

CONSISTENCY OF STRESS HORMONE PROFILES AND ASSOCIATIONS BETWEEN
PERSONALITY AND HORMONE LEVELS IN A WILD BREEDING BIRD

by

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Abstract

CONSISTENCY OF STRESS HORMONE PROFILES AND ASSOCIATIONS BETWEEN PERSONALITY AND HORMONE LEVELS IN A WILD BREEDING BIRD

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Differences in personalities similar to those in humans have been documented in wild animals; individuals differ consistently in suites of correlated traits within and across contexts. Little is known about how hormones profiles may be related to animal personality. Because animals face ecological challenges, how an individual's endocrine system responds and adapts to stressors can affect their survivorship and reproductive output. Although stress hormones might mediate behavior, researchers have rarely tested whether stress hormone profiles are consistent within individual animals. In the summer of 2014, I studied a breeding population of wild tree swallows (*Tachycineta bicolor*) to address the following questions: 1) Do individuals express parental defense aggression that is consistent when measured multiple times during the breeding season? 2) Are blood glucocorticoid levels consistent within individual animals when measured multiple times during the breeding season? 3) Is the bird's personality (i.e. aggression) correlated with glucocorticoid levels during breeding season? I predicted that personality and glucocorticoid levels would be consistent (statistically repeatable) and correlated with parental defense aggression. I measured parental defense behavior towards models of nest predators twice during the nestling rearing season. I found high repeatability of female aggression; females that were aggressive toward mock predators during the first trial were also aggressive one week later. Moreover, I found

that baseline glucocorticoid levels were repeatable and that females with lower baseline glucocorticoid levels defended their nestlings more vigorously. These data suggest that stress hormone profiles are predictable within females and that females may experience hormone-mediated tradeoffs between self-preservation and caring for young. Finally, my research suggests that hormone levels may be an important proximate determinant of animal personality.

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Introduction

In the past decade, behavioral ecologists and evolutionary biologists have been increasingly interested in why animals of the same species, within the same population exhibit variation in behavior towards similar stimuli. That is, within a population, individuals show different responses, but within individuals, animals are often consistent in their behavioral profiles. Animal personality is defined as the tendency for individual animals to exhibit consistent behavioral tendencies within and across contexts (Clark and Ehlinger, 1987; Gosling, 2001; Sih et al., 2004).

Consistency in phenotypes is quantified by comparing repeated measures of among-individual and within-individual variance (Lessells and Boag, 1987). Animals that display personality are expected to be consistent in behavior, that is, to show low within-individual variance in behavior compared to the larger among-individual variance measured at the population level. Of course, individual animals are expected to also change their behavior in response to different stimuli or when environmental conditions change; phenotypic plasticity represents within-individual variation that is a consequence of the individual's ability to change from internal (i.e. genetic) and external (i.e. weather) environmental cues (Stearns, 1989). However, personality research has demonstrated that consistency in behavior limits the degree to which they are phenotypically plastic (Betini and Norris, 2012).

To assess personality, researchers often use a common suite of behaviors, such as aggression, risk taking, and exploration (Carere et al., 2005; Koski, 2014), which are often correlated with each other. Animal personality also often involves measuring multiple behavioral traits (e.g. aggression and exploratory behavior) and those traits

are expected to be correlated; these suites of correlated traits are referred as behavioral syndromes (Sih et al., 2004). The result is that individual animals show suits of behaviors thought to be under selection, because they represent strategic behavioral profiles (or personality types or coping styles) that enable individuals to cope with difficult situations in their environment (Cockrem, 2007; Koolhaas et al., 1999; Baugh et al., 2013). These personality types have been shown to be heritable, related to fitness, and shaped by evolution (Bell et al., 2009; Cockrem, 2005; Ouyang et al., 2011).

Aggression associated with breeding, such as territorial defense and protection of offspring, is one of the most commonly studied personality traits in wild birds (Montgomerie and Weatherhead, 1988). In obligate secondary cavity-nesting birds (i.e. those that do not excavate their own nesting cavities), nest sites are often limited (Newton, 1994). This results in extreme competition for nesting cavities and, as a consequence, both males and females of many secondary cavity-nesters are extremely aggressive (Robertson et al., 1992; Krist, 2004). Among socially monogamous birds, aggression is also an important component of parental care and predation is one of the most important factors affecting nestling survival (Ricklefs, 1969). Aggression is an important component to territorial defense but behaviors associated with defending offspring are often risky and can result in injury, or even death, of the parents.

