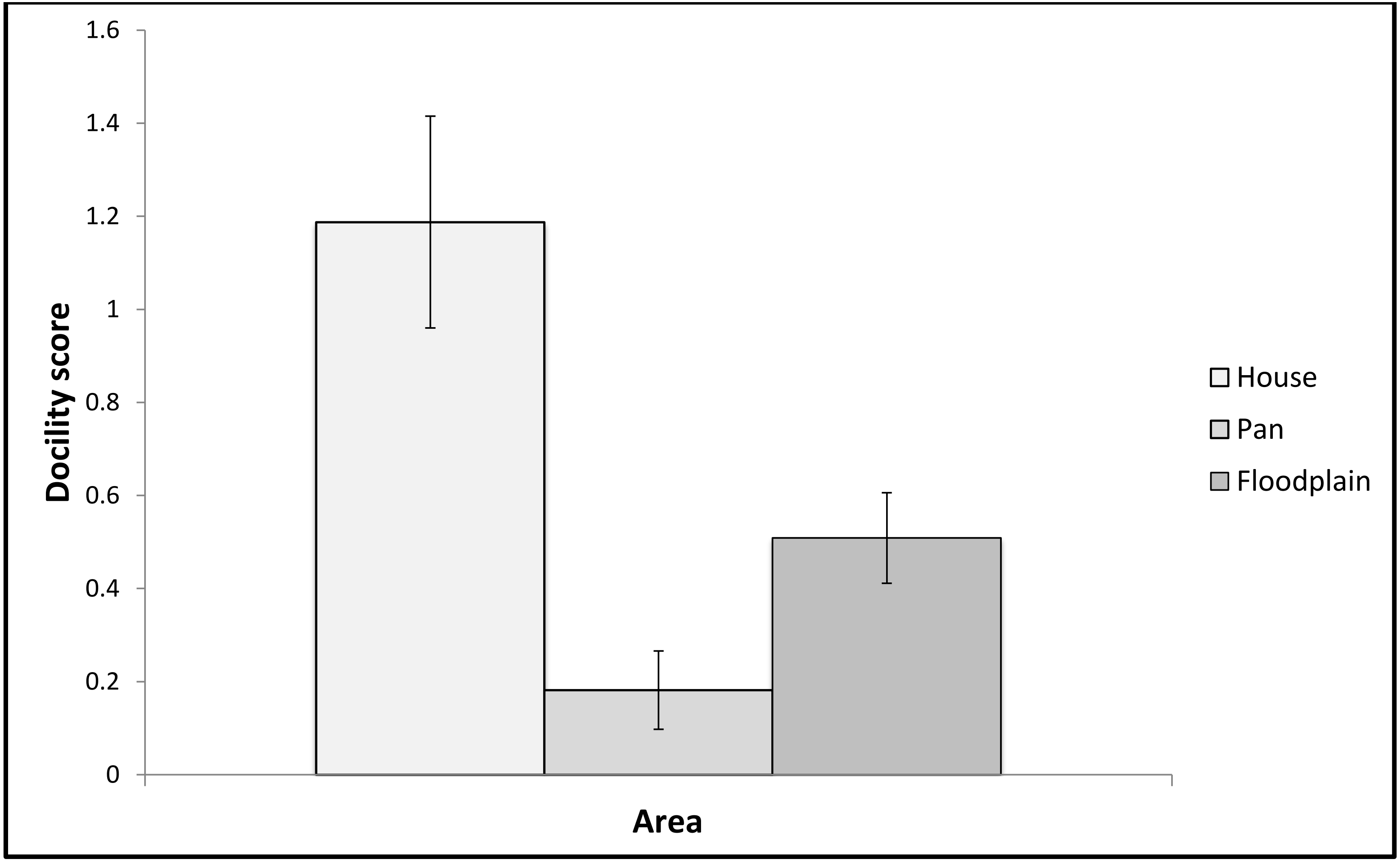


Figure 2.7. Body condition and activity/exploration scores for Cape ground squirrels (Pearson's product moment correlation; $t_{37} = -2.00$, $p = 0.05$).

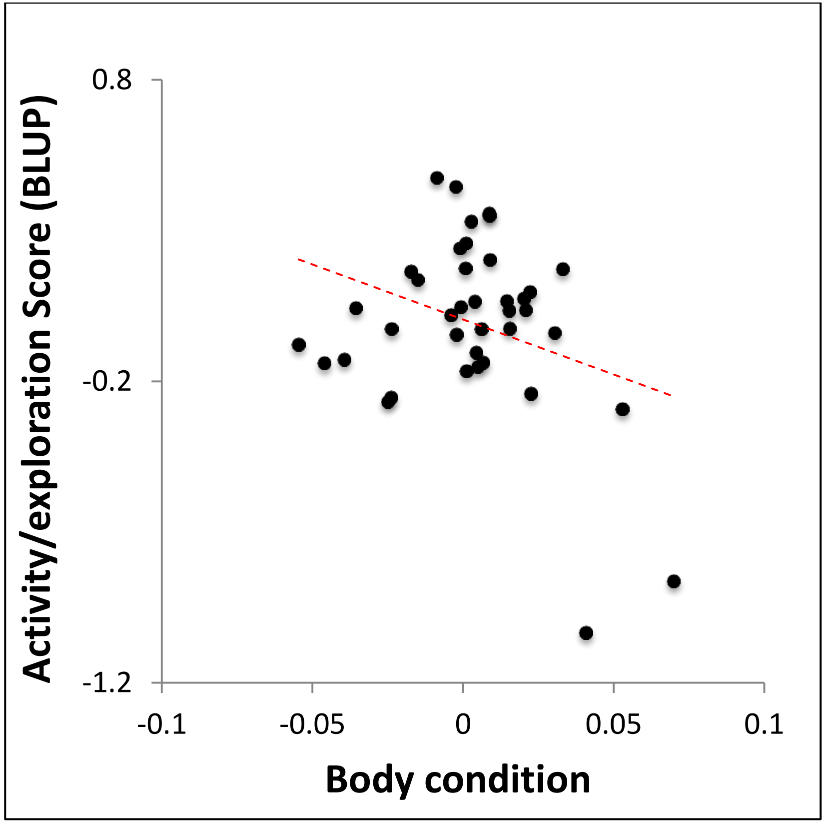
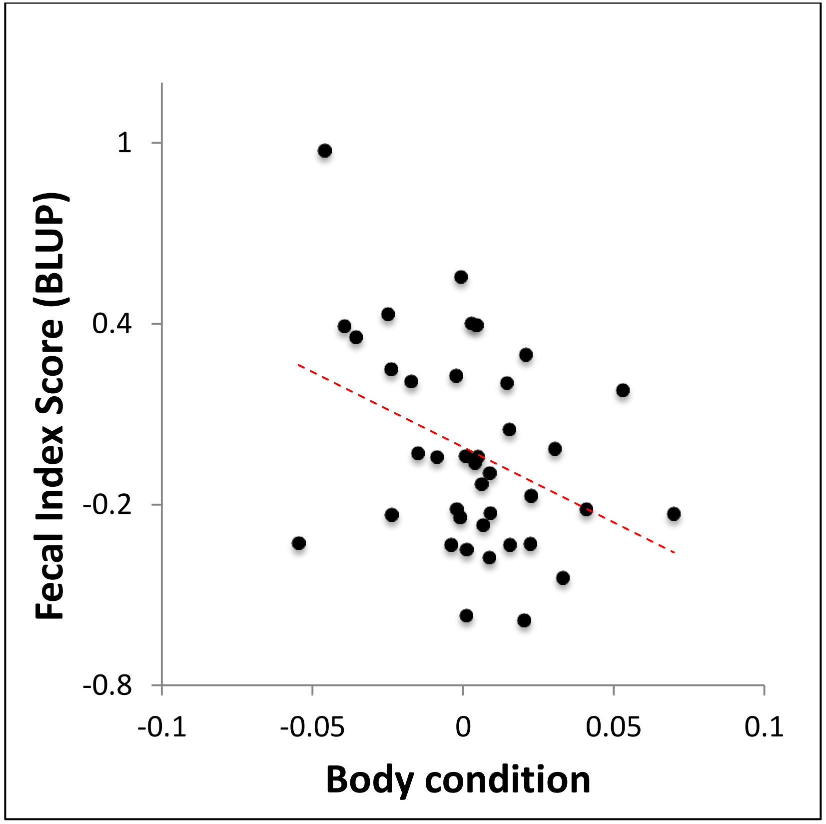


Figure 2.8. Body condition and fecal index scores for Cape ground squirrels (Pearson's product moment correlation; $t_{37} = -2.45$, $p = 0.02$).



