DEVELOPMENT OF THE STRESS RESPONSE IN FAST (*COTURNIX JAPONICA*) VERSUS SLOW (*CALLIPEPLA GAMBELII*) GROWING SPECIES

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Development of the stress response in fast (*Coturnix japonica*)

versus slow (*Callipepla gambelii*) growing species

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ABSTRACT

In this study, we evaluated the development of stress response in (1) two different species of quail, one species that is relatively fast-growing (Japanese quail, *Coturnix japonica*) and one that is relatively slow-growing (Gambel's quail, *Callipepla gambelii*) and (2) two strains of a single species, the Japanese quail (wild-type and domesticated), that differ considerably in final size. Our data indicate that wild-type Japanese quail and Gambel's quail have experienced trade-offs between growth and the stress response (e.g. Gambel's quail have slower growth rates, but greater levels of CORT). However, the domesticated strain of Japanese quail used in this study seem to violate some predictions based on the life history theory (e.g. the domestic strain has faster growth rates and greater levels of CORT). The data in this study contribute to the understanding of differences in the stress response between species that exhibit different life history strategies.

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LITERATURE REVIEW

Conceptual Background

An organism's fitness depends on surviving long enough to successfully reproduce. An individual's ability to survive is continuously challenged by the environment surrounding it. Simply put, environmental stressors can be divided into two groups: abiotic (non-biological) stressors and biotic (biological) stressors (Lovejoy and Barsyte 2011). Since the time required to reach sexual maturity is much longer than the time required for the actual act of reproduction, a number of systems and strategies have evolved that increase survival probability when challenged with environmental stressors (Roff 1992; Stearns 1992).

Common examples of abiotic stressors include daily and seasonal changes in weather such as air or water temperature, atmospheric gases, radiation exposure, and water availability. The organism must deal with these stressors appropriately in order to survive (Lovejoy and Barsyte 2011). For example, terrestrial animals usually cope with a greater amount of temperature fluctuations than aquatic animals since water is better at buffering temperature changes than air. Homeothermy evolved as a strategy for dealing with these more extreme temperature changes. A downfall of homeothermy is that it is metabolically costly, requiring a certain amount of oxygen and energy. To reduce the costs of homeothermy during cool temperatures, some animals produced insulation in the form of feathers, hair, and fat deposits. Furthermore, behavioral changes such as shivering and huddling have been acquired to reduce the amount of energy needed to maintain correct internal body temperatures. Alternatively, to reduce homeothermic costs in warmer temperatures, some animals may molt, sweat, pant, or seek shade.

Common examples of biotic stressors include population density, disease, dominance hierarchies, nutrient availability, and competition among individuals for resources such as food and shelter. However, the production of biotic stressors is not strictly exogenous, an organism might create its own set of stressors internally (Lovejoy and Barsyte 2011). Using homeothermy as an example again, the energetic cost of physiologically maintaining a certain internal body temperature is much higher than observed in poikilotherms. The metabolic rate of the homeothermic animal must be high enough to meet the energetic costs associated with homeothermy. As metabolic rates increase, the amount of reactive oxygen species (ROS) produced also increases. ROS are by-products of multiple metabolic oxidation-reduction reactions and are necessary for certain tasks such as immune defense, apoptosis, and stress acclimation. However, if the level of ROS is not maintained correctly and becomes too high, ROS can be destructive to cells and lead to more rapid aging and ultimately reduce lifespan by continual damage to RNA, DNA, and proteins.

Energy organizes living systems and ultimately living organisms are limited by their ability to acquire, process and transfer energy into work. Organisms must balance the costs of acquiring energy by means of foraging and digestion with the benefits of reproduction and survival (Zera and Harshman 2001). In very general terms, energy is allocated differentially among three broad functions: growth, reproduction, and survival promoting mechanisms (Figure 1). Growth includes mechanisms and systems that regulate somatic and skeletal growth. Reproduction includes mechanisms and systems responsible for all physical, physiological, and behavioral requirements for successful reproduction like those associated with sexual maturity, breeding, and parental care. Survival-promoting mechanisms include systems that directly increase the survival of the organism such as immune function, energy storage, tissue repair and stress responses.

Energy allocated among these broad functions is straightforward when the amount of energy available matches the energy needed to meet all the demands placed on the individual. However, when the energy acquisition is lower than the amount of energy the individual requires, there are trade-offs in energy distribution among these functions and some functions are relatively neglected (Roff 1992). Though the exact mechanisms that controls energy allocation are unclear, hormones are thought to be mediators of these tradeoffs (Harshman and Zera 2006).

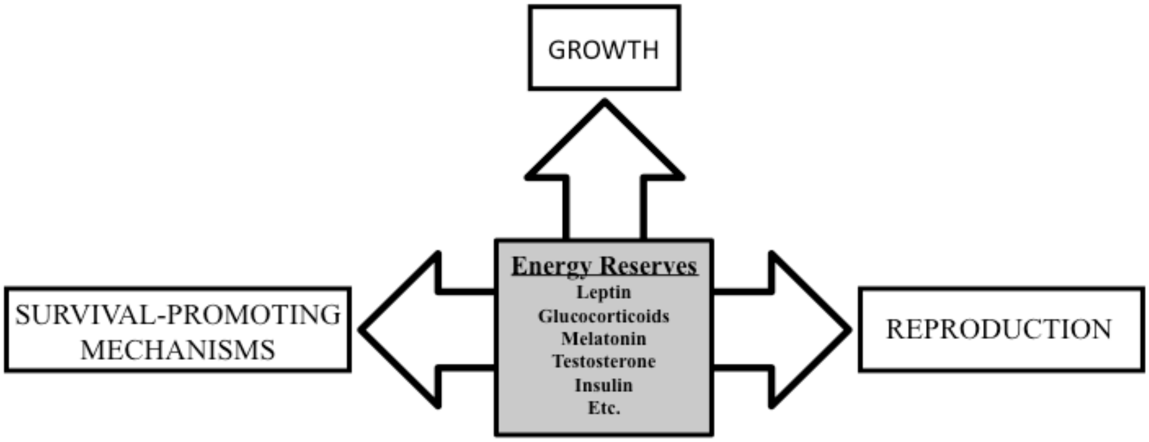

Figure 1. Demonstration of physiological trade-offs. Energy is allocated differentially to these three broad physiological systems (growth, reproduction, and survival-promoting mechanisms). When energy is limited, competition among these three physiological systems arises, as investment in one system limits the amount of energy available to invest in another system. Examples of hormones thought to mediate these trade-offs are listed inside the shaded box. (Developed from Demas et al. 2012)

Allocation of energy not only depends on the amount of energy available, it also depends on the demands of the life stage an individual is experiencing (Stearns 1992; Roff 1992). According to the terminal investment hypothesis energy is expected to be concentrated on growth and survival-promoting mechanisms during the early stages of an organism's life because the individual must reach a certain size and age before it can become sexually mature and produce offspring (Williams 1966; Trivers 1972; Pianka and Parker 1975). As growth slows and individuals approach adult size, energy allocation is expected to shift from growth to reproduction. The amount of energy needed to reach adult size and sexual maturity depends on the amount of time the individual requires to grow as well as the actual size the individual reaches as an adult (Stearns 1992; Roff 1992). The amount of time the individual requires to reach adult size and sexual maturity is largely based on environmental factors, such as the amount of resources available, and how much competition is present for these resources (Zera and Harshman 2001).

As individuals continue to age after sexual maturity is reached, energy is expected to be diverted away from survival-promoting mechanisms that may interfere with reproduction as the chances for future reproductive opportunities become more uncertain (Lavoie 2005; Ottinger and Lavoie 2007; Vleck et al. 2007). For example, allocation away from self-maintenance functions was measured as age-related declines in antibody titers in response to *in vitro* challenges with sheep red blood cells were found in female collared flycatchers (*Ficedula albicollis*) (Cichon et al. 2003). In another study, when males of a long-lived tropical seabird, the blue-footed boobie (*Sula nebouxii*), were exposed to lipopolysaccharide challenges that usually elicit strong immune responses in animals, older males nearly doubled their reproductive effort compared to younger males (Velando et al. 2006). The results from these studies support the terminal investment hypothesis, as they demonstrate both a decrease in self-maintenance investments with age and an increase in reproductive effort when reproductive prospects are threatened in older individuals that have less chances to breed in the future compared to younger individuals who have more chances to breed in the future.

Since reproduction takes a toll on internal energy reserves, it is important that the individual reproduces under the correct conditions, because effort dedicated to current reproduction reduces future reproduction and survival (Harshman and Zera 2006; Norris and Evans 2000; Zera and Harshman 2001; Stearns 1992; Roff 1992). For example, an organism that is experiencing a surplus of energy might increase current reproductive effort at the expense of future reproductive success and survival. Alternatively, if the organism is experiencing an energy deficit and the cost of reproduction is too high, one strategy to maximize fitness is to conserve energy reserves, focus energy on mechanisms that increase survival, and reproduce during the next breeding season. A core objective of life history studies is to understand how these reproductive decisions evolve (Roff 1992).

Life History Theory

The amount of energy available in an environment has the potential to shape key life characteristics in individuals and populations (Stearns 1992; Roff 1992; Zera and Harshman 2001). Life history theory is used as a framework to understand variation in these key life characteristics in the context of natural selection, fitness, adaptation, tradeoffs and constraints (Stearns 1992; Roff 1992). If demographic characteristics of a population such as age, size, and stage-specific schedules of reproduction and mortality are known, inferences about the strength of selection for certain life history traits can be made. Life history theory categorizes these demographic characteristics along a continuum of life-history traits from fast to slow (Figure 2). For example, organisms that lie on the fast end of the continuum are typically characterized by high adult mortality rate, short life expectancy, fast growth rates as juveniles, and early sexual maturity (Stearns 1992; Roff 1992). Organisms that at the slow end of the life history spectrum are typically characterized by low adult mortality rate, long life expectancy, slow growth as juveniles, and late sexual maturity relative to organisms on the fast end of the spectrum (Stearns 1992; Roff 1992). Additionally, slow-paced species typically have smaller clutch sizes (Skutch 1949), longer development time, and longer duration of parental care (Ricklefs and Wikelski 2002; Stutchbury and Morton 2008) than fast-paced species.

Figure 2. A simplified version of the life history continuum.