Recently, behavioral ecologists have become interested in the proximate physiological determinants of animal personality and hypothesize that personality is related to how individuals respond physiologically to their environment (Ouyang et al., 2013). Researchers have proposed that endocrine signals are involved in the underlying mechanism of personality variation because hormones are key to

understanding the physiological mechanisms that organize behavioral traits (Baugh et al., 2012). If personality types are hormonally mediated, hormone profiles should be repeatable (Angelier et al., 2010; Koolhaas et al., 1999; Ouyang et al., 2011) and hormone and behavioral profiles should be correlated (Baugh et al., 2012; Koolhaas et al., 1999; Ouyang et al., 2011; Ouyang et al., 2013).

Although studies of how wild birds respond hormonally to their environment and how hormones mediate behavior are well studied (Arnold and Breedlove, 1985; Wingfield, 1987), fewer studies have focused on measuring within-individual variation in hormones and correlations between hormone profiles and personality types (Baugh et al., 2014; Bonier et al., 2009). Understanding the extent to which animals display consistent hormonal profiles will allow researchers to better understand the individual's physiological phenotype within a wild population (Angelier et al., 2010; Rensel and Schoech, 2011) and whether the hormonal profile plasticity can respond to selection (Ouyang et al., 2011; Williams, 2008). However, detecting significant correlations between personality and endocrine profiles may be difficult if animals have low repeatability or animals are not maintaining consistent behaviors or hormone profiles. Indeed, because hormone concentrations can fluctuate quickly in response to environmental stimuli (Williams, 2008), it may be too simplistic to assume that a single blood sample to measure hormones represents the individual's entire hormonal phenotype (Bonier et al., 2009). Therefore, researchers need to sample hormone concentrations concurrently with behavioral observations, or at least within the same life history stage, otherwise high within individual variation in hormones might obscure biologically meaningful relationships (Ouyang et al., 2011).

Glucocorticoids hormones play an important role in mediating how individuals respond to environmental changes and can result in multiple simultaneous physiological effects on the body and the behavioral phenotype (Romero, 2004). Glucocorticoids are the primary mediators of allostasis and can vary rapidly in response to both internal and external environments cues (Ketterson and Nolan, 1999). Corticosterone (CORT) is the main glucocorticoid steroid hormone of birds and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. The HPA maintains baseline concentrations of CORT as well as unpredictable and stressful events, which allows birds to respond appropriately to unexpected ecological conditions (Grace and Anderson, 2014; Lendvai et al., 2011), predators (Cockrem and Silverin, 2002) and unfavorable weather conditions (Breuner and Hahn, 2003; Ouyang et al., 2011) by increasing plasma levels of CORT available to the body (Baugh et al., 2013). High levels of CORT can stimulate foraging when individuals have low on energy levels, but at the same time, high CORT can suppress immune activity and reproductive functions (Baugh et al., 2012; Sapolsky et al., 2000). Due to the regulation of the HPA from various environmental conditions, different levels of CORT maintain many mechanisms involved with behavior and homeostasis. Moreover, because the HPA axis regulates behavioral components and because CORT has the ability to act on multiple physiological mechanisms, CORT and behavior should correlate (Baugh et al., 2012; Ketterson and Nolan, 1999; Korte et al., 2005). Thus, glucocorticoid physiology could be a fundamental proximate component of animal personality (Korte et al., 2005).

Here, I studied a population of wild tree swallows (*Tachycineta bicolor*) to address the following questions: 1) Is territorial defense aggression repeatable within

individuals when measured multiple times during the breeding season? 2) Are baseline concentrations of CORT individually repeatable when measured multiple times during the breeding season? 3) Is baseline CORT response correlated with aggression? I hypothesize that both behavior and CORT profiles are repeatable and that behavior and CORT are correlated. I predict that that females with lower baseline CORT, because they less likely to be experiencing food stress and should be better able and willing to devote energy toward protecting their offspring.