Chapter 3 — Personality and life history trade-offs in female Cape ground squirrels (*Xerus inauris*)

Abstract

Life history theory predicts that trade-offs exist between current and future reproduction. When applied to the study of animal personality this hypothesis suggests that bolder, exploratory individuals should prioritize current reproductive success whereas their more risk-averse counterparts should favor future reproductive success, or survival. In the present study, we examine personality traits and their correlations, or behavioral syndromes, and how they relate to survival and reproductive success in female Cape ground squirrels (*Xerus inauris*), a highly social and rarely aggressive species. We quantified boldness, docility and exploration using standardized tests and compared individual's scores in those tests to yearly survival and total number of offspring. We found evidence for two behavioral syndromes, but found no relationship between survival or reproductive success and any of the measured personality traits. We suggest that the distinctive, non-aggressive social structure of Cape ground squirrels offers a new perspective in the study of personality that does not rely on aggressive behaviors or dominance hierarchies.

Introduction

Differential reproductive success and survival are the cornerstones of natural selection, and as a result, behavioral ecologists have spent considerable time investigating which behaviors affect these processes (Hall et al. 2008). Traditionally, behavior has been seen as infinitely plastic, with individuals able to express the most adaptive behavior in any given context (Sih et al. 2004a). However, this plasticity does not always prove to be the case and research over the past twenty years has suggested that individuals are constrained in their behavioral response by consistent individual differences (Sih et al. 2004b; Réale et al. 2007). This cross-contextual consistency, also known as animal personality and its correlations, or behavioral syndromes, predicts that individuals should retain certain traits, such as relative aggressiveness or boldness, across functional behavioral categories like mating, foraging and parenting, as well as through time (Sih et al. 2004a; Sih & Bell 2008; Stamps & Groothuis 2010).

The life-history trade off hypothesis, as applied to animal personality, predicts that personality differences should correlate with differences in life-history strategies (Wolf et al. 2007). In the most simplistic view of this hypothesis, trade-offs between current and future reproduction should cause two strategies to emerge; those individuals with a ‘fast’ strategy should be bolder, more exploratory and more aggressive and should sacrifice survival for higher reproductive output. Meanwhile, a ‘slow’ strategy involves more risk-averse behavior that should lend itself to higher survival. This fast/slow paradigm has also been extended to include physiological reactivity to stressful events where proactive (fast) individuals have a low stress reactivity compared to reactive (slow) individuals (Koolhaas et al. 1999, 2010).

Most of the research on personality has focused on species that exhibit dominance and aggression. Many of the most well-studied species in animal personality have established dominance relationships and engage in aggressive interactions (Armitage 1991; Festa-Bianchet 1991; Slagsvold 1993). Although dominance does not always predict aggression (Réale et al. 2000), both dimensions have been linked to reproductive success and survival (Smith & Blumstein 2008) as well as to other personality traits (Riechert & Hedrick 1993; Boogert et al. 2006; Dahlbom et al. 2011). However, not all species use aggression and dominance in their interactions, yet little is known about how personality in these egalitarian species is linked to fitness.

Cape ground squirrels (*Xerus inauris*) are highly social rodents found in arid savannah regions of southern Africa (Waterman 1995). Although females in this species show very low levels of aggression and no dominance hierarchy, previous research has demonstrated significant variation in reproduction among females (Waterman 1995; Pettitt & Waterman 2011). Nearly 70% of females that enter estrus fail to produce offspring (Waterman 1996; Pettitt & Waterman 2011). Furthermore, of 40 females, 13% of individuals raised multiple litters in a year, while 43% produced none at all (Waterman 1996). Research examining the cause of this differential reproduction has yielded mixed results; resource availability seems to have no effect on measures of fitness, while parasitism almost certainly plays a role as removal increased reproductive success fourfold (Pettitt et al. 2008; Hillegass et al. 2010). However, even within groups where parasites were experimentally removed, individual success still varied widely (Hillegass et al. 2010). In this study, we hypothesized that personality plays a role in reproductive skew among adult females. We tested the following predictions in a free-living population of Cape ground squirrels:

1. Personality traits correlate to form behavioral syndromes. Specifically, individuals who demonstrate relative boldness have higher exploration and lower docility constituting a fast/proactive behavioral syndrome.
2. Proactivity is associated with higher reproductive success.
3. Individuals who are more proactive have lower survival.

Methods

Trapping and Handling

We conducted all experiments at SA Lombard Nature Reserve (27°35’S, 25°35’E) near Bloemhof, South Africa during the austral winter (April-September 2014). Squirrels at this site have been monitored since 2002. The trapping area is split into three distinct areas, the house, the floodplain and the pan, which differ in levels of human contact and predation pressure (both low in the pan and floodplain and high in the house area). Previous research has also found that squirrels in the floodplain area invest more time in vigilance behavior then those in the house area (Unck et al. 2009). Twice a day, we trapped squirrels using live traps (Tomahawk Live Trap Inc., WI; 15 cm high X 15 cm wide X 50 cm long) baited with peanut butter and birdseed (Thokomon Foods SA LTD, Pretoria, SA; Kinko Birdfeed, D. Kingsbury, Johannesburg, SA). Once trapped, squirrels were transferred to a canvas, cone-shaped handling bag (Koprowski 2002). During handling, we injected passive integrated transponders (PIT) tags (FECAVA, 125 KHz, AVID Inc. Noroco, LA) and applied freeze marks (Quick Freeze; Rood & Nellis 1980) on individuals for permanent identification. We weighed squirrels using a 1000g spring scale (Pesola AG, Baar, Switzerland) and took spinal measurements from the base of the neck to the base of the tail using a measuring tape to estimate body condition (Schulte-Hostedde et al. 2005).

We determined the age class of females by the state of the nipples; subadults have small, inconspicuous nipples, whereas adults have longer, swollen nipples (Waterman 1995). Age was further estimated using long-term trapping data. Squirrels were considered juveniles from emergence to six months old, whereas squirrels were classified as subadults between six months and the time of sexual maturity (Waterman 1996; Pettitt et al. 2008). Females that were first captured as juveniles were aged counting back from 2014 (i.e. a juvenile first caught in 2012 is 2 years old) (Pettitt et al. 2008). We determined that any female first captured as a subadult would have been born in the preceding 6-9 months (Waterman 1995) and therefore added 0.5 years to their age estimate. This method allowed us to estimate the ages of 25/49 females in our sample. To estimate the age of the remaining individuals, who were first captured as adults, we added 1 year to the time elapsed since first capture. Because social groups tend to occupy similar areas, female emigration is rare and trap success is high, we are confident that these individuals were born in the preceding year (Waterman 1996).

Reproductive success and survival

Female reproductive success was measured by determining the maternity of all emerged juveniles and subadults captured from 2011-2015. During pregnancy and lactation, female Cape ground squirrels isolate from their social groups (Waterman 1996). Upon emergence and weaning of offspring, females return to their social groups with their offspring, where multiple adults interact with and care for the young (Waterman 1995). Because of this alloparental care, maternity can be difficult to assign based on behavioral observations alone. We therefore determined maternity using microsatellite primers and the computer program CERVUS 3.0 (Kalinowski et al. 2007).

During trapping, we collected a small amount of skin from the tip of the tail of all individuals and stored it in 95% ethanol (Manjerovic & Waterman 2015). We extracted DNA from tail-tip tissue samples using an E.Z.N.A ® Tissue DNA Kit (Omega Bio-tek, Norcross, GA). We amplified the DNA using eight previously identified primers (Abercrombie et al. 2009) as well as 12 newly developed primers. We used an Applied Biosystems 3130xl genetic analyzer to fingerprint the amplified DNA, and we scored each allele using Genemarker (v.2.6.0).

With the program CERVUS 3.0 (Marshall et al. 1998), we used microsatellites from 462 individuals (both male and female) to perform an allele frequency analysis, which calculated the number of alleles at each microsatellite locus, determined heterozygosity and verified that the selected microsatellites were acceptable for use in parentage analyses. We then conducted a simulation of parentage analysis (mother alone, no known father) of 100,000 offspring to determine how likely parentage is to be assigned correctly (Kalinowski et al. 2007). Using behavioral observations and trapping data, we estimated that 99% of candidate mothers were sampled with an average of 5 candidate mothers per offspring (Waterman 1996). We excluded 9 offspring and 10 potential mothers who had fewer than 15 loci genotyped. Using trapping and observational data, we constructed lists of adult females in the social group that were candidate mothers for each juvenile (mean = 5 candidate mothers/juvenile). We calculated a measure of long-term reproductive success by totaling the number of offspring that each female in our sample produced over the four years. We measured survival by determining whether individuals were present during the following three trapping seasons (survival to 2015, survival to 2016, and survival to 2017).

Personality

We measured and analyzed personality following the methods outlined in Chapter 2. Briefly, we measured three commonly identified personality traits: exploration (decomposed into three components using principal components analysis: exploration/activity, vigilance and fecal index), defined as an animal's response to a novel situation (Réale et al. 2007) and measured by an open-field test (Walsh & Cummins 1976); boldness, defined as an animal's response to any non-novel potentially dangerous situation (Réale et al. 2007) and measured by a flight initiation distance test (Blumstein 2003); and docility, defined as a an animal's response to handling and measured by a handling index (Réale et al. 2000)**.** We used these measures and environmental variables (date, time of day, trial number, area within the study site, cloud cover, wind speed) from each of these tests to construct linear mixed-effects models with squirrel ID as the random effect. From these models, we extracted best linear unbiased predictors (BLUPs), which gave us a single score for each individual for each personality trait.