Trade-offs Among Life History Traits

Trade-offs between survival and key life history traits are likely to occur differentially among species that lie on different points along the fast-slow continuum. If a few demographic characteristics of a species or population are known, life history theory can help to predict phenotypes and trade-offs that the majority of a population will most likely express (Stearns 1992). Variation in trade-offs between fecundity and survival along the fast-slow continuum is supported by theory and well documented empirically (Ricklefs and Wikelski 2002). Organisms that lie on the fast end of the continuum are typically limited by the number of opportunities to breed and they tend to allocate resources preferentially to current reproduction instead of adult survival (future reproductive opportunities) (O'Neal and Ketterson 2012). In contrast, organisms that lie on the slow end of the continuum typically have more opportunities to breed and they tend to allocate resources preferentially to adult survival (future reproductive opportunities) instead of current reproduction. For example, characteristics of slow-paced species include lower basal metabolic rates (Wikelski et al. 2003; Wiersma et al. 2007), more robust immune responses (Martin et al. 2004; Tieleman et al. 2005; Addison et al. 2009), lower testosterone during breeding (Wingfield et al. 2001), and increased corticosterone levels in response to a stressor (Bókony et al. 2009; Malisch and Breuner 2010), which collectively reflect a larger investment to self-maintenance in contrast to reproduction (O'Neal and Ketterson 2012).

The current life-stage of an individual can differentially affect allocation of energy, and life history theory predicts that as an individual ages, hormone and immune responses that can interfere with reproduction may decline (O'Neal and Ketterson 2012). For example, the response to a stressor in a long-lived species, the common tern (*Sterna hirundo*), has been found to decrease with age, as young adults are more responsive to stressors than old adults (Heidinger et al. 2006). Heidinger et al. (2006) attributed this difference to the greater number of reproductive attempts likely remaining to a younger adult than an older adult. Declines in cell-mediated immune responses to PHA with age have been demonstrated in several avian species (Tella at al. 2002; Haussmann et al. 2005). Furthermore, trade-offs among different branches within the immune system have been documented and individuals age (Lavoie et al. 2007). Lavoie et al. (2007) found that 28 month-old Japanese quail (*Coturnix japonica*) had reduced cell-mediated response to PHA compared to six week- and 10 month-old Japanese quail, but did not significantly differ with respect to humoral responses to chuckar red blood cells or avian influenza virus (H9N2). These studies show the importance of considering life-stages when creating hypotheses from the life history theory.

Trade-offs between survival-promoting mechanisms and growth are also well documented (e.g., immune response versus growth, and stress response versus growth). For example, when infected with ectoparasites, male lizards (*Lacerta vivipara*) showed significant decreases in growth rates compared to control males (Uller and Olsson 2003). Similarly, barn swallow (*Hirundo rustica*) nestlings heavily infected with ectoparasites exhibited slower rates of tarsus growth and body mass growth compared to control nestlings (Saino et al. 1998). These nestlings also exhibited increases in feather growth, which may enable earlier fledging from infected nests (Saino et al. 1998). Undoubtedly, the costs of investing energy into the immune response can be large in growing animals.

Similar to the immune response, hormones associated with the physiological response to stress are capable of altering growth. For example, growing chickens (broilers) that received daily injections of a hormone associated with stress (corticosterone) experienced suppressed growth rate (as measured by body mass) and decreased leg muscle mass, as well as increased abdominal fat and liver mass compared to control birds (Siegel and Van Kampen 1984). Likewise, juvenile alligators (*Alligator mississippiensis*) that received corticosterone treatments over a 3-month period weigh significantly less than the control group (Morici et al. 1997). Moreover, chronically elevated stress hormones are known to affect other key life history characteristics in a wide range of free-living and captive animals, ultimately decreasing fitness (Barja et al. 2007; Bonier et al. 2004; Partecke et al. 2006; Pride 2005; Silverin 1998; Wingfield and Silverin 1986; Pettite and Etches 1991; Morici et al. 1997). Because the impact of trade-offs associated with the physiological response to stress on fitness can be large, we were particularly interested in trade-offs associated with the development of the stress response from hatch to sexual maturity in two precocial avian species that are closely related, but exhibit different life history strategies in this study.

What is the Stress Response?

Generally stated, stress is any event that disrupts homeostasis and the set of neural and endocrine-associated adaptations that help re-establish homeostasis is the stress response. One major axis that controls the physiological response to stress is the hypothalamic-pituitary-adrenal (HPA) axis (Figure 3). The HPA axis is activated in response to a stressor and is initiated when the paraventricular nuclei (PVN) of the hypothalamus secretes corticotropin releasing hormone (CRH). Corticotropin releasing hormone travels through the hypophyseal portal system to the pituitary, which stimulates the production of adrenocorticotropic hormone (ACTH). Adrenocorticotropic hormone is then secreted from the pituitary into the circulatory system, ultimately stimulating the synthesis and secretion of glucocorticoids from the adrenal cortex; in mammals and fish cortisol is the primary glucocorticoid active and in birds, amphibians and reptiles, corticosterone (CORT) is the primary glucocorticoid released.

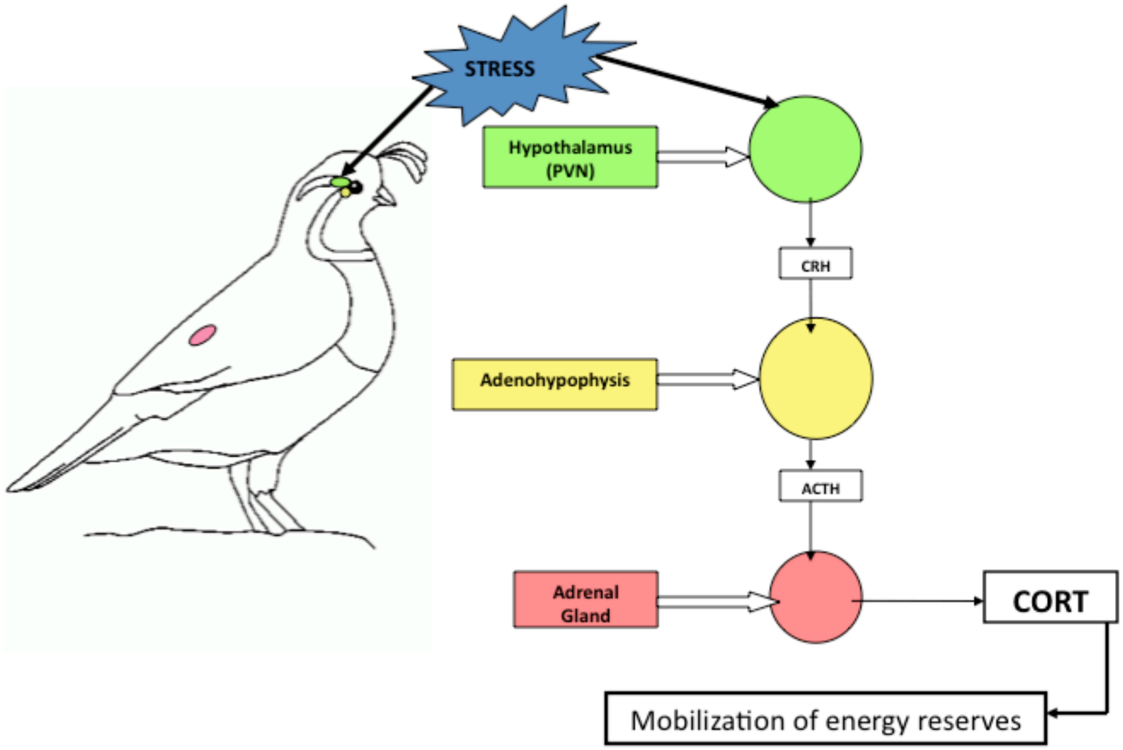

Figure 3. The hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is activated in response to a stressor and is initiated in the hypothalamus, where CRH is produced and secreted. CRH stimulates the adenohypophysis resulting in the production and secretion of ACTH. ACTH flows through the body, ultimately stimulating the adrenal gland to produce and secrete CORT, which mobilizes energy reserves.

Glucocorticoids are involved in many physiological processes (Figure 4), including the regulation of protein, carbohydrate and lipid metabolism (Sapolsky et al. 2000; Stewart 2003) resulting in benefits of short-term elevated levels of CORT, providing quick boosts of energy at critical moments. However, when CORT is elevated chronically, these responses are energetically expensive and secretion of ACTH and CORT depletes energy reserves, leading to suppression of growth, development and the immune system, ultimately decreasing fitness (Siegel and Van Kampen 1984; Pettite and Etches 1991;

Morici et al. 1997; Silverin 1998; Sapolsky et al. 2000; Stewart 2003; Pride 2005; Demas et al. 2012).

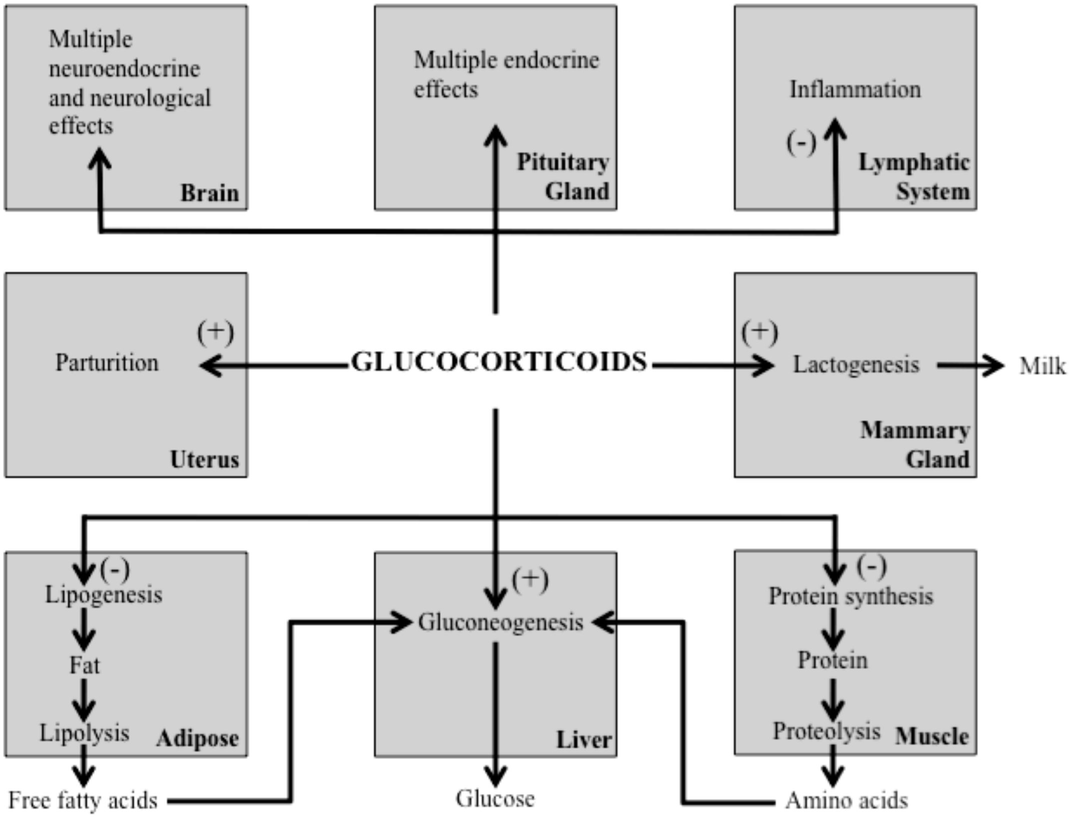

Figure 4. Actions of glucocorticoid hormones. (From Lovejoy and Barsyte 2011)

Physiological responses to stressors are a function of active hormone levels, carrier proteins and receptor dynamics (Reviewed in Malisch and Bruener 2010; Bruener and Boonstra in press). In order for corticosterone to be transported throughout the body, it must bind to corticosterone binding globulins (CBGs), which are secreted from the liver (Rosner et al. 1988). Corticosterone binding globulin binds to CORT in a hydrophobic pocket (Klieber et al. 2007) with high affinity, low capacity, and high specificity (Westphal 1971; Siiteri et al. 1982). Corticosterone binding globulin and CORT bind and unbind at equilibrium in a continuous fashion (Malisch and Breuner 2010). According to the free hormone hypothesis, when CORT is bound to CBGs, it is biologically inactive until it reaches the target tissue, where it is enzymatically unbound (free) from the CBG and becomes active (presented in Mendel 1989; reviewed in Malisch and Breuner 2010). Therefore, the total level of corticosterone circulating in the blood does not reflect the actual portion that is biologically active. Although measures of circulating glucocorticoids provide estimates of an animal's capacity to handle stress, they can be biased when bound and free levels are not considered separately.