Methods

Study Species

Wild breeding tree swallows are an ideal species to explore relationships between hormones and behavior and are one of the most well-studied passerines in North America (Robertson et al., 1992). Tree swallows are socially monogamous, readily use nesting boxes, exhibit bi-parental care, aggressively mob predators, and readily defend their nesting cavity against conspecifics and heterospecifics (Robertson et al., 1992; Winkler, 1992). Aggression in tree swallows is repeatable when measured as behavioral responses associated with 1) guarding the cavity against conspecifics nest usurpers (Rosvall, 2008) and 2) mobbing potential predators of their nestlings (Betini and Norris, 2012).

General Field Methods:

I studied breeding tree swallows by monitoring 180 nest boxes during in the spring-summer of 2014 at a field site in Watauga County, NC. I recorded dates of nest

construction, egg laying, and hatching and noted the sizes of clutches and broods and the number of offspring that successfully fledged each nest. I captured females and obtained two separate blood samples for CORT assays. I also quantified parental defense aggression towards mock predators two times.

I measured baseline concentrations of CORT using the standardized protocol for capturing and handling wild animals (Baugh et al., 2014; Romero and Romero, 2002). First, using first egg date and clutch size and typical incubation period (15 days), I targeted capturing females on the day before the eggs hatched (± 1 day) and again when nestlings were 8 days (\pm day) old. I used nest box trap (Romero and Romero, 2002) and collected the initial blood sample within 3 min of first handling each bird and recorded the latency time from handling to blood collection (sec). Upon first capture, I fitted each adult female with a U.S.G.S. numbered band and one colored leg band. Additionally, I marked each female with Sharpie® marker on her abdominal feathers for subsequent behavioral observations such that mated pairs could be differentiated during behavioral trials.

Behavioral Assays

I measured parental response to mock predators two times; when nestlings were 3 days old (± 1 day) and 11 days old (± 1 day). I presented a model American crow (*Corvus brachyrhynchos*) positioned 0.3 m above the nest box. From 20-30-m away, I observed the behavior of both parents (Harris and Siefferman, 2014). During the 5 min trial period, I recorded number attacks per min for females and defined an attack as a complete dive within a 10-meter radius of the nest box.

Corticosterone Assays

CORT assays were measured by Alexandria Bentz at the University of Georgia. Serum CORT was extracted using cellite column chromatography following methods modified by Schwable (1993). Briefly, 20ul of serum was mixed with 3ml diethyl ether, vortexed and allowed to settle for 20min. Samples were snap frozen and the liquid portion containing CORT was reserved and dried using N₂. Samples were re-suspended in 1 mL of 10% ethyl acetate in isooctane and then eluted through columns in varying fractions of ethyl acetate in isooctane. Samples were dried using N₂ and CORT quantified using a competitive binding radioimmunoassay following Wingfield and Farner (1975).

Statistical Analyses

I log transformed corticosterone and aggression data to ensure that the data conformed to normality tests. I measured repeatability of corticosterone and of aggression using intraclass correlations (Lessels and Boag, 1987). I used Pearson correlations to test relationships between corticosterone levels and aggression and how corticosterone levels varied with human handling time prior to blood collection. I used paired t-tests to determine whether aggression or CORT varied significantly from week 1 to week 2 within individual females.

Results

I monitored 76 tree swallow nests and collected blood samples from 40 females in week 1 and collected blood from 16 of the same females in week 2. Additionally, I measured parental defense behavior of 52 females in week 1 and from 40 of the same females in week 2.

Timing of blood collection (time lapse between first handling and blood collection) was not significantly correlated with CORT concentration in week 1 ($r = 0.18$, $n = 37$, $p = 0.28$) or in week 2 ($r = 0.12$, $n = 12$, $p = 0.72$). Corticosterone levels were marginally significantly repeatable within individual females between week 1 and week 2 (intraclass $r = 0.44$, $n = 13$, $p = 0.056$; Fig. 1). Moreover, individual females exhibited significantly higher CORT in week 2 compared to week 1 (paired $t = 4.72$, $n = 13$, $p = 0.001$; Fig. 1).

Aggression level (attack rate) was significantly repeatable within individual females measured in week 1 and week 2 (intraclass $r = 0.62$, $n = 40$, $p < 0.001$; Fig. 2). Analyzing the difference in female aggression within individuals captured in week 1 and week 2 revealed no significant change in aggression (paired $t = 0.77$, $n = 40$, $p = 0.44$; Fig. 2).