We compared personality BLUPs to reproductive success using linear models with age as a fixed effect. Residuals were examined for normality. We used logistic regressions to compare personality BLUPs to survival. All calculations were performed in R (Revelle 2010; R Development Core Team 2015) and had significance set to $\alpha = 0.05$. Descriptive statistics are reported as mean ± SE.

Results

We found that all 20 microsatellites had sufficient polymorphic information content (mean = 0.64 ± 0.03) and did not deviate from the Hardy-Weinberg equilibrium. Of 462 individuals, an average of 91.6% loci were typed. Using this information, we were able to assign maternity to 204/242 juveniles and subadults born from 2011-2015 with 95% confidence (Kalinowski et al. 2017; Manjerovic & Waterman 2015). Of these, 111 were assigned to females from whom we were able to collect personality data. We found that increased docility was associated with increased activity (Figure 2) and decreased vigilance (Figure 3). No other correlations between personality traits were significant (Table 1). However, under high predation (pan and floodplain females pooled) boldness and fecal index scores were positively correlated (Pearson's product moment correlation; $t_{24} = 2.20$, p-value = 0.04). We found no relationships between exploration traits (activity/exploration, vigilance and fecal index) under low predation (house area). We did not have an adequate sample size to examine docility and boldness in the house area.

Females in this study were an average of 2.16 ± 0.14 years old and had an average of 2.15 ± 0.30 offspring. Overall, 30% of individuals left no offspring over the five-year period, while 20% left five or more. We found that reproductive success was affected by age (linear model; $t_{47} = 4.54$, $p < 0.001$, Figure 1), with the oldest individuals producing significantly more offspring over their lifetime than the youngest. We therefore included age in subsequent comparisons of personality and reproductive success. However, we found no significant effects of personality scores on reproductive success (activity, $t_{36} = -0.797$, $p = 0.43$; vigilance, $t_{36} = -0.809$, $p = 0.42$; fecal index, $t_{35} = -0.182$, $p = 0.85$; docility, $t_{35} = -0.087$, $p = 0.93$; boldness, $t_{31} = -1.12$, $p = 0.23$).

Overall, 12/49 individuals did not survive to the 2015 trapping season, while only 19 females of our original sample were trapped in 2016. Eleven females were still alive in 2017. We found that females lived to an average of 4.09 ± 0.19 years. Age did not affect the likelihood of survival to 2015, 2016, 2017 or beyond (Kruskal-Wallis test; $\chi^2_3 = 0.79$, $p = 0.85$). We did not find any relationship between personality traits and survival to any subsequent year: **2015** (logistic regression; activity, $z_{37} = -0.49$, $p = 0.62$; vigilance, $z_{37} = 0.65$, $p = 0.51$; fecal index, $z_{37} = 0.153$, $p = 0.88$; docility, $z_{31} = -1.20$, $p = 0.23$; boldness, $z_{32} = -0.67$, $p = 0.50$), **2016** (logistic regression; activity, $z_{37} = 1.06$, $p = 0.29$; vigilance, $z_{37} = 1.77$, $p = 0.08$; fecal index, $z_{37} = 0.43$, $p = 0.67$; docility, $z_{31} = -0.71$, p 0.48; boldness, $z_{32} = 1.81$, $p = 0.07$), or **2017** (logistic regression; activity, $z_{37} = 0.86$, $p = 0.39$; vigilance, $z_{37} = 1.48$, $p = 0.14$; fecal index, $z_{37} = 0.37$, $p = 0.71$; docility, $z_{31} = -0.71$, $p = 0.77$; boldness, $z_{32} = 0.99$, $p = 0.32$.

Discussion

We examined the relationship between three commonly measured personality traits (exploration, boldness and docility) and reproductive success and survival to understand more about the causes of reproductive skew in adult females of this species. Our results were consistent with previous work on Cape ground squirrels that has demonstrated that females have high reproductive skew (Waterman 1996). We found support for two behavioral syndromes. However, we found no evidence that personality was related to either reproductive success or survival.

We found that increased docility was associated with decreased vigilance and increased activity. Dosmann et al. (2014) found a negative relationship between docility and activity and exploration in Belding's ground squirrels (*Urocitellus beldingi*). However, other studies have found no evidence for behavioral syndromes involving docility (Martin & Réale 2008a, 2008b; Petelle et al. 2013). Like many ground squirrels, Cape ground squirrels rely on vigilance behavior to avoid predation (Unck et al. 2009; Edwards & Waterman 2011). Individuals who are tame, or docile, in their reaction to humans may be able to increase their overall activity and decrease their vigilance and thus increase food intake.

Behavioral syndromes may arise as a result of similar underlying physiological mechanisms governing each trait (Sih et al. 2004a; Réale et al. 2007). Previous research has found no evidence that personality traits are related to long-term measures of cortisol in Cape ground squirrels (chapter 1). However, more data are required to determine which physiological mechanisms (e.g. short-term cortisol response, other hormonal responses) regulate individual personality traits and therefore behavioral syndromes in this species.

Previous studies examining the relationship between reproductive success and personality in other species have reported mixed results. In a meta-analysis of 31 studies, Smith and Blumstein (2008) found that bolder and more aggressive individuals had higher reproductive success. Additionally, activity has been linked to secondary reproductive success indicators such as offspring growth rate and territory quality in red squirrels (*Tamiasciurus hudsonicus*; Boon et al. 2007, 2008). In contrast, other studies have found that less bold, more aggressive, more exploratory and more docile individuals enjoyed higher survival (Smith & Blumstein 2008; Réale et al. 2009). However, there remains no consensus on the topic with more recent studies finding the opposite relationship (i.e. bolder, less exploratory individuals have higher survival, Hall et al. 2015; Haage et al. 2017) while others have detected no links between personality and survival (Bijleveld et al. 2014).

Some research suggests that the effects of personality on reproductive success may vary over time and therefore differences in the link between personality and reproduction may depend on the length of time examined. For example, Patrick and Weimerskirch (2014) found no relationship between boldness or foraging score and fledgling success of black-browed albatrosses (*Thalassarche melanophrys*) in a single season. However, when looking at a longer period (23 years), bold females and those with a low foraging personality had a higher fitness, which was particularly strong in years with high resource availability. Interestingly, Cape ground squirrels may engage in both short and long-term inhibition of reproduction during energetically stressful events such as drought (Waterman & Fenton 2000; Pettitt et al. 2008). Although this study examined a relatively long time period (five years of reproductive data), due to a long life-span (up to 10 years; JM Waterman, unpublished data), if females were restraining or inhibiting their reproduction at the time of our study, we might not have collected sufficient data to give an accurate estimate of functional reproductive success.

We also found no evidence for a relationship between personality and survival. In contrast, Smith and Blumstein (2008) found that overall higher exploration led to higher survival whereas more recent studies have found that exploratory, bold and active individuals had lower survival (Boon et al. 2008; Hall et al. 2015; Niemelä et al. 2015). Bold common roaches (*Rutilus rutilus*) were more likely to be consumed directly by avian predators compared to shy individuals (Hulthén et al. 2017). Personality traits can vary over ontogeny and certain traits may produce higher survival during different life stages (Stamps 2007; Petelle et al. 2013; Ballew et al. 2017). Cape ground squirrels have high infant mortality (Pettitt & Waterman 2011) and because we only tested adult females, we may have missed the effects that personality exerts on survival at younger life stages.

The highly social nature of Cape ground squirrels means they also benefit greatly from enhanced predator avoidance through the dilution effect and collective detection (Edwards & Waterman 2011). Adult female squirrels spend about 50% of their time within 10m of another member of their social group (Waterman 1995). ‘Collective personality’ traits and behavioral syndromes have been demonstrated at the colony level in eusocial insects (Chapman et al. 2011; Wray et al. 2011) and these traits are linked to fitness outcomes (Blight et al. 2016). Although not colonial per se, the complexity of ground squirrel social systems may mean that many factors interact to affect fitness outcomes (Bengston & Jandt 2014). For instance, a shy, yet vigilant individual might ‘protect’ a bold individual and even its offspring by providing alloparental care. Consequently, it would be beneficial for future research to examine the full composition of personality within groups to determine if certain behavioral types interact to allow females to achieve better fitness outcomes.