Study Objective

The purpose of this study is to evaluate the development of the stress response from hatch to sexual maturity in species that are closely related, but exhibit different life history strategies. To measure the development of the stress response, we quantified corticosterone and CBG from Gambel's quail and two strains of Japanese quail (wild-type and domesticated). Gambel's quail are slow growing relative to the Japanese quail strains, which provided us with a model to evaluate the interaction between stress and life history. Levels of total CORT, free CORT, and CBG were calculated and analyzed from hatch to sexual maturity in two different analyses. (1) CORT and CBG levels from Gambel's quail and wild-type Japanese quail were compared for a life history evaluation of potential trade-offs in two naturally occurring species. Based on the life history theory, we predicted that the capacity to handle stressors (i.e., low CORT response and high CBG levels) is greater in slow-growing Gambel's quail and less in the fast-growing wild-type Japanese quail. (2) CORT and CBG levels from wild-type Japanese quail and domestic Japanese quail were compared for an evaluation of potential trade-offs resulting from artificial selection for increased body size and growth rate. Based on the life history theory, we predicted that the capacity to handle stressors (i.e., high CORT response and low CBG levels) is greater in wild-type Japanese quail and less in domestic Japanese quail.

Investigational Model

Birds have served an important model for the development of mechanistic understanding of life history diversification in natural contexts has relied heavily and is well documented across birds living in diverse environments worldwide (Ricklefs and Wikelski 2002). Additionally, the use of quail as a model for research, namely domesticated Japanese quail, has become quite common. Japanese quail are used in a variety of scientific fields, including but not limited to physiology, endocrinology, toxicology, genetics, nutrition, embryology and pathology. Their fairly small size, short life cycle, and ability to produce five to seven eggs per week are a few of their highly desirable traits. Furthermore, these birds also require relatively small amounts of animal care from birth since they are a highly precocial species. Though Gambel's quail is at present not as widely used in scientific research as Japanese quail, they make for an interesting life history comparison as they are in the same order as Japanese quail, but exhibit different life history characteristics. Research has shown that animals with different life histories show variation in stress response (Hayward et al. 2005).

Gambel's quail and Japanese quail are precocial species that belong to the order Galliformes. Both Japanese quail and Gambel's quail have precocial young and their hypothalamic-pituitary-adrenal (HPA) axis is highly developed at birth (Wada et al. 2009). The trade-offs among life history traits (growth vs. maturation) combined with a highly developed HPA axis at birth makes them an interesting model to study the development of the stress response from hatch to sexual maturity.

Though both species have a relatively short lifespan, they differ in the rate at which they grow and mature. Gambel's quail are New World quail that require approximately 7 months to reach adult size and sexual maturity (Brown et al. 1998). Alternatively, Japanese quail are Old World quail that require approximately eight weeks to reach adult size and sexual maturity (Wilson et al. 1971). For the present study, we identify Gambel's quail with the slow end of the life history spectrum since they have slower growth and maturation rates than Japanese quail. Likewise, we identify Japanese quail with the fast end of the life history spectrum since they have faster growth and maturation rates than Gambel's quail.

Figure 5. Japanese quail strains. Size comparison of a male wild-type (right) and domesticated (left) Japanese quail used in this study.

Furthermore, we have included two different strains of Japanese quail in this study. Large breast and egg size were selected for in the domesticated strain, so the wild-type strain is much smaller (about 1/3 of the body mass, Figure 5). Because strong associations between growth and the stress response exist, the two strains selected allow us to analyze possible consequences to the stress response due to increased investments in growth.

Gambel's Quail Life History

Gambel's quail (*Callipepla gambelii* Figure 6A) are a dimorphic non-migratory New World quail in the family Odontophoridae. Currently, Gambel's quail populations found throughout the desert southwest in North America (Figure 6B) and are large enough to sustain a controlled hunting season (Nevada Dept. of Wildlife 2012). In the Mojave Desert, these quail experience 43°C summers and often time below freezing winters (Nevada Dept. of Wildlife 2012).

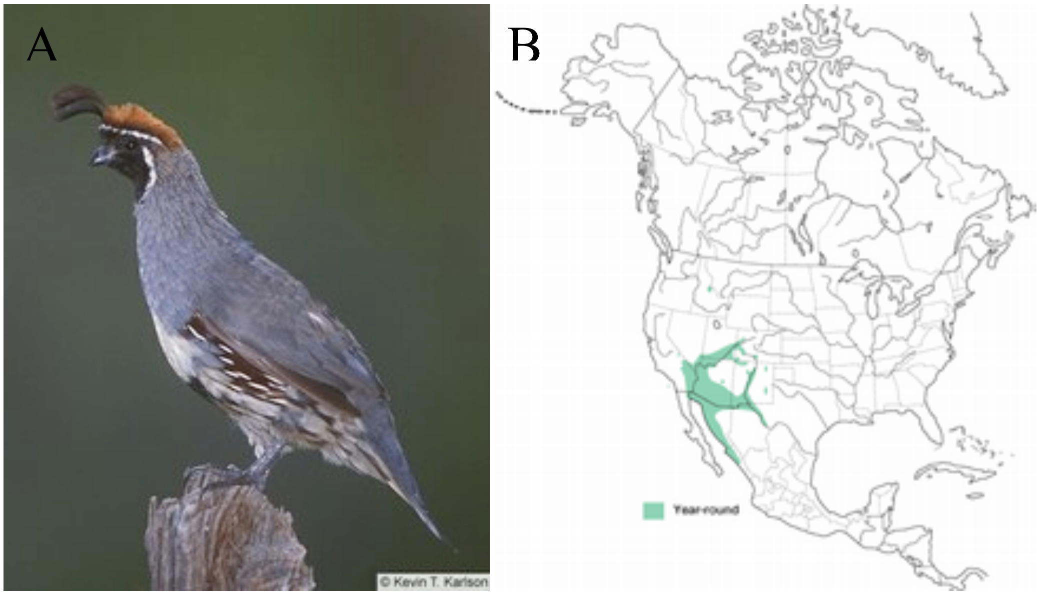

Figure 6. Gambel's quail and species distribution. Left: A male Gambel's quail. (Photo credit: Kevin T. Karlson). Right: Gambel's quail distribution in North America. (Figure taken from Brown et al. 1998)

Males and females in this species are generally not territorial, but breeding pairs will keep space between each other when foraging in a food-rich area (Brown et al. 1998). Additionally, family coveys typically merge with other family coveys over the winter months, resulting in several dozen birds (Brown et al. 1998). However, males usually maintain a dominance hierarchy in these merged coveys.

Males usually seek out females to pair with for spring in late winter. Though Gambel's quail are considered to be a socially monogamous species, it has been suggested that behavior from some experienced females resembles serial polyandry (Johnsgard 1973; Brown et al. 1998). Females typically lay between 10 and 12 eggs in each clutch, laying an egg every 25 to 28 hours and reach a full clutch in approximately three cycles of four eggs, each cycle separated by a day (Brown et al. 1998). However, clutch size depends on rainfall, because broods are much smaller or non-existent during drought years (Nevada Dept. of Wildlife 2012). It remains unclear if the clutches laid late in the breeding season are due to renesting attempts, second nestings or delayed nestings (Brown et al. 1998). The female incubates the eggs for 21 to 23 days after the final egg was laid and clutches can hatch as early as May and as late as September. The young fledge at 3 months and become sexually mature between seven and eight months of age (Brown et al. 1998). The juvenile mortality is typically 40% higher than adult mortality rates (Gallizioli 1967). Though Gambel's quail have a mean life expectancy of roughly 1.5 years, they are reported to live up to three or four years in the wild (Brown et al. 1998; Nevada Dept. of Wildlife 2012).

Gambel's quail are diurnal (Brown et al. 1998). A half of a typical day is spent foraging for food. They are relatively inactive for the other half of the day and can be found under brush that shields them from sun and predators. At night, they are inactive and roost off the ground in leafy shrubs or trees with many branches. When threatened in the open, Gambel's Quail prefer to outrun the predator, only bursting into flight if the predator gets too close. When threatened while brooding, the male will most often flush while the female continues to sit on the nest. However, if the predator continues to approach, the hen may flush as well. If young have hatched, they will scatter to safety in nearby brush.

Japanese Quail Life History

Japanese quail (*Coturnix japonica,* Figures 5 and 7), belong to the family Phasianidae, and subfamily Perdicinae. Currently, these quail are thought to have originated as sub-species of common quail (*Coturnix coturnix*), which are abundant and can be found throughout Asia, Africa and Europe (Chang et al. 2005). However, native wild Japanese quail populations are endangered and exist only in China (Chang et al. 2005).

Figure 7. A pair of Japanese quail. The female is on the left and the male is on the right.

Because males in this species mate with multiple females, males are very territorial (Chang et al. 2009). Though this species is sexually dimorphic, differences are limited when compared to other sexually dimorphic species (Chang et al. 2009). Adult males roughly weigh 92 grams, while adult females roughly weigh 100 grams (Wilson et al 1971; Chang et al. 2009). The median age at sexual maturity for Japanese quail is 59 days (Wilson et al. 1971). Females lay between 7 and 14 eggs per year, each egg weighing about 7.3% of her total body mass (Chang et al. 2009).