Females that were more aggressive in week 2 exhibited significantly lower corticosterone levels in week 2 ($r = -0.58$, $n = 13$, $p = 0.039$; Fig. 3). However, female aggression in week 1 was not significantly associated with CORT in week 1 ($r = -0.16$, $n = 37$, $p = 0.34$). Moreover, female aggression in week 1 was not significantly associated with CORT levels in week 2 ($r = -0.01$, $n = 15$, $p = 0.96$) nor was aggression in week 2 significantly associated with CORT in week 1 ($r = -0.01$, $n = 30$, $p = 0.99$).

Discussion

My field data suggests that both aggressive behavior and CORT were consistent within wild breeding tree swallows. However, CORT levels were only marginally significantly repeatable while female aggression was highly significantly repeatable during the same timeframe, suggesting that behavior is more consistent than baseline CORT levels. Indeed, individual females did not become more aggressive as their offspring aged from newly hatched chicks to near fledgling age (11 days old), but those same females expressed higher CORT when nestlings were older. My data also revealed that CORT predicted aggressive behavior; females with lower baseline CORT behaved more aggressively toward the mock predator when nestlings were approximately 11 days old. Interestingly, I found no significant association between female CORT and behavior during the late incubation and early nestling rearing stage (one week earlier).

Individual female tree swallows showed consistent parental defense aggression between two consecutive weeks, corroborating research from a Canadian population of tree swallows that demonstrated repeatable parental defense aggression (towards humans; Betini and Norris, 2012) and from a population in Pennsylvania that showed repeatable territorial intra-sexual aggression (Rosvall, 2008). Together, these studies suggest that tree swallows exhibit personality (Betini and Norris, 2012; Rosvall, 2008). However, aggressive behaviors are highly energetically expensive, particularly during unexpected environmental conditions or when food availability is low or fluctuating greatly (Sih et al., 2004). Although tree swallows experience varying environmental condition, the general behavioral phenotype of individuals remains generally consistent and therefore, predictable (Betini and Norris, 2012). I found no evidence that

individuals exhibited plasticity in aggression; that is, the paired t-test that compared the behavior of individuals in week 1 and week 2 was not significant. These data differ from those of Betini and Norris (2012), who found that individual aggression within tree swallows is repeatable but also increases with ambient temperature (and presumably as a consequence of greater prey abundance). Indeed, a plethora of research demonstrates that animals adjust their behavior in response to changing environmental conditions (Komers, 1997). For example, in eastern bluebirds (*Sialia sialis*), individuals defend their offspring with more aggression as the nestlings increase in age (Siefferman et al., in review). The discrepancy in my results and those of Betini and Norris (2012) might be a consequence of differences in plasticity between the two populations of tree swallows or could result from having measured different environmental variables (weather and nesting timing).

My data also show some weak evidence of repeatability in baseline CORT concentration, even with a small sample size ($n = 16$). Few studies have reported repeatability in baseline CORT concentrations within individuals in a breeding population (Baugh et al., 2014; Ouyang et al., 2011). Female tree swallows in Ontario show repeatable baseline CORT during the breeding season (Ouyang et al., 2011) while great tits (*Parus major*) do not show repeatability either when measured within the non-breeding season (Baugh et al., 2014) or when measured across the breeding and non-breeding seasons (Ouyang et al., 2011). It is possible that repeatable behavior in the breeding season could be a consequence of more consistent endocrine profiles when investing in reproduction (Bonier et al., 2009).

To my knowledge, my data are the first to compare repeatability of baseline CORT with repeatability of behavior in the same population of birds. However, great tits show repeatable personality types and researchers have not documented consistent baseline CORT, however those datasets were collected in different populations at different times (Baugh et al., 2014; Ouyang et al., 2011). A possible reason for the lower repeatability of CORT compared to aggression in my study could be that individual birds quickly elevate CORT in response to acute stress (Baugh et al., 2014). CORT is metabolic hormone that responds to variation in environmental conditions including unexpected energy losses, variation in food availability, weather patterns and social interactions (McEwen and Wingfield, 2003; Ouyang et al., 2011). During a high-stress response from a perceived stimulus, CORT levels are elevated, which influence neurological pathways and therefore influence behavior (Rensel and Schoech, 2011). Also it is possible that when researchers capture birds repeatedly, individual birds may respond differently to this stress (differences in behavioral plasticity) and this could add noise to the dataset (Dohm, 2002; Love et al., 2003).