A key assumption of Wolf et al.'s (2007) model is that resources are heterogeneously distributed throughout the landscape. Cape ground squirrels feed primarily on seeds and grasses that are abundantly available throughout the area surrounding burrow openings, known as a burrow cluster (Herzig-Straschil 1978; Skurski & Waterman 2005). Indeed, previous research has demonstrated that females spend a majority of their above-ground time in close proximity to their home burrow cluster (Waterman 1995). Furthermore, a single burrow cluster can have several dozen openings, increasing the chances of successfully escaping predation (Herzig-Straschil 1978; Waterman 1995; Skurski & Waterman 2005). As a result, individuals may not have to make foraging decisions that significantly affect survival. Pulsing resources that differ among years (i.e. masting) are also suggested to be a mechanism that could drive the maintenance of personality through the life history trade-off hypothesis, as different traits may be adaptive in different years (Dingemanse et al. 2004). As a result, some personality profiles may have a relatively higher reproduction in resource-sparse years.

This could explain yearly differential reproductive success in female Cape ground squirrels but would not necessarily result in differential reproductive success when examined over an individual's lifespan. While we do not explicitly refute this hypothesis, resource availability does not affect reproductive success in this species (Pettitt et al. 2008).

Cape ground squirrels are cooperative breeders that do not have dominance hierarchies and rarely interact aggressively within their social groups (Waterman 1995, 1996; Pettitt & Waterman 2011). Aggression is common in other sciurid species and has been shown to be important in reproductive success. For instance, Viblanc et al. (2016) found that the most aggressive female Columbian ground squirrels (*Urocitellus columbianus*) were able to spend more energy on reproduction while Boon et al. (2007) found that more aggressive red squirrel (*Tamiasciurus hudsonicus*) mothers had young who were more likely to survive the winter. In general, aggressive individuals are often more dominant (Kim & Zuk 2000; Kralj-Fiser et al. 2009; McGhee & Travis 2010) and have higher reproductive success (Lill 1965; Cheng & Burns 1988). The link between aggression and other traits is particularly well documented (e.g., boldness: Bell 2005; Johnson & Sih 2005; Dingemanse et al. 2007; Dochtermann & Jenkins 2007; Reaney & Backwell 2007; Sih & Bell 2008; Martins & Bhat 2014). If traits are governed by similar physiological mechanisms, strong selection for aggressive behavioral types may mean that other proactive traits are selected for. There is seemingly no selective pressure for Cape ground squirrels to develop aggression, as resources are abundant and males rely on post-copulatory sperm competition (Herzig-Straschil 1978, Skurksi and Waterman

2005, Waterman 1998). Because of their unique social structure, individuals may be more behaviorally plastic in changing functional contexts, such as interacting with humans and foraging. Long-term behavioral testing of personality (i.e. between years and life stages) could confirm the stability or plasticity of personality in Cape ground squirrels.

Overall, we found that female Cape ground squirrels have several consistently repeatable personality traits (boldness, activity/exploration, fecal index and docility) and behavioral syndromes, yet we found no evidence that these traits were related to reproductive success or survival. These results suggest that, over the time period monitored in this study, the life history trade-off hypothesis as applied to animal personality is not be operating to select for traits and syndromes in this species. We suggest that that future research in Cape ground squirrels examine if traits are consistently repeatable over longer timeframes and if they relate to lifetime reproductive success. Furthermore, while societies with such low aggression are undoubtedly rare (but see Strier 1992; Macdonald et al. 2002), more research is needed in these species to determine if personality and behavioral syndromes follow the same principles as in other more aggressive species.

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Figures and Tables

Figure 3.1. Overall offspring produced by adult female Cape ground squirrels and age (linear model; $t_{47} = 4.54$, $p < 0.001$).

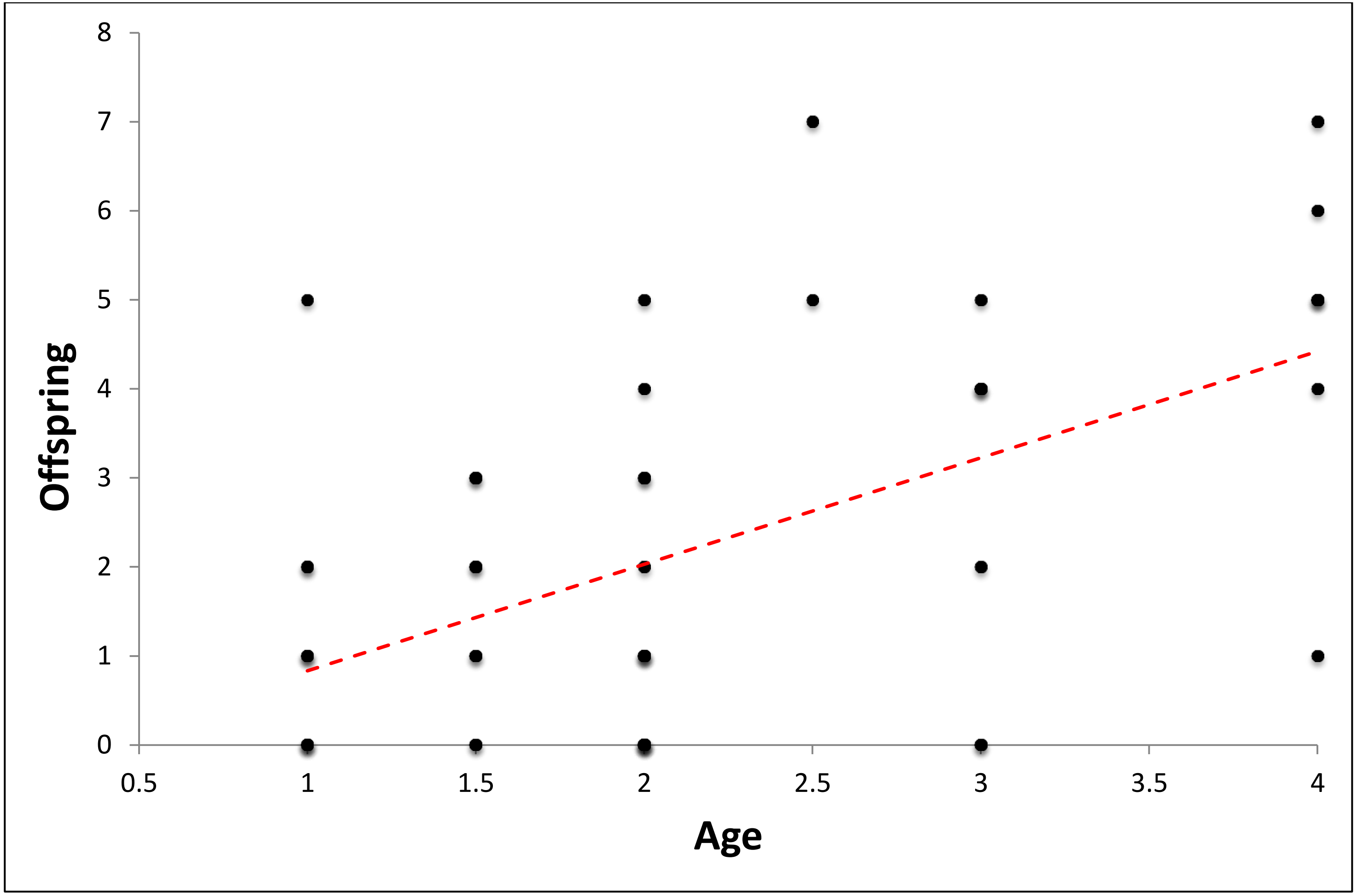

Figure 3.2. Behavioral syndrome between activity and docility scores for female Cape ground squirrels. Increasing activity/exploration score is characterized by decreasing proportion of time walking, total squares visited and percent of squares visited. Increased docility score is associated with increased difficulty of handling.

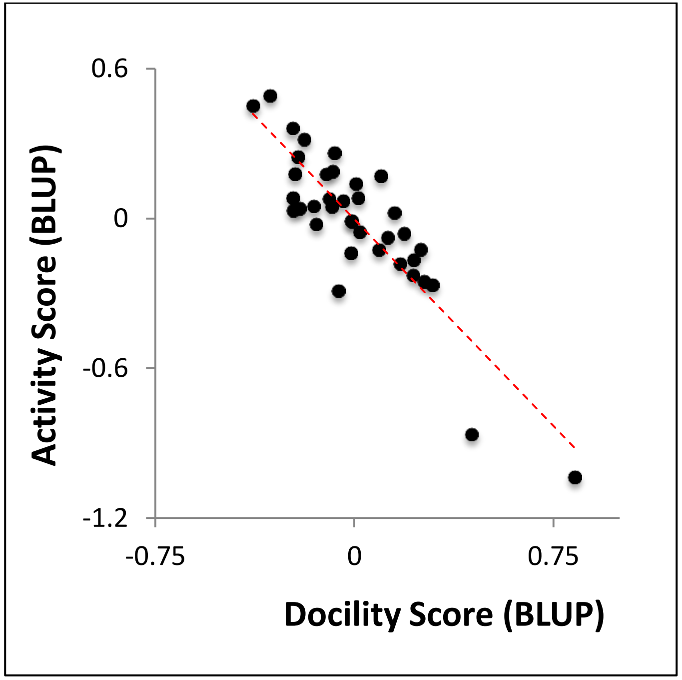

Figure 3.3. Behavioral syndrome between vigilance and docility scores for female Cape ground squirrels. Increasing vigilance score is characterized by more time spent in high vigilance (rearing up) and less time spent walking. Increased docility score is associated with increased difficulty of handling.