This migratory species prefers habitats sparsely covered with grass, primarily inhabiting plains, coastal regions and foothills throughout East Asia (Chang et al. 2005). Though flying ability is weak in this species, they still successfully migrate hundreds and, in some cases, thousands of kilometers (Chang et al. 2005). Generally, they arrive in western Xinjiang Province and east of Inner Mongolia in March and April to breed there during June and July, migrating back south for the winter (Figure 8). In a few cases, individuals stay south the entire year, as these birds favor the warmer climate and moist conditions (Chang et al. 2005). Japanese quail hide in shrubs and grasses during daylight and prefer to fly together at night (Chang et al. 2005). When threatened, the birds tend to run, often clustering with others under nearby vegetation (Chang et al. 2005; Chang et al. 2009).

Figure 8. The distribution and migration routes of wild Japanese quail. Generally, the quail migrate to northwest China in March and April to breed in June and July. During winter months, Japanese quail reside in coastal and southern regions of China. Distribution and migration routes are illustrated by red dotted lines. (Figure adapted from Change et al. 2005).

Domestication of Japanese Quail

Around the 11th century, Japanese quail were either domesticated in Japan or domestic quail were brought to Japan from China (Cain and Cawley 1972). At present, phylogenetic relationships support the hypothesis that domestication first occurred in China, since some strains of domesticated Japanese quail are more closely related to wild quail in China than wild quail in Japan (Chang et al. 2001). Selection on body size in

Japanese quail led to notably larger quail capable of producing larger eggs. Domesticated Japanese quail have since been used extensively in the commercial quail industry for meat and egg production. China has the largest quail industry in the world, followed by Japan (Chang et al. 2005).

Studies have revealed that domesticated Japanese quail and wild Japanese quail differ more than just by body and egg size. Domestic Japanese quail are considered fully mature at 50 days of age (Cain and Cawleyn 1972), compared to 59 days of age for wild Japanese quail (Wilson et al. 1971). Domestic Japanese quail also lay around 280 eggs per year, while wild Japanese quail usually lay 7-14 per year (Chang et al. 2009). Domestic Japanese quail are significantly more vocal and aggressive than the wild-type (Chang et al. 2009). However, domesticated Japanese quail are less restless and are less likely to take flight than the wild Japanese quail (Wilson et al. 1971). Domestic Japanese quail also exhibit greater mating frequencies, fertility rates, hatching rates, and hatching rates of fertilized eggs than wild Japanese quail (Chang et al. 2009). When all these differences are taken together, it is clear that domestication has drastically changed this strain of Japanese quail.

Conclusions

Being able to mount the appropriate response to a stressor is vital to maintaining or increasing an animal's fitness. The appropriate response to a stressor varies between individuals, populations, and species, depending on the life history strategy of the individual and what life stage it is experiencing (Stearns 1992). Likewise, the trade-offs that may occur in order to preserve the appropriate stress response also occur differentially along the life history continuum (Roff 1992). Thus, life history theory can help to predict phenotypes and trade-offs that the majority of a population will most likely express if a few demographic characteristics of a species or population are known (Stearns 1992). Based on the demographic characteristics presented in literature, we know that Gambel's quail and Japanese quail are closely related species that utilize different life history strategies. Because strong associations between growth and the stress response are evident, the two strains of Japanese quail (wild-type and domesticated) allow us to analyze possible consequences to the stress response due to increased investments in growth in the domesticated strain. Collectively, the species and strains within species chosen for the model in this study present a valuable opportunity to explore potential trade-offs (growth versus maturation) associated with the stress response.

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PAPER. DEVELOPMENT OF THE STRESS RESPONSE IN FAST (*COTURNIX JAPONICA*) VERSUS SLOW (*CALLIPEPLA GAMBELII*) GROWING SPECIES [1]

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Abstract

Life-history theory predicts that growing animals are constrained in the amount of energy available for systems that promote survival (i.e., stress response). Based on principles from life-history theory, one prediction is that fast growing animals have lower stress responses than slow growing animals, which have more energy to dedicate to survival promoting mechanisms. Species of comparable size in the Order Galliformes can

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[1] The material in this chapter was co-authored by Michelle Gastecki, Wendy Reed, Mark Clark and Creagh Breuner. Michelle Gastecki had the primary responsibility for raising quail, collecting samples, quantifying hormone/protein from samples, and analyzing results. Michelle Gastecki was the primary developer of this chapter, including the conclusions that are advanced here. Michelle Gastecki also drafted and revised all versions of this chapter. Wendy Reed served as secondary developer, technical and statistical support, proofreader, and checked math in the statistical analysis conducted by Michelle Gastecki. Mark Clark served as statistical support for complex curves fit to growth data and checked math in the statistical analysis conducted by Michelle Gastecki. Creagh Breuner served as technical support and helped quantify hormone and protein from samples collected by Michelle Gastecki.

vary considerably in growth and developmental rate and provide a system for evaluating these predictions. In this study, we evaluated the development of stress response in (1) two different species of quail, one species that is relatively fast-growing (Japanese quail, *Coturnix japonica*) and one that is relatively slow-growing (Gambel's quail, *Callipepla gambelii*) and (2) two strains of a single species, the Japanese quail (wild-type and domesticated), that differ considerably in final adult size. Changes in mass and tarsus length were used to measure growth, and stress response was evaluated by measuring corticosterone release in response to an ACTH challenge. Corticosterone binding globulin was also quantified from plasma to estimate free and total levels of corticosterone. Our data indicate that wild-type Japanese quail and Gambel's quail have experienced trade-offs between growth and the stress response (e.g. Gambel's quail have slower growth rates, but greater levels of CORT). However, the domesticated strain of Japanese quail used in this study seem to violate some predictions based on the life history theory (e.g. the domestic strain has faster growth rates and greater levels of CORT).

Introduction

An organism's physiological response to stressful events is determined by both genetic and environmental influences. Stress is any event that disrupts homeostasis and the set of neural and endocrine-associated adaptations that help re-establish homeostasis is the stress response. The release of glucocorticoids in response to a stressor via activation of the hypothalamic-pituitary-adrenal (HPA) axis is highly conserved (Mormède et al. 2007). Glucocorticoids are involved in many physiological processes, including the regulation of protein, carbohydrate and lipid metabolism, and short-term elevations in glucocorticoids are beneficial, giving animals a quick boost in energy at critical moments (Sapolsky et al. 2000; Stewart 2003). For example, if an animal is not able to initiate a stress response, it may become vulnerable to a predator or other environmental conditions. Alternatively, an overactive stress response can also be detrimental to the organism, as chronically elevated glucocorticoid levels are known to increase susceptibility to disease, suppress growth, and decrease fitness (Siegel and Van Kampen 1984; Wingfield and Silverin 1986; Pettite and Etches 1991; Morici et al. 1997; Silverin 1998; Bonier et al. 2004; Pride 2005; Partecke et al. 2006; Barja et al. 2007).

Trade-offs between survival and key life history traits are likely to occur differentially among species that lie on different points along the fast-slow continuum of life-history traits (Stearns 1992; Roff 1992). Organisms that lie on the fast end of the continuum (fast-paced) are typically characterized by high adult mortality rate, short life expectancy, fast growth rates as juveniles, and early sexual maturity (Stearns 1992; Roff 1992). Organisms that at the slow end (slow-paced) of the life history continuum are typically characterized by low adult mortality rate, long life expectancy, slow growth as juveniles, and late sexual maturity relative to organisms on the fast end of the continuum (Stearns 1992; Roff 1992). Additionally, slow-paced species typically have smaller clutch sizes (Skutch 1949), longer development time, and longer duration of parental care (Ricklefs and Wikelski 2002; Stutchbury and Morton 2008) than fast-paced species. If a few demographic characteristics of a species or population are known, life history theory can help determine the pace of life (Stearns 1992). For example, baseline corticosterone levels were highly correlated with the pace of life in garter snakes (*Thamnophis elegans*) that exhibit slow and fast pace of life strategies (Palacios et al. 2012). In agreement with the authors' predictions from the life history theory, slow-paced garter snakes had higher levels of baseline corticosterone than fast-paced garter snakes (Palacios et al. 2012). Thus, the life history theory can help predict phenotypes and trade-offs that the majority of a population will most likely express (Stearns 1992).

The development of mechanistic understanding of life history diversification in natural contexts has relied heavily on birds since life history variation is well documented in numerous birds living diverse environments worldwide (Ricklefs and Wikelski 2002). In this study, we were interested potential trade-offs associated with the development of the stress response from hatch to sexual maturity in two precocial avian species, Gambel's quail (*Callipepla gambelii*) and Japanese quail (*Coturnix japonica*). Because both species of quail are precocial animals, their hypothalamic-pituitary-adrenal (HPA) axis is more developed at hatch than birds with an altricial mode of development (Sapolsky and Meaney 1986; Starck and Ricklefs 1998; Wada et al. 2009). Though Gambel's quail and Japanese quail are from the same order (Galliformes), they differ considerably with respect to their life history. Gambel's quail require approximately 7 months to reach adult size and sexual maturity (Brown et al. 1998) and Japanese quail only require approximately 8 weeks to reach adult size and sexual maturity (Wilson et al. 1971). We also compared two different strains of Japanese quail in this study, a wild-type strain (*Coturnix japonica*) and a domesticated strain (*Coturnix japonica*) that differ about 3-fold in adult mass. The trade-offs among life history traits (growth vs. maturation) combined with a highly developed HPA axis at hatch makes these two species and strains within species an interesting model to study the development of the stress response from hatch to sexual maturity (Stearns 1992).

To measure the development of the stress response, we quantified corticosterone (CORT; the main glucocorticoid in avian species) levels before and after a challenge with adrenocorticotropic hormone (ACTH) in Gambel's quail and the two strains of Japanese quail (wild-type and domesticated). Additionally, we quantified corticosterone binding globulin (CBG), because measures of circulating CORT provide estimates of an animal's capacity to handle stress, they are often biased when bound and free levels are not considered separately ("free hormone hypothesis", proposed in Mendel 1989; reviewed in Malisch and Breuner 2010). Patterns in the levels of total CORT, free CORT, and CBG capacity were analyzed from hatch to sexual maturity in each species and strain within species. Furthermore, the differences in the patterns of said stress response parameters were investigated between species and strains within species and associations with life history strategies and domestication were made, respectively. These comparisons were chosen to focus on life history differences associated with growth and body size of two different species (Gamble's versus wild-type Japanese quail) and differences in growth and body size associated with domestication of a species (wild-type versus domesticated Japanese quail).

Based on demographic information gathered from literature, we identified Gambel's quail at the slower end of the life history continuum because they require more time to reach adult size and become sexually mature than Japanese quail. Likewise, we identified Japanese quail at the faster end of the life history continuum because they require less time to reach adult size and become sexually mature than Gambel's quail. Based on principles of life history theory, species that invest more energy into growth will likely have less energy to invest into other systems, such as the stress response (Stearns 1992).