Another possibility is that seasonality influences the degree to which endocrine traits are consistent within individuals. During the breeding season, the endocrine system is expected to closely track reproductive investment (Ouyang et al., 2011) and thus may be expected to show lower repeatability. In recent studies, breeding female birds that achieve higher fitness experience greater increases in baseline CORT levels during the breeding season, suggesting that CORT is an accurate predictor of reproductive investment or the stress associated with rearing young (Bonier et al., 2009; Ouyang et al., 2011). Indeed, I found that individuals exhibited significantly

higher CORT profiles when rearing 11 day-old nestlings compared to when CORT was measured within a few days of egg hatching.

I also found that when rearing 11 day-old nestlings, females with lower baseline CORT were more aggressive to models of potential predators. To my knowledge, mine is the only study to demonstrate such a relationship. However, wild great tits with less exploratory personalities elevate their CORT response more quickly than do more exploratory (bolder) birds (baseline CORT was measured within 3 minutes of handling (Baugh et al., 2013)). Moreover, within great tits that have been selectively bred for personality traits, those that had less exploratory behavior (shier) exhibited higher acute stress response (higher CORT), suggesting that these two traits are genetically correlated (Baugh et al. 2012). I suggest that females with higher baseline CORT could be food stressed and when challenged with a nest predator, may be more likely to shunt their limited energy towards self-preservation and away from parental care. Indeed, elevated CORT stimulates foraging when food reserves are low but can also suppress reproductive investment (Sapolsky et al., 2000). Future research on this population will include habitat analyses to better characterize territory quality; it may be that females in lower quality territories exhibit higher baseline CORT.

My data showed significant correlations between baseline CORT and aggressive behaviors during week 2, when females were rearing older nestlings but I found no significant co-variation during the first week of sampling despite much larger samples sizes (Week 1, n = 40; week 2, n = 13). I also found no significant relationships between CORT and behavior when those parameters were compared across timeframes (e.g. week 1 CORT and week 2 behavior and vice versa). Perhaps during the window of

sampling in week 1 (from late incubation to chick rearing), females exhibited changes in CORT profiles- indeed CORT profiles in week 1 were only marginally repeatable with week 2. If baseline CORT changes with energy expenditure, then we might expect that females would experience different CORT profiles when primarily spending their time incubating eggs versus feeding young. This variation in CORT could have created noise in either or both the CORT and behavior datasets, making correlations between behavior and hormone profiles undetectable.

It is interesting that my data do not show parallel relationships between aggression and CORT when females are tending their newly hatched young versus tending 11 day-old nestlings. During the second week of nestling rearing (11 day-old nestlings), reproduction is expected to be more costly both because nestlings need to be fed more often and females' energy reserves are more depleted. During this time, the female is under higher physiological stress, which increases CORT levels (indeed the females I studied had higher CORT when offspring were older). Furthermore, because the nestlings are older and are closer to independence, they are more valuable to the parents (Trivers, 1972), and thus females should follow strategies in which they are more willing to experience physiological stress to ensure the survival of their young (Bonier et al., 2009). Indeed, other studies have found that female breeding birds that invest more in reproduction tend to exhibit higher baseline CORT concentrations (Love et al., 2003; Ouyang et al., 2011).

One limitation of my dataset is that I did not measure elevated corticosterone and some studies have shown that elevated CORT is a better predictor of behavior than is baseline (reviewed in Cockrom, 2007). However, my study provides evidence that

there are linkages between endocrine profiles and animal personality (i.e. behavioral traits). To date, little research has focused on the extent to which hormone profiles are consistent within individuals, the heritability of endocrine traits, and how hormones relate to personality traits (Ouyang et al., 2011; reviewed in Cockrom, 2007). Future studies should manipulate behavior using predator simulations and measure stress responses- including elevated stress.