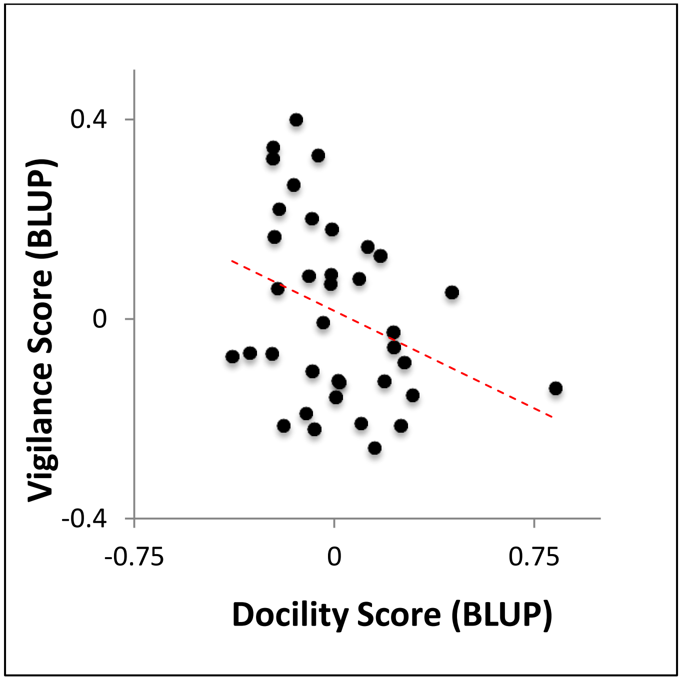

Table 3.1. Results of Pearson's product moment correlations for behavioral syndromes in a population of female Cape ground squirrels. Significant results are in bold.

	Activity/exploration	Vigilance	Fecal Index	Boldness
Vigilance	$t_{38} = -0.36$, $p = 0.72$			
Fecal Index	$t_{38} = -0.24$, $p = 0.81$	$t_{38} = -0.15$, $p = 0.88$		
Boldness	$t_{28} = 0.22$, $p = 0.82$	$t_{28} = 0.02$, $p = 0.99$	$t_{28} = 1.42$, $p = 0.16$	
Docility (Transfer)	**$t_{35} = -10.46$, $p < 0.001$**	**$t_{35} = -2.10$, $p < 0.05$**	$t_{35} = -0.64$, $p = 0.52$	$t_{29} = -0.53$, $p = 0.60$

Chapter 4 — Thesis Conclusions

Although I found support for several consistently repeatable personality traits including docility, boldness, activity and a fecal index in Cape ground squirrels, the proximate and ultimate aspects of personality traits were less clear. I did not find any support for the hypothesis that long-term cortisol levels are related to personality traits. Furthermore, I found no evidence that personality is ultimately maintained in this species by influencing reproductive success and survival.

My work found no evidence that long-term cortisol was related to any personality traits. This result may be because cortisol levels do not mediate personality traits. However, it is likely that differences in short- and medium-term cortisol responses, detected in the blood and feces respectively, may more accurately reflect differences in personality traits. Comparison of cortisol in blood, fecal and hair samples concurrently would be the next step in testing this prediction (Øverli et al. 2002; Voellmy et al. 2014). Experimentally lowering or increasing hormones at varying time scales and measuring personality traits would also test the role that cortisol may play in mediating individual differences in behavior in this species. I hypothesized that hair may better reflect cortisol levels under natural conditions in this population because humans are not present during the time of hair growth (Herzig-Straschil 1978). A validation study using exogenous cortisol would also clarify if cortisol is incorporated into the hair in a proportional fashion (Touma & Palme 2005).

I found no evidence for a relationship between personality traits and reproductive success or survival. This result raises an important question: how are personality traits in this species ultimately maintained and why do they exist in the first place? Personality traits can vary over life stages (Stamps 2007; Petelle et al. 2013; Ballew et al. 2017), and further work could determine if certain traits affect life stages differently or increase the survival of juveniles to adulthood. Further monitoring at this study site will also allow us to determine the lifespan of individuals. The number of offspring left by a single individual may also not be sufficient to determine reproductive success. 'Collective personality' refers to traits that emerge at the group-level and may be more than just the sum of personalities of individuals that make up the group (Bengston & Jandt 2014). Cape ground squirrels form strong social groups (Waterman 1995) that may have key individuals, or leaders, that influence the collective expression of personality in the group (Conradt & Roper 2003; Bengston & Jandt 2014). It would be of interest to determine how the personalities of all individuals in a group interact to influence the persistence of social groups. These interactions could be measured by how the colony as a unit moves or reacts to predators, as opposed to individual responses (Bengston & Dornhaus 2014).

Extrinsic factors like date, time of day and area within the study site all significantly affected personality tests (Chapter 2). Weather conditions and other environmental factors significantly affect behavior in general, so they should influence personality as well. In Cape ground squirrels, high winds and temperatures lower overall activity, while predation risk influences vigilance (Herzig-Straschil 1979; Unck et al. 2009). Many studies of personality fail to justify their use of environmental variables, or even exclude them altogether. Some of these variables certainly affect the expression of personality, whereas others do not, and the influence of factors like predation risk could change over time or season (Petelle et al. 2013). It would be beneficial to conduct experimental manipulation, especially on free-living vertebrates to determine which factors uniformly affect behaviors. Furthermore, it would be interesting to determine if influential environmental factors affect within or among individual variation in the expression of traits (Dosmann et al. 2014).

Finally, female Cape ground squirrels are unique in that they have extremely low levels of aggression, do not hold territories and have no measurable dominance hierarchy (Waterman 1995, 1996; Pettitt & Waterman 2011). Resources are abundant and males rely on post-copulatory sperm competition (Herzig-Straschil 1978; Waterman 1998; Skurski & Waterman 2005), allowing groups to form that benefit from collective vigilance (Edwards & Waterman 2011). In other social species, aggression and dominance are linked to reproductive success (Lill 1965; Dunbar & Dunbar 1977; Lamprecht 1986; Cheng & Burns 1988; Paull et al. 2010). Thus, there may be strong selection for aggressive behavioral types and as a result, other proactive traits may be selected for. More research could determine if Cape ground squirrels may be able to be more behaviorally plastic in changing functional contexts than species that are constrained by aggression. Furthermore, other personality traits that I did not measure, such as social tolerance (Cote et al. 2008), may exert significant influence on reproductive success and survival in this species. It is unclear if the lack of aggression affects other ultimate and proximate mechanisms that maintain personality but it would be of interest to compare this work to studies conducted in other low aggression species, such as muriquis (*Brachyteles arachnoides*; Strier 1992) or European badgers (*Meles meles*; Macdonald et al. 2002). Future work in these areas could help to elucidate the proximate and ultimate mechanisms that maintain personality.

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Waterman, J. M. 1998. Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Animal Behaviour*, **56**, 459–466.

Appendix A – Chapter 1 supplemental graphs

Figure A1. Long-term cortisol and boldness scores for Cape ground squirrels (Pearson's product moment correlation; t_{32} = 0.69, *p* = 0.50).

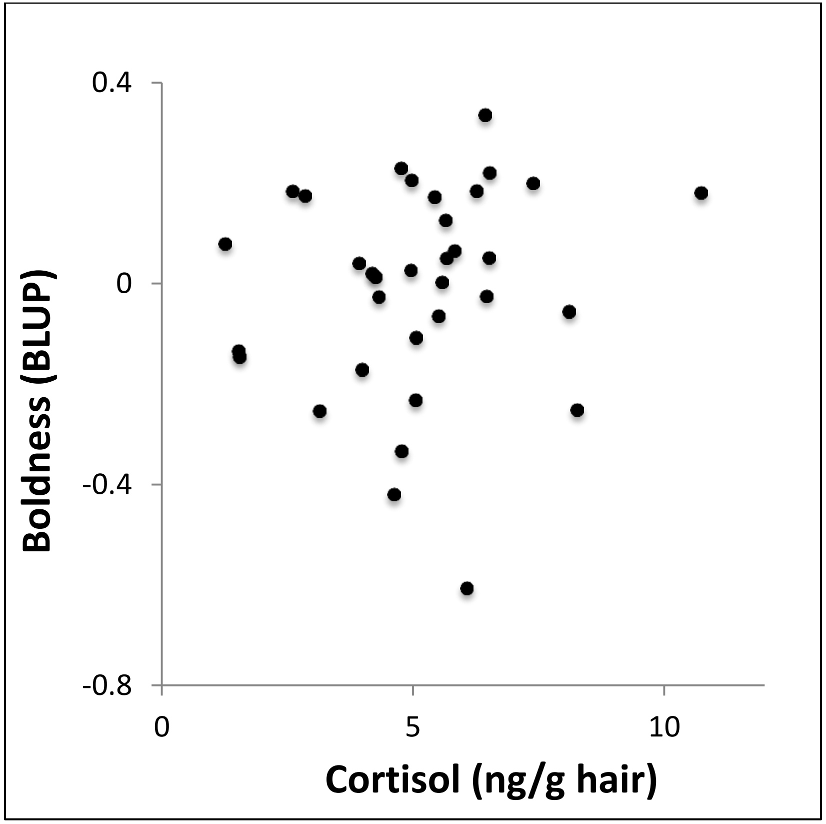


Figure A2. Long-term cortisol and docility scores for Cape ground squirrels (Pearson's product moment correlation; t_{31} = 0.28, *p* = 0.78).