Therefore, we expected to see a greater trade-off between growth and the stress response with age in the fast-growing Japanese quail than the slow-growing Gambel's quail. Additionally, we predicted that the capacity to handle stressors is (1) greater in slow-growing Gambel's quail and less in the fast-growing wild-type Japanese quail and (2) greater in the smaller (wild-type) strain of Japanese quail and less in larger (domestic) strain of Japanese quail. High capacity to handle stress will be measured as high CORT response to stress paired with low CBG levels.

Methods and Materials

All methods were approved by North Dakota State University's Institution Animal Care and Use Committee (#A10033).

Egg Collection

Eggs were collected from breeding colonies of wild-type Japanese quail, domesticated Japanese quail, and Gambel's quail every 2-3 days. The eggs were incubated at 37°C and 50% humidity in forced air incubators and turned automatically 4 times daily.

Growth Measurement

Once chicks hatched, they were placed in brooders maintained at 32°C and provided with *ad libitum* access to food and water. The temperature of the brooder was gradually reduced over the course of about 21 d after which the chicks were able to thermoregulate and maintain body temperature. Body mass and tarsus lengths were used as measures of growth. Body mass was measured to the nearest 0.1 g on an electric balance and tarsus length was measured to the nearest 0.1 mm with digital calipers. Growth was measured until the CORT response to an ACTH challenge was evaluated, after which a final body mass and tarsus measurements were taken and birds were euthanized.

Adrenocorticotropin Hormone Challenge

Corticosterone response to an ACTH challenge was measured for all three quail species/strains from hatch to maturity. Maturity was based on reaching adult body size (see below). To administer ACTH challenges, individuals were weighed immediately after capture and a blood sample to measure basal CORT levels was taken from the brachial (adults) or iliac (chicks) vein before injecting ACTH. After this blood sample either ACTH (100 IU/kg body mass) in saline solution (Wada et al. 2007) or an equivalent volume of saline-only solution was injected into the breast muscle. After injection, individuals were isolated for 30 minutes before a second blood sample was taken to estimate the amount of total CORT in response to ACTH challenge. Approximately 50-150 µl of blood was collected from chicks and 300-400 µl of blood was collected from adults. Blood samples were collected in 300 µl capillary collection tubes treated with sodium heparin (Microvette CB 300), and centrifuged at 8,000 g for 10 minutes to separate blood cells from plasma. Plasma was stored at -20°C until analysis. CORT response was measured in Gambel's quail between the ages of two and 22 weeks, and measured in domestic and wild-type Japanese quail between the ages of one and nine weeks.

Corticosterone Quantification

Total corticosterone levels in plasma were quantified with radioimmunoassay (RIA; Wingfield et al. 1992). In brief, tritiated corticosterone (approximately 1000 cpm) was added to 20-40 µl plasma samples to estimate the amount hormone recovered after steroid extraction. After incubating the samples for 12 hours at 4°C, 5 ml of distilled dichloromethane was added to each tube as a solvent to extract CORT from the plasma. The samples were vortexed and incubated at room temperature (~24°C) for 2 hours. The supernatant was then decanted into a new test tube and dried under nitrogen in a 40°C water bath. The extracted CORT was reconstituted in 500 µl phosphate buffered saline containing gelatin (PBSg) and 300 µl of the sample was used for RIA (duplicates of 150 µl). Each duplicate was incubated with 100 µl CORT antibody (Abcam #ab77798) and 100 µl tritiated CORT (approximately 10,000 cpm) for approximately 12 hours. After incubation the free hormone was separated from bound hormone by incubating each sample with 500 µl activated charcoal for 12 minutes then spinning them at ~500 g (2,000 rpm) for 10 minutes at 4°C. Supernatants were transferred to scintillation tubes and 500 µl scintillation fluid (PerkinElmer #6013329) was added to each sample and counted for 5 minutes on a scintillation counter. Each sample was corrected for individual plasma volume and recovery after extraction. Standard curves used to estimate CORT concentrations were run in each assay. Standards were run at the beginning and end of each assay. In total, 18 assays were run. Species and ages were run at random in each assay. The average recovery value was 73%. The average intra-assay variation was 12% and inter-assay variation was 20%.

Corticosterone Binding Globulin Quantification

Corticosterone binding globulin was quantified using RIA (Breuner et al. 2003). Briefly, hormones were stripped from plasma samples by incubating them with an activated charcoal solution (0.1% dextran, 1% Norit A charcoal in 50 mM Tris) at room temperature (~24° C) for 20 minutes. After incubation, the samples were spun in a centrifuge ~4,500 g (7,000 rpm) for 10 minutes at 4ºC. The supernatant containing stripped CBG was transferred to a new tube for quantification. The final plasma dilutions used in the assay were optimized for all species, 1:900 for wild-type Japanese quail and 1:630 for both Gambel's quail and domesticated Japanese quail. Three total binding tubes and three non-specific binding tubes were then set up for each sample and incubated at 4ºC for two hours. Non-specific binding tubes consisted of 1µM unlabled CORT, 5nM tritiated CORT and stripped plasma. Total-binding tubes contained 50mM Tris buffer in place of unlabled CORT. Bound and free steroid were separated by rapid vacuum filtration over glass-fiber filters presoaked in a polycationic solution (25mM Tris with 0.3% polyethylenimine). The filters containing bound steroid were soaked in 100µl EtOH prior to being saturated with scintillation fluid (PerkinElmer #6013329). Samples used for analysis of trends in CBG between Gambel's quail, domestic Japanese quail and wild-type Japanese quail were run in a single assay, separate from the assay used to estimate individual variation among ages. Intra-assay variation for the point sample was 18%.

Free hormone levels were estimated using an equation by Barsano and Baumann (1989):

$$H_{free} = 0.5 \times \left[H_{total} - B_{\max} - 1/K_a \pm \sqrt{\left(B_{\max} - H_{total} + 1/K_a\right)^2 + 4\left(H_{total}/K_a\right)} \right]$$

where K_a is $1/K_d$ (nM), K_d is affinity of corticosterone for CBG, B_{max} is total CBG capacity, and H_{total} is the total plasma hormone concentration. K_d was determined in equilibrium binding analysis using pooled plasma from individuals within each species and strain within species.

Statistical Analysis

General linear models were used to statistically analyze chick growth, corticosterone and CBG characteristics (JMP version 10.0), both to establish patterns with age and sex within species/strains as well as to compare patterns between species/strains. Analyses within species/strains specifically characterized patterns in growth and CORT dynamics with respect to sex and sample age from hatch to adult. The comparison between species was limited to Gamble's quail versus wild-type Japanese quail. The comparison between strains was limited to wild-type Japanese quail versus domesticated Japanese quail.

To best compare age in these two analyses, individuals were categorized into two life stage groups, either juveniles or adults. We used tarsus length as a measure of skeletal size to categorize individuals by life stage. Birds were considered adult once they reached 90% of their asymptotic tarsus length calculated from the growth curve that included all individuals within each species/strain. Growth rates for body mass and tarsus length for each individual, species, and strain within species were characterized using Janoschek growth curves:

$$\left(A_{final} - \left(A_{final} - A_{initial}\right) * Exp\left(-\left(k - Age^{p}\right)\right)\right)$$

where $A_{initial}$ is the lower asymptotic body mass or tarsus length, A_{final} is the upper asymptotic body mass or tarsus length, and k and p growth parameters affect the slope and inflection point of the curve. Maximum growth rates for body mass and tarsus length and the age at which that maximum growth occurred were estimated using parameters calculated from the Janoschek curve. If a curve could not be fit to an adult individual because the bird had not reached its asymptotic mass or tarsus length before being euthanized, the individual was excluded from analyses of growth.

All data were log transformed to meet requirements of normality of residuals (Shapiro-Wilk tests for normality). Means are reported as mean of untransformed data ± standard error mean. A one-way ANOVA was used to analyze differences in growth parameters between species/strain. The full model contained species/strain, life stage (juvenile or adult), and the interaction. This model was used to investigate all CORT and CBG levels, but not growth parameters. Post- hoc student-t-test was used to compare least-square means between species/strains. Significance was based on $\alpha = 0.05$. To allow for more individuals to be analyzed by the full model, sex was not included because we lacked sex information for some juvenile individuals. However, a one-way ANOVA was run to see if there was an effect of sex within each species on all growth, CORT, and CBG parameters. ANOVAs for the effect of sex are only reported for those that were significant ($p < 0.05$).

Results

Individuals with baseline CORT levels below the sensitivity of our RIA (< 1 pg CORT per 1μl plasma) or those with no response to ACTH injection (difference between

30 minute sample and baseline sample < 10 pg CORT per 1 µl plasma) were excluded from CORT analyses. In total, seven Gambel's quail, 18 domestic Japanese quail, and two wild-type Japanese quail were excluded for low baseline CORT levels while one Gambel's quail, four domestic Japanese quail, and one wild-type Japanese quail were excluded for the lack of CORT response to ACTH injection.

In some domestic Japanese and Gambel's quail individuals, we lacked enough plasma from baseline samples to estimate CBG. Since the amount of CBG in baseline and 30-minute samples did not differ for wild-type and domestic Japanese quail (wild-type: $F_{1,24} = 0.27$, $p = 0.61$, $r^2 = 0.01$; domestic: $F_{1,8} < 0.01$, $p = 0.95$, $r^2 < 0.01$), we estimated baseline CBG levels from 30-minute CBG levels to calculate free CORT in baseline samples. In juvenile Gambel's quail, CBG increased in 30-minute samples ($F_{1,5} = 8.09$, $p < 0.05$, $r^2 = 0.66$) compared to baseline CBG levels, thus free CORT analyses were limited to 30-minute samples in these birds.

Within Species/Strain Within Species Analysis of Morphometric Measures of Growth

All means and ranges of morphometric measures of growth for species and strains within species are summarized in Table 1. A total of four Gambel's quail and one wild-type Japanese quail were excluded from tarsus analyses while 18 Gambel's quail, 10 wild-type Japanese quail, and 21 domestic Japanese quail were excluded from mass analyses because the individuals did not reach their asymptotic mass and growth curves could not be fit for these individuals. There were no sex differences in tarsus length or tarsus growth rate within any of the species/strains ($p \geq 0.06$), but differences in body mass between the sexes did exist (all non-significant measures of mass, $p \geq 0.08$). Gambel's quail males had significantly greater asymptotic masses than females ($F_{1,30} = 5.07$, $p = 0.03$, $r^2 = 0.14$; males = 161.07 ± 2.79 g, females = 153.18 ± 2.51 g). Wild-type Japanese quail females had significantly greater asymptotic masses than males ($F_{1,18} = 14.57$, $p < 0.01$, $r^2 = 0.45$; females = 108 ± 7.28 g, males = 89.08 ± 1.68 g) and experienced faster maximum mass growth rates than males ($F_{1,26} = 12.86$, $p < 0.01$, $r^2 = 0.33$; females = 3.13 ± 0.09, males = 2.75 ± 0.06). Domesticated Japanese quail females had significantly greater asymptotic masses than males ($F_{1,8} = 7.59$, $p < 0.03$, $r^2 = 0.49$; female = 277.07 ± 10.77 g, male = 218.00 ± 9.20 g), but the sexes did not differ in their maximum growth rate ($p \geq 0.07$).