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Figures

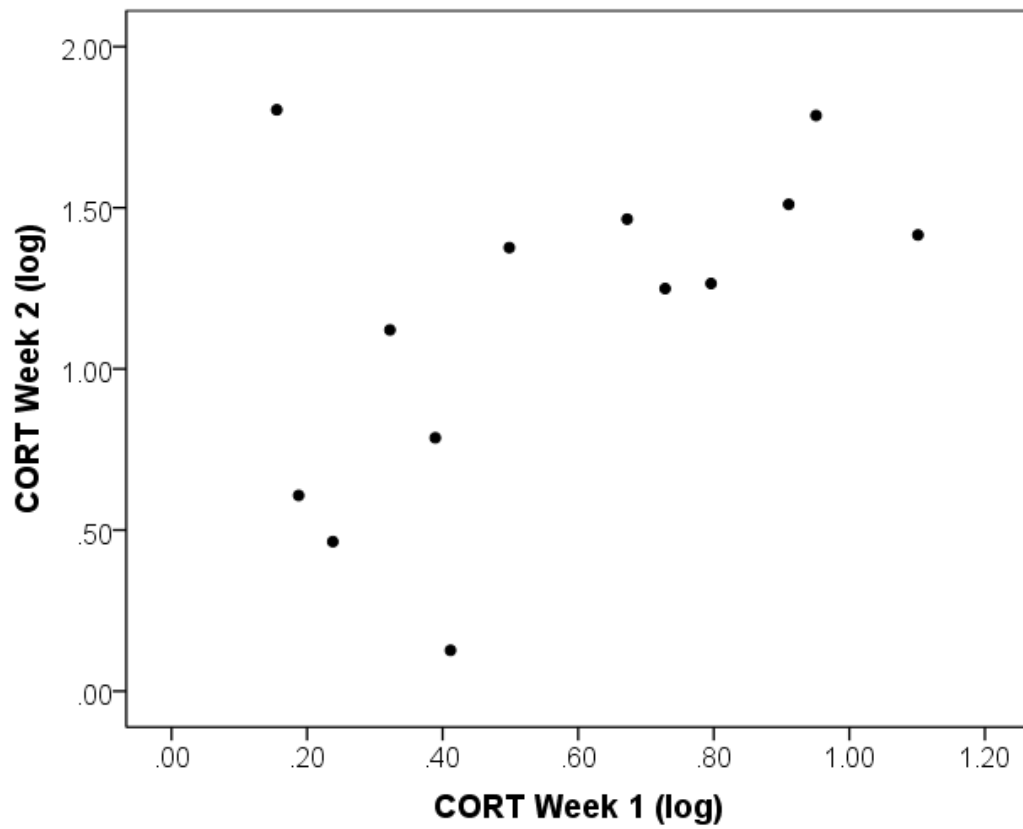


Figure 1. Female tree swallows exhibited marginally significantly repeatable baseline corticosterone levels when measured in week 1 and week 2 (intraclass $r = 0.44$, $n = 13$, $p = 0.056$).