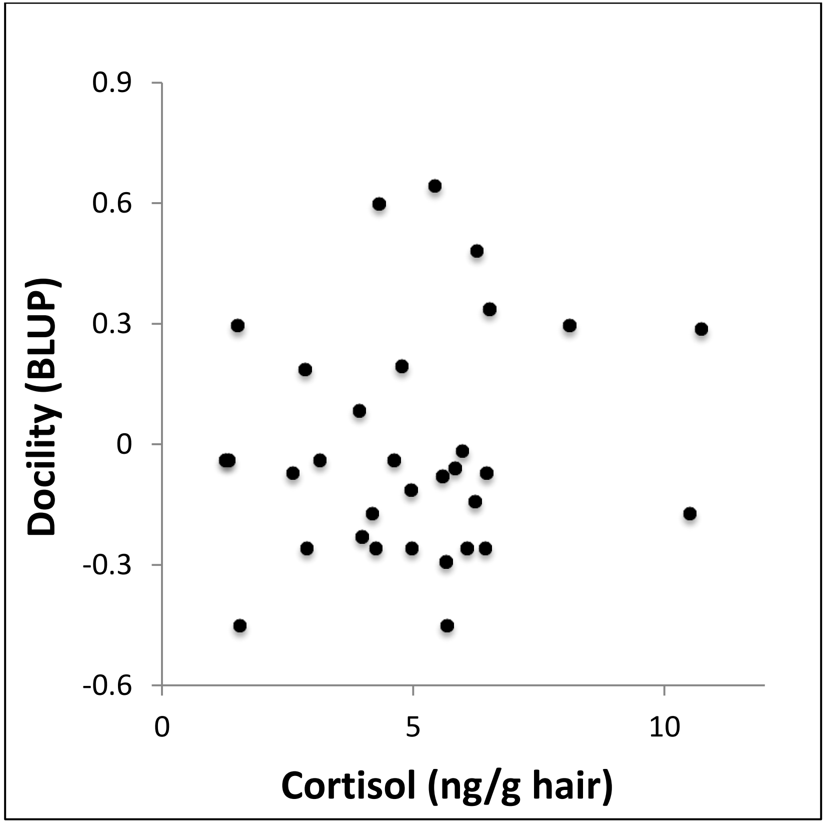


Figure A3. Long-term cortisol and activity scores for Cape ground squirrels (Pearson's product moment correlation; t_{38} = 0.53, *p* = 0.61).

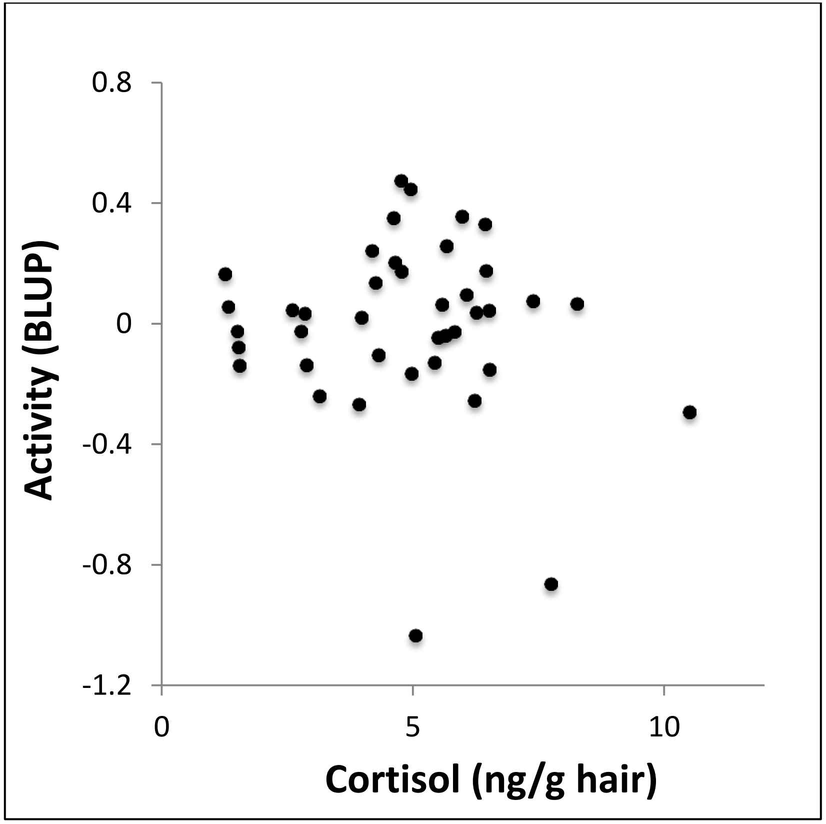


Figure A4. Long-term cortisol and vigilance scores for Cape ground squirrels (Pearson's product moment correlation; t_{38} = 1.37, *p* = 0.18).

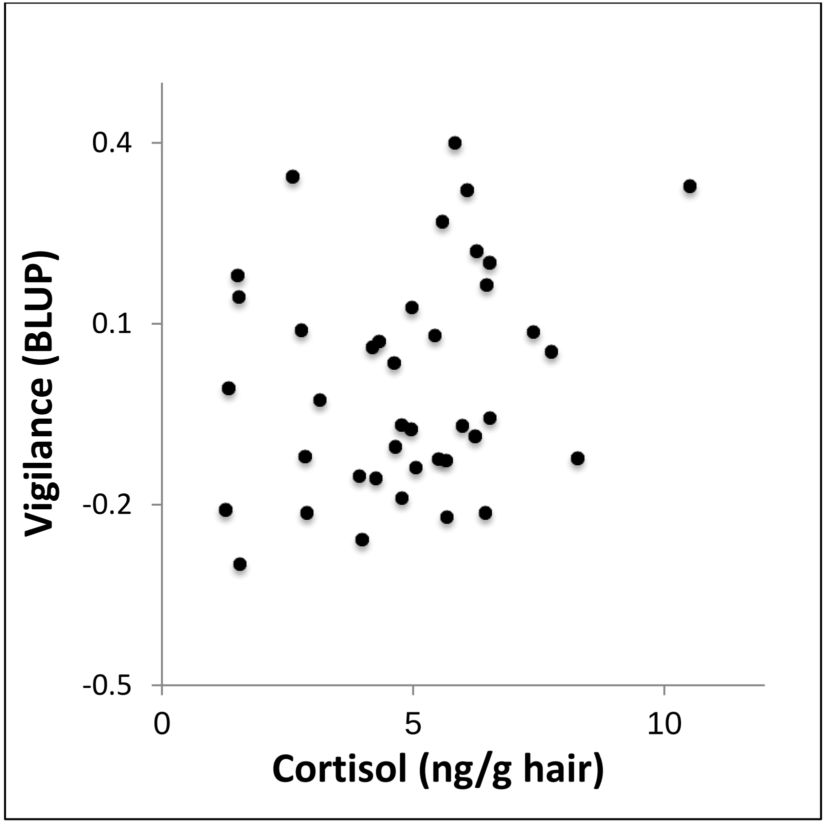


Figure A5. Long-term cortisol and fecal index scores for Cape ground squirrels (Pearson's product moment correlation; $t_{38} = 0.59$, $p = 0.56$).