Table 1. Summary of morphometric measures of growth for all species. Untransformed means are reported for Gambel's quail (GaQu), wild-type Japanese quail (JaQu), and domesticated Japanese quail (JaQu-Dom). (mean ± SE$_{n=\text{sample size}}$; data range)

Parameter	GaQu	JaQu	JaQu-Dom
Tarsus:			
Hatch Length (mm)	$13.55 \pm 0.09_{n=31}$ (12.30-14.80)	$12.16 \pm 0.07_{n=69}$ (10.50-14.10)	$14.27 \pm 0.11_{n=107}$ (12.50-18.70)
Asymptotic Length (mm)	$29.81 \pm 0.23_{n=54}$ (25.20-33.90)	$26.28 \pm 0.18_{n=40}$ (24.10-28.60)	$33.71 \pm 0.21_{n=49}$ (30.40-36.80)
Max. Growth Rate (mm/day)	$0.41 \pm 0.01_{n=42}$ (0.34-0.49)	$0.59 \pm 0.01_{n=38}$ (0.34-0.82)	$0.84 \pm 0.02_{n=47}$ (0.49-1.12)
Age at Max. Growth Rate (day)	$19.24 \pm 0.78_{n=42}$ (9.81-29.43)	$11.98 \pm 0.49_{n=38}$ (5.56-18.79)	$8.99 \pm 0.52_{n=47}$ (1.72-13.57)
Body Mass:			
Hatch Mass (g)	$7.30 \pm 0.09_{n=32}$ (6.20-8.23)	$6.00 \pm 0.08_{n=69}$ (4.51-7.40)	$8.92 \pm 0.22_{n=107}$ (6.49-19.14)
Asymptotic Mass (g)	*$156.99 \pm 1.93_{n=32}$* *(133.90-176.23)*	*$93.50 \pm 2.06_{n=20}$* *(78.35-114.05)*	*$230.30 \pm 10.77_{n=10}$* *(191.37-287.83)*
Max. Mass Growth Rate (g/day)	$2.47 \pm 0.04_{n=34}$ (2.05-2.88)	*$2.93 \pm 0.06_{n=28}$* *(2.19-3.82)*	$6.09 \pm 0.15_{n=29}$ (4.97-7.76)
Age at Max Mass Growth Rate (day)	$39.57 \pm 0.62_{n=34}$ (32.85-46.41)	$22.69 \pm 0.42_{n=28}$ (17.67-27.09)	$32.37 \pm 1.43_{n=29}$ (19.90-46.21)

Italicized mean ± SE$_{n=\text{sample size}}$ and data ranges denotes an effect of sex ($p < 0.05$).

Within Species/Strain Analysis of Stress Response

Total CORT levels measuring the baseline, 30-minute post-ACTH injection, and CORT response (difference between baseline and 30 minute value) showed no change with age in Gambel's quail (Figure 9A). Likewise, free CORT levels at 30-minute post-ACTH injection showed no change with age in Gambel's quail (Figure 9D). Corticosterone binding globulin levels, however, did decrease with age in Gambel's quail (Table 2). There were no differences in total and free CORT levels or CBG levels between the sexes in Gambel's quail (p ≥ 0.27).

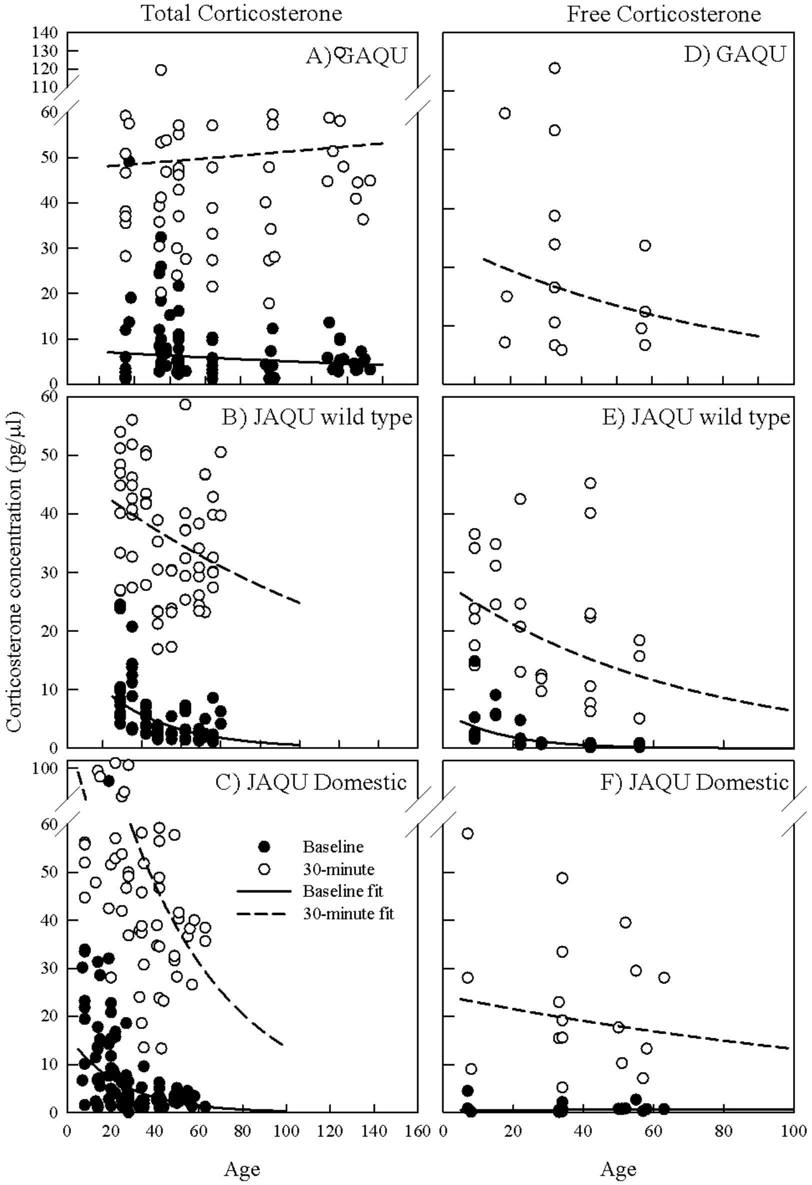

Figure 9. Relationships between CORT and age. There were no significant changes in CORT parameters with age in Gambel's quail (A and D), however, there were significant decreases in total and free baseline and 30-minute CORT with age in wild-type Japanese quail (B and E) and total baseline and 30-minute CORT as well as total CORT response, but not free CORT parameters in domesticate Japanese quail (C and F).

Table 2. Summary of age and CORT parameters. Results from One-way ANOVAs of the relationship between age and CORT levels at baseline, 30-minute post ACTH injection, response to ACTH challenge and CBG levels for Gambel's quail (GaQu), wild-type Japanese quail (JaQu), and domesticated Japanese (JaQu-Dom).

Parameter	GaQu	JaQu	JaQu-Dom
Total CORT (pg/μl):			
Baseline	$F_{1,71} = 2.57$ $p = 0.11$ $r^2 = 0.03$	**$F_{1,65} = 38.80$ $p < 0.01$** **$r^2=0.37$**	**$F_{1,91} = 48.26$ $p < 0.01$** **$r^2=0.35$**
30-Minute	$F_{1,71} = 0.36$ $p = 0.55$ $r^2 < 0.01$	**$F_{1,64} = 5.26$ $p = 0.03$** **$r^2 = 0.08$**	**$F_{1,91} = 38.17$ $p < 0.01$** **$r^2 = 0.30$**
Response	$F_{1,71} = 2.36$ $p = 0.13$ $r^2 = 0.03$	$F_{1,64} = 0.11$ $p = 0.74$ $r^2 < 0.01$	**$F_{1,91} = 17.86$ $p < 0.01$** **$r^2 = 0.16$**
Free CORT (pg/μl):			
Baseline	-----	**$F_{1,26} = 45.40$ $p < 0.01$** **$r^2 = 0.64$**	$F_{1,15} = 0.06$ $p = 0.81$ $r^2 < 0.01$
30-Minute	$F_{1,13} = 0.76$ $p = 0.40$ $r^2 = 0.06$	**$F_{1,25} = 5.13$ $p = 0.03$** **$r^2 = 0.17$**	*$F_{1,15} = 0.41$ $p = 0.53$* *$r^2 = 0.03$*
Response	-----	$F_{1,25} = 2.51$ $p = 0.13$ $r^2 = 0.09$	*$F_{1,15} = 0.44$ $p = 0.52$* *$r^2 = 0.03$*
CBG (nM)	**$F_{1,15} = 10.35$ $p < 0.01$** **$r^2 = 0.44$**	$F_{1,26} = 0.09$ $p = 0.76$ $r^2 < 0.01$	*$F_{1,15} = 0.60$ $p = 0.45$* *$r^2 = 0.04$*

Bolded ANOVAs denotes an effect of age ($p < 0.05$). *Italicized* ANOVAs denotes an effect of sex ($p < 0.05$).

Total CORT levels measuring baseline and 30-minute post-ACTH injection decreased with age in wild-type Japanese quail (Figure 9B). Likewise, free CORT levels measuring baseline and 30-minute post-ACTH injection decreased with age in wild-type Japanese quail (Figure 9E). Corticosterone levels for total and free responses did not change with age, nor did CBG levels change with age in wild-type Japanese quail. There were no differences in any of the stress response parameters between the sexes in wild-type Japanese quail ($p \geq 0.33$).

Total CORT levels measuring baseline, 30-minute post-ACTH injection, and CORT response significantly decreased with age in domestic Japanese quail (Figure 9C). However, CBG and free CORT levels measuring baseline, 30-minute post-ACTH injection and CORT response did not change with age in domestic Japanese quail (Figure 9F). There were no differences in total CORT levels measuring baseline, 30-minute post-ACTH injection, and response CORT, or free CORT levels measuring baseline between the sexes in domestic Japanese quail ($p \geq 0.65$). However, male domestic Japanese quail had greater 30-minute free CORT ($F_{1,12} = 21.03$, $p < 0.01$, $r^2 = 0.64$), free CORT response ($F_{1,12} = 22.34$, $p < 0.01$, $r^2 = 0.65$), and CBG capacity ($F_{1,12} = 5.01$, $p < 0.05$, $r^2 = 0.29$) levels than female domestic Japanese quail.