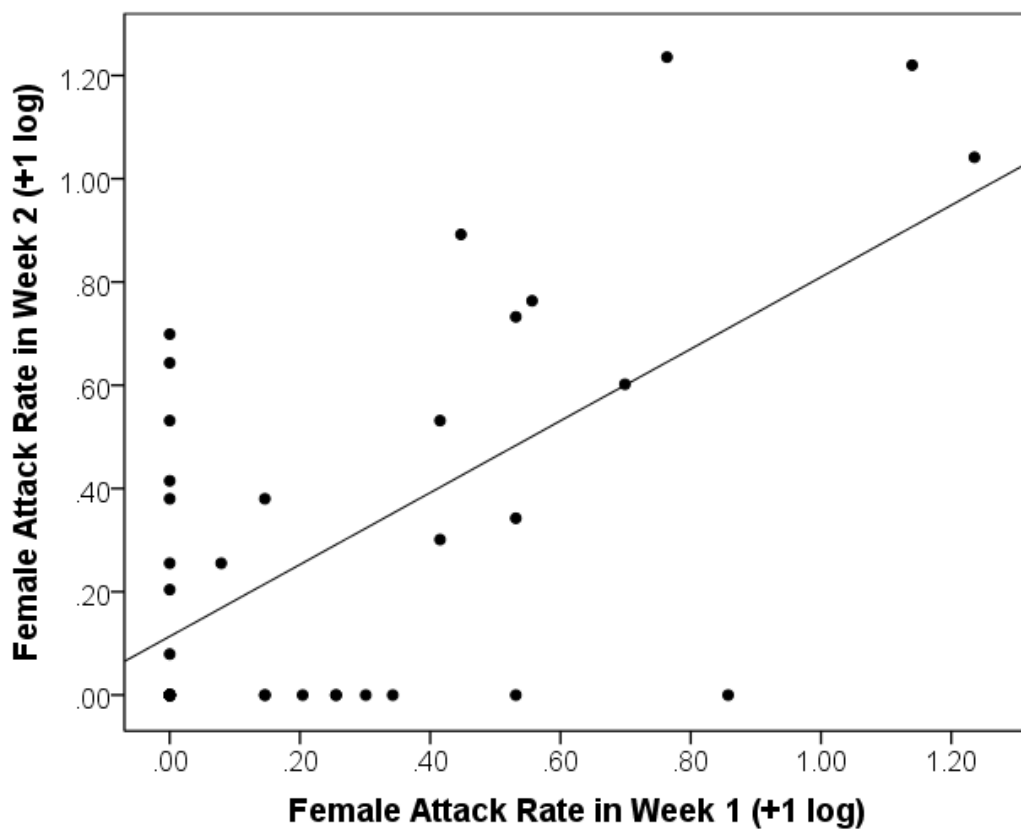


Figure 2. Female tree swallows exhibited significantly repeatable parental defense aggression (attack rate) when measured in week 1 and week 2 (intraclass $r = 0.62$, $n = 40$, $p < 0.001$).

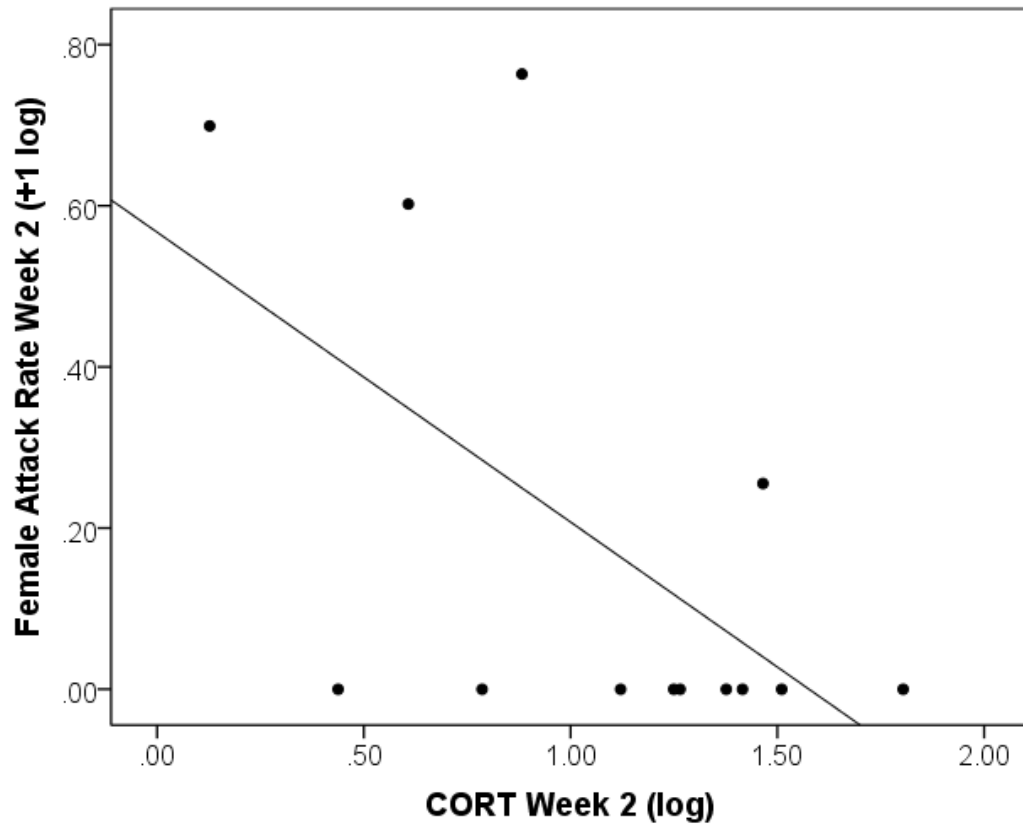


Figure 3. Highly aggressive *Tachycineta bicolor* females during week 2 had significantly lower levels of baseline corticosterone in week 2 ($r = -0.58$, $n = 13$, $p = 0.039$).