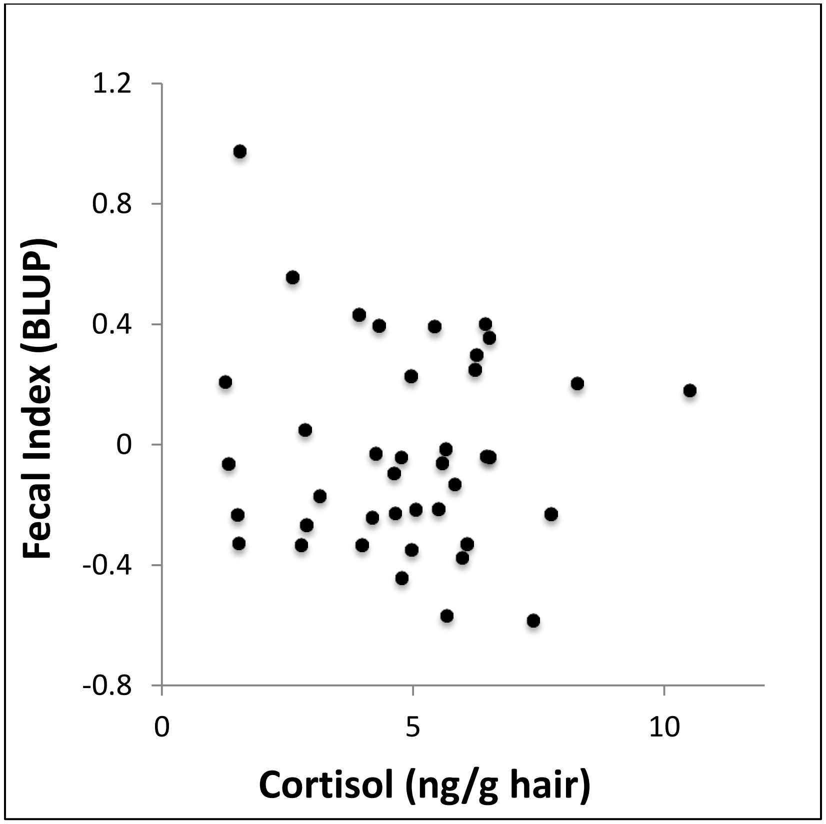


Figure A6. Body condition and boldness scores for Cape ground squirrels (Pearson's product moment correlation; $t_{32} = -1.21$, p = 0.23).

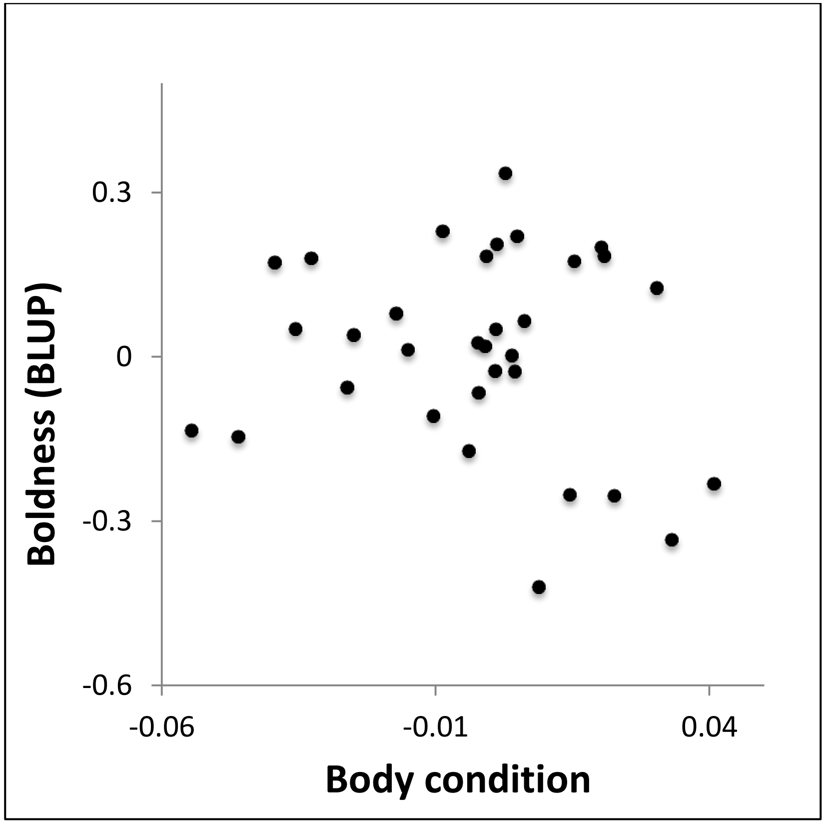


Figure A7. Body condition and docility scores for Cape ground squirrels (Pearson's product moment correlation; $t_{30} = 1.21$, p = 0.24).

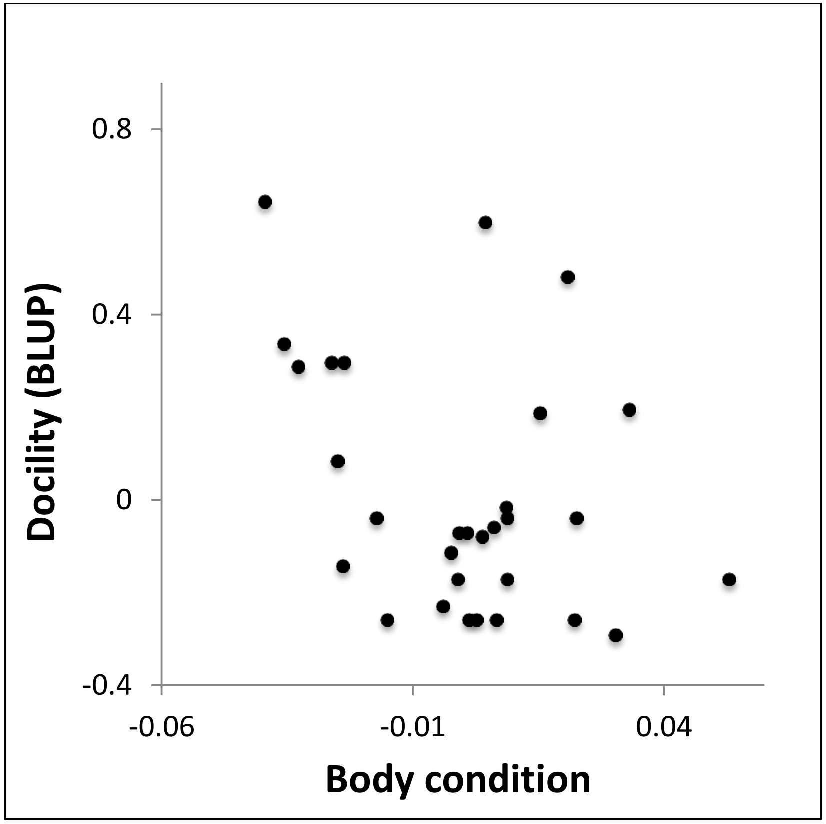
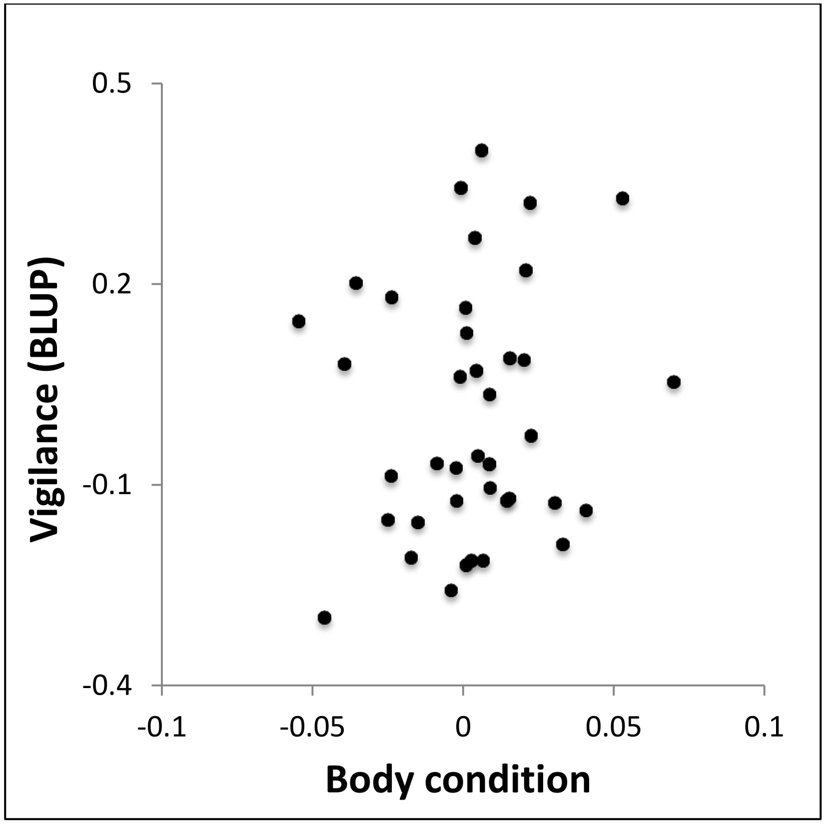
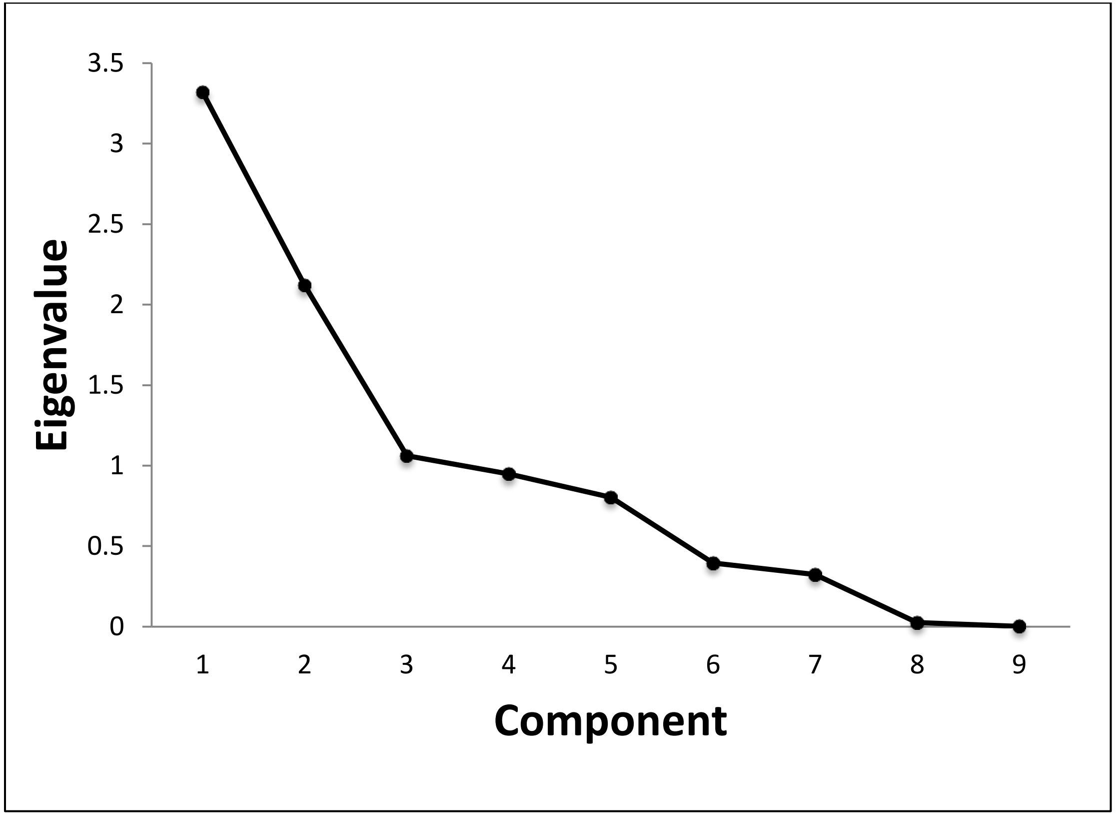