Life History Comparison: Gambel's Quail versus Wild-Type Japanese Quail

All morphometric growth parameters were significantly different between the two species. Gambel's quail had longer tarsi at hatch and at their asymptotic length than wild-type Japanese quail. However, wild-type Japanese quail had faster maximum tarsus growth rates and experienced those growth rates at earlier ages than Gambel's quail (Table 3 and Figure 10 A). Gambel's quail also had significantly greater hatch and asymptotic masses than wild-type Japanese quail, although wild-type Japanese quail had faster maximum mass growth rates and experienced those growth rates at earlier ages than Gambel's quail (Table 3 and Figure 10 B). Furthermore, wild-type Japanese quail reached their asymptotic tarsus length and body mass in nearly half the time it took Gambel's quail (tarsus lengh: GaQu = 45 days, JaQu = 26 days; mass: GaQu = 79 days, JaQu = 42 days).

Table 3. Life history comparison of morphometric measures of growth. All growth parameters differed significantly ($p < 0.05$) between Gambel's quail (GaQu) and wild-type Japanese quail (JaQu). (mean ± SE $_{n=\text{sample size}}$)

Parameter	GaQu	JaQu	ANOVAs
Tarsus:			
Hatch Length (mm)	$13.55 \pm 0.09_{n=31}$	$12.16 \pm 0.07_{n=69}$	$F_{1,99} = 117.33$ $p < 0.01$ $r^2=0.54$
Asymptotic Length (mm)	$29.81 \pm 0.23_{n=54}$	$26.28 \pm 0.18_{n=40}$	$F_{1,92} = 136.55$ $p < 0.01$ $r^2 = 0.60$
Max. Growth Rate (mm/day)	$0.41 \pm 0.01_{n=43}$	$0.59 \pm 0.01_{n=38}$	$F_{1,79} = 172.02$ $p < 0.01$ $r^2 = 0.69$
Age at Max. Growth Rate (day)	$19.24 \pm 0.78_{n=43}$	$11.98 \pm 0.49_{n=38}$	$F_{1,79} = 60.04$ $p < 0.01$ $r^2 = 0.43$
Body Mass:			
Hatch Mass (g)	$7.30 \pm 0.09_{n=32}$	$6.00 \pm 0.08_{n=69}$	$F_{1,99} = 90.00$ $p < 0.01$ $r^2 = 0.48$
Asymptotic Mass (g)	$156.99 \pm 1.93_{n=32}$	$93.50 \pm 2.06_{n=20}$	$F_{1,50} = 512.60$ $p < 0.01$ $r^2 = 0.91$
Max. Growth Rate (g/day)	$2.47 \pm 0.04_{n=34}$	$2.93 \pm 0.06_{n=28}$	$F_{1,60} = 39.51$ $p < 0.01$ $r^2 = 0.40$
Age at Max. Growth Rate (day)	$39.57 \pm 0.62_{n=34}$	$22.69 \pm 0.42_{n=28}$	$F_{1,60} = 500.17$ $p < 0.01$ $r^2 = 0.89$

A one-way ANOVA was used to investigate the effect of species on growth parameters.

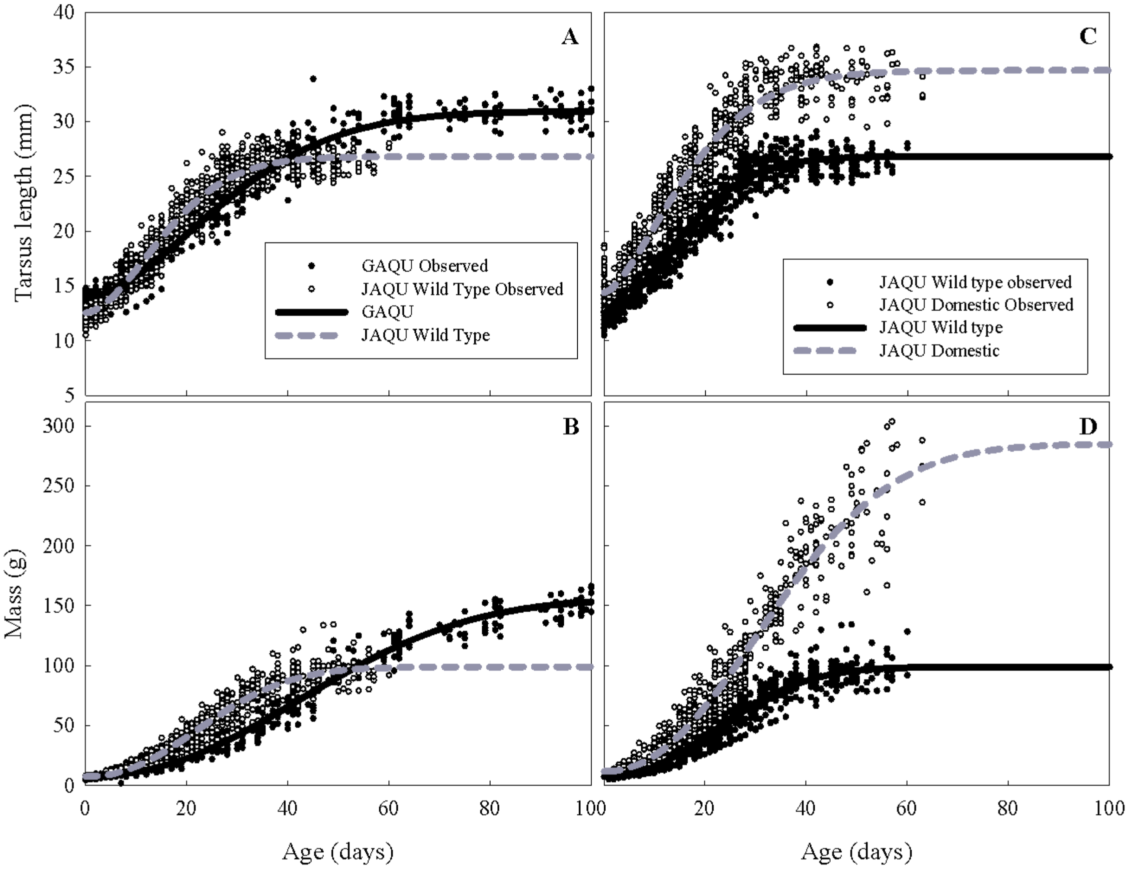

Figure 10. Growth curves for species/strains. Janoschek growth curves fit to growth data for (A) tarsus length and (B) body mass by day from Gambels' quail and wild-type Japanese quail (life-history comparison), and (C) tarsus length and (D) body mass by day from wild-type Japanese quail and domesticated Japanese quail (domestication comparison).

According to the full model, Gambel's quail had higher total CORT levels at baseline, 30-minute post-ACTH injection, response to ACTH challenge, and CBG levels than wild-type Japanese quail (Table 4). Free levels CORT at 30-minutes post-ACTH injection did not differ between Gambel's quail and wild-type Japanese quail, but there were significant interactions between species and life stage (juvenile/adult) ($p = 0.03$) on the levels of free CORT at 30 minutes post-ACTH injection and total levels of CORT at baseline ($p = 0.02$).

Table 4. Life history comparison of stress response parameters. The differences in stress response parameters for all ages and two life stages (juvenile and adult) between Gambel's quail (GaQu) and wild-type Japanese quail (JaQu). Means that are statistically different between species are in bold. (mean ± SE$_{n=\text{sample size}}$)

Parameter	GaQu	JaQu	ANOVAs		
ALL AGES					
Total CORT (pg/µl):					
Baseline	**$8.16 \pm 0.95_{n=73}$**	**$5.71 \pm 0.64_{n=67}$**	$F_{3,136} = 16.63$	$p < 0.01$	$r^2 = 0.27$
30-Minute	**$54.67 \pm 2.67_{n=73}$**	**$38.54 \pm 1.70_{n=66}$**	$F_{3,135} = 12.99$	$p < 0.01$	$r^2 = 0.22$
Response	**$46.52 \pm 2.49_{n=73}$**	**$32.80 \pm 1.56_{n=66}$**	$F_{3,135} = 6.75$	$p < 0.01$	$r^2 = 0.13$
Free CORT (pg/µl):					
30-Minute	$20.65 \pm 4.08_{n=15}$	$21.93 \pm 2.18_{n=27}$	$F_{3,38} = 1.98$	$p = 0.13$	$r^2 = 0.14$
CBG (nM)	**$115.82 \pm 14.93_{n=15}$**	**$74.79 \pm 7.17_{n=28}$**	$F_{3,39} = 4.04$	$p = 0.01$	$r^2 = 0.24$
JUVENILES					
Total CORT (pg/µl):					
Baseline	$11.74 \pm 2.16_{n=27}$	$9.18 \pm 1.13_{n=29}$	$F_{1,54} < 0.01$	$p = 0.95$	$r^2 < 0.01$
30-Minute	**$58.04 \pm 4.65_{n=27}$**	**$45.22 \pm 2.37_{n=28}$**	$F_{1,53} = 4.44$	$p = 0.04$	$r^2 = 0.08$
Response	$46.30 \pm 4.00_{n=27}$	$35.85 \pm 2.40_{n=28}$	$F_{1,53} = 3.16$	$p = 0.08$	$r^2 = 0.06$
Free CORT (pg/µl):					
30-Minute	$22.77 \pm 11.94_{n=3}$	$25.97 \pm 2.33_{n=14}$	$F_{1,15} = 1.48$	$p = 0.24$	$r^2 = 0.09$
CBG (nM)	**$187.43 \pm 45.40_{n=3}$**	**$75.95 \pm 8.60_{n=15}$**	$F_{1,16} = 8.57$	$p < 0.01$	$r^2 = 0.35$
Adults					
Total CORT (pg/µl):					
Baseline	**$5.94 \pm 4.12_{n=46}$**	**$3.06 \pm 0.30_{n=38}$**	$F_{1,82} = 21.28$	$p < 0.01$	$r^2 = 0.21$
30-Minute	**$52.11 \pm 3.21_{n=46}$**	**$33.62 \pm 2.07_{n=38}$**	$F_{1,82} = 24.91$	$p < 0.01$	$r^2 = 0.23$
Response	**$46.17 \pm 03.18_{n=46}$**	**$30.55 \pm 1.99_{n=38}$**	$F_{1,82} = 16.97$	$p < 0.01$	$r^2 = 0.17$
Free CORT (pg/µl):					
30-Minute	$20.12 \pm 15.45_{n=12}$	$17.57 \pm 3.47_{n=13}$	$F_{1,23} = 0.21$	$p = 0.65$	$r^2 < 0.01$
CBG (nM)	$97.92 \pm 10.75_{n=12}$	$73.45 \pm 12.21_{n=13}$	$F_{1,23} = 3.05$	$p = 0.09$	$r^2 = 0.12$

The full model was used to investigate the influence of species, life stage (juvenile or adult), and the interaction between the two on stress responses. A one-way ANOVA was used to investigate the effect of species on stress response parameters in juveniles and adults.