Figure A8. Body condition and vigilance scores for Cape ground squirrels (Pearson's product moment correlation; $t_{37} = 0.71$, $p = 0.48$).



Appendix B – Supplemental principal components analysis graphs

Figure B1. Scree plot for components in principal components analysis. Only components with eigenvalues greater than one were retained for further analysis.



Appendix C – Supplemental cortisol graphs

Figure C1. Results of wash test for cortisol on five samples of Cape ground squirrel hair.

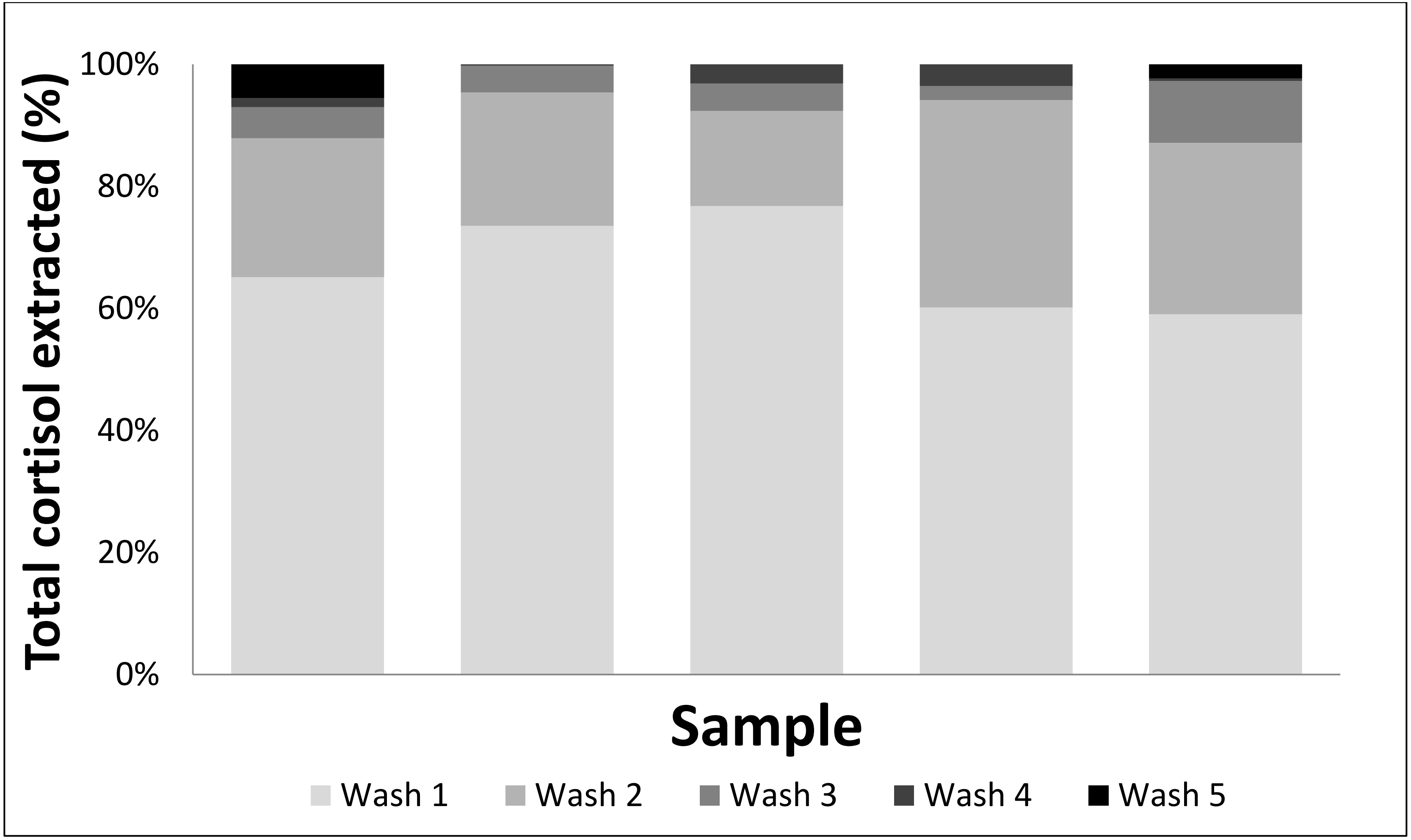


Figure C2. Example standard curve of cortisol concentrations

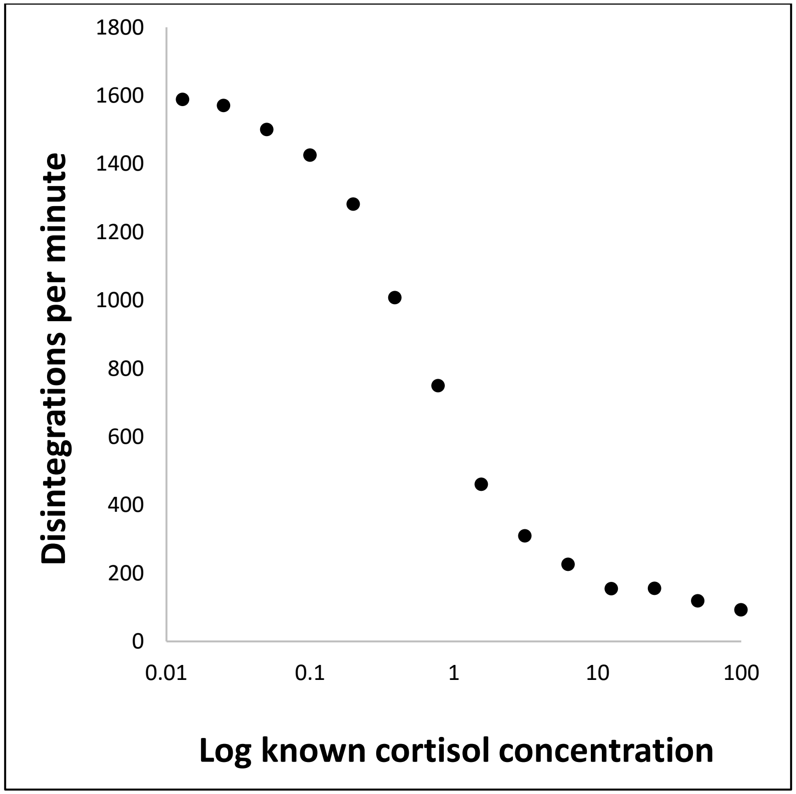


Figure C3. Results from parallelism validation (linear model; $p < 0.05$, $r^2 = 0.94$).