The majority of juvenile stress response parameters did not differ between Gambel's quail and wild-type Japanese quail (Table 4). The only stress parameters that differed were total CORT levels at 30-minute post-ACTH injection and CBG levels. In both cases, juvenile Gambel's quail had greater levels than juvenile wild-type Japanese quail (Table 4). At the adult stage multiple stress response parameters differed between Gambel's quail and wild-type Japanese quail. Adult Gambel's quail had greater total CORT levels at baseline, 30-minutes post ACTH injection, and response to ACTH injection, as well as greater free CORT levels at baseline than adult wild-type Japanese quail (Table 4).

Domestication Comparison: Wild-Type Japanese Quail versus Domesticated Japanese Quail

Domesticated Japanese quail had a longer tarsus at hatch and longer asymptotic tarsus length, greater body masses at hatch and at their asymptotic size, and faster maximum tarsus and body mass growth rates than wild-type Japanese quail (Table 5, Figure 10 C). Domestic Japanese quail experienced their maximum tarsus growth rate at earlier ages than wild-type Japanese quail, and wild-type Japanese quail experienced their maximum growth rate for body mass earlier than domestic Japanese quail (Table 5, Figure 10 D). However, the wild-type strain reached asymptotic tarsus length slightly earlier (wild-type = 26 days, domesticated= 29 days) and asymptotic body mass much earlier (wild-type = 42 days, domesticated = 56 days) than the domesticated strain. Furthermore, at asymptotic masses, the domesticated strain has nearly twice as many grams body mass per millimeter tarsus length (means: domesticated = 6.96 ± 0.28, wild-type = 3.69 ± 0.11; ANOVA: $F_{1,30} = 170.51$, $p < 0.01$, $r^2 = 0.85$).

Table 5. Domestication comparison of morphometric measures of growth. All growth parameters significantly differed ($p < 0.05$) between wild-type Japanese quail (JaQu) and domesticated Japanese quail (JaQu-Dom). (mean ± $\text{SE}_{n=\text{sample size}}$)

Parameter	JaQu	JaQu-Dom	ANOVAs
Tarsus:			
Hatch Length (mm)	$12.16 \pm 0.07_{n=69}$	$14.27 \pm 0.11_{n=107}$	$F_{1,174} = 230.59 p < 0.01\ r^2=0.57$
Asymptotic Length (mm)	$26.28 \pm 0.18_{n=40}$	$33.71 \pm 0.21_{n=50}$	$F_{1,88} = 603.86\ p < 0.01\ r^2 = 0.87$
Max. Growth Rate (mm/day)	$0.59 \pm 0.01_{n=38}$	$0.84 \pm 0.02_{n=47}$	$F_{1,83} = 98.50\ p < 0.01\ r^2 = 0.54$
Age at Max. Growth Rate (day)	$11.98 \pm 0.49_{n=38}$	$8.99 \pm 0.52_{n=47}$	$F_{1,83} = 13.95\ p < 0.01\ r^2 = 0.14$
Body Mass:			
Hatch Mass (g)	$6.00 \pm 0.08_{n=69}$	$8.92 \pm 0.22_{n=107}$	$F_{1,174} = 190.76\ p < 0.01 r^2 = 0.52$
Asymptotic Mass (g)	$93.50 \pm 2.06_{n=20}$	$230.30 \pm 10.77_{n=10}$	$F_{1,28} = 406.42\ p < 0.01\ r^2 = 0.94$
Max. Growth Rate (g/day)	$2.93 \pm 0.06_{n=28}$	$6.09 \pm 0.15_{n=29}$	$F_{1,55} = 516.30\ p < 0.01\ r^2 = 0.90$
Age at Max. Growth Rate (day)	$22.69 \pm 0.42_{n=28}$	$32.37 \pm 1.43_{n=29}$	$F_{1,55} = 45.19\ p < 0.01\ r^2 = 0.45$

A one-way ANOVA was used to investigate the effect of species on growth parameters.

According to the full model, domesticated Japanese quail had greater total CORT levels at 30-minutes post-ACTH injection and in their response to ACTH injection than wild-type Japanese quail (Table 6). An interaction between strain of Japanese quail and life stage (juvenile/adult) were also found in total CORT levels at 30-minutes ($p < 0.01$) and in their response to ACTH injection ($p < 0.01$).

Juvenile domestic Japanese quail had greater total CORT levels at 30-minutes post ACTH-injection, in their response to ACTH injection, and CBG levels than juvenile wild-type Japanese quail (Table 6). Likewise, adult domestic Japanese quail had total CORT levels at 30-minutes post ACTH-injection and in their response to ACTH injection than adult wild-type Japanese quail (Table 6).

Table 6. Domestication comparison of stress response parameters. The differences in stress response parameters for all ages and two life stages (juvenile and adult) between wild-type Japanese quail (JaQu) and domesticated Japanese quail (JaQu-Dom). Means that are statistically different between species are in bold. (mean ± SE $_{n=\text{sample size}}$)

Parameter	JaQu	JaQu-Dom	ANOVAs		
ALL AGES					
Total CORT (pg/µl):					
Baseline	$5.71 \pm 0.64_{n=67}$	$8.81 \pm 1.29_{n=93}$	$F_{3,156} = 33.06$	$p < 0.01$	$r^2 = 0.39$
30-Minute	**$38.54 \pm 1.70_{n=66}$**	**$70.75 \pm 4.94_{n=93}$**	$F_{3,155} = 48.03$	$p < 0.01$	$r^2 = 0.48$
Response	**$32.80 \pm 1.56_{n=66}$**	**$61.94 \pm 4.19_{n=93}$**	$F_{3,155} = 30.78$	$p < 0.01$	$r^2 = 0.37$
Free CORT (pg/µl):					
Baseline	$2.43 \pm 0.62_{n=28}$	$0.93 \pm 0.28_{n=17}$	$F_{3,41} = 12.00$	$p < 0.01$	$r^2 = 0.47$
30-Minute	$21.93 \pm 2.18_{n=27}$	$23.57 \pm 3.61_{n=17}$	$F_{3,40} = 2.09$	$p = 0.11$	$r^2 = 0.14$
Response	$19.46 \pm 1.98_{n=27}$	$22.63 \pm 3.42_{n=17}$	$F_{3,40} = 1.37$	$p = 0.27$	$r^2 = 0.09$
CBG (nM)	$74.79 \pm 7.17_{n=28}$	$107.75 \pm 18.33_{n=17}$	$F_{3,41} = 2.59$	$p = 0.07$	$r^2 = 0.16$
JUVENILES					
Total CORT (pg/µl):					
Baseline	$9.18 \pm 1.13_{n=29}$	$13.52 \pm 2.09_{n=52}$	$F_{1,79} = 0.40$	$p = 0.53$	$r^2 = 0.01$
30-Minute	**$45.22 \pm 2.37_{n=28}$**	**$91.93 \pm 7.32_{n=52}$**	$F_{1,78} = 44.26$	$p < 0.01$	$r^2 = 0.36$
Response	**$35.85 \pm 2.40_{n=28}$**	**$78.41 \pm 6.32_{n=52}$**	$F_{1,78} = 35.90$	$p < 0.01$	$r^2 = 0.32$
Free CORT (pg/µl):					
Baseline	$4.07 \pm 0.98_{n=15}$	$1.77 \pm 1.35_{n=3}$	$F_{1,16} = 4.12$	$p = 0.06$	$r^2 = 0.20$
30-Minute	$25.97 \pm 2.33_{n=14}$	$31.67 \pm 14.26_{n=3}$	$F_{1,15} < 0.01$	$p = 0.99$	$r^2 < 0.01$
Response	$21.70 \pm 1.96_{n=14}$	$29.90 \pm 12.96_{n=3}$	$F_{1,15} = 0.21$	$p = 0.66$	$r^2 = 0.14$
CBG (nM)	**$75.95 \pm 8.60_{n=15}$**	**$185.77 \pm 25.33_{n=3}$**	$F_{1,16} = 9.91$	$p < 0.01$	$r^2 = 0.38$
Adults					
Total CORT (pg/µl):					
Baseline	$3.06 \pm 0.30_{n=38}$	$2.84 \pm 0.28_{n=41}$	$F_{1,77} = 0.45$	$p = 0.50$	$r^2 < 0.01$
30-Minute	**$33.62 \pm 2.07_{n=38}$**	**$43.89 \pm 2.89_{n=41}$**	$F_{1,77} = 6.89$	$p = 0.01$	$r^2 = 0.08$
Response	**$30.55 \pm 1.99_{n=38}$**	**$41.05 \pm 2.76_{n=41}$**	$F_{1,77} = 7.99$	$p < 0.01$	$r^2 = 0.09$
Free CORT (pg/µl):					
Baseline	$0.53 \pm 0.08_{n=13}$	$0.75 \pm 0.20_{n=14}$	$F_{1,25} = 0.11$	$p = 0.74$	$r^2 < 0.01$
30-Minute	$17.57 \pm 3.47_{n=13}$	$21.83 \pm 3.38_{n=14}$	$F_{1,25} = 1.01$	$p = 0.32$	$r^2 = 0.04$
Response	$17.04 \pm 3.50_{n=13}$	$21.08 \pm 3.29_{n=14}$	$F_{1,25} = 1.04$	$p = 0.32$	$r^2 = 0.04$
CBG (nM)	$73.45 \pm 12.21_{n=13}$	$91.03 \pm 18.96_{n=14}$	$F_{1,25} = 0.08$	$p = 0.78$	$r^2 < 0.01$

The full model was used to investigate the influence of strain, life stage (juvenile or adult), and the interaction between the two on stress responses. A one-way ANOVA was used to investigate the effect of species on stress response parameters in juveniles and adults.

Discussion

In agreement with our predictions for the life history analysis between the two species, our data suggest trade-offs between growth and the stress response in wild-type Japanese quail and Gambel's quail from hatch to sexual maturity. The effects of domestication and selection for large body size and increased reproductive effort on the Japanese quail stress response were not completely in agreement with predictions based on the life history theory. Of all the measures of growth and stress response the maximum growth rate and baseline corticosterone levels seem to characterize the life-history axes, whereas asymptotic size and corticosterone response to an ACTH challenge appear to distinguish a domestication axis. For example, Figure 11 illustrates how the life history axis between the two species (Gambel's and wild-type Japanese quail) is in line with predictions from life-history theory (Figure 11 A and B), whereas the life history axes between wild-type and domesticated Japanese quail are not different. The expected trade-offs between growth parameters (determined by pace-of-life) and the stress response between Gambel's quail and wild-type Japanese quail are clear. Though Gambel's quail are larger (Figure 11A and 11C), they grow at a slower pace (Figures 11B and 11D), ultimately allowing them more energy to devote to the stress response (Figure 11C and 11D). The domesticated strain of Japanese quail are larger (Figures 11A and 11C), they experience faster growth rates (Figures 11B and 11C), and, surprisingly, they are able to mount a response to stress at greater magnitudes than the wild-type strain (Figures 11C and 11D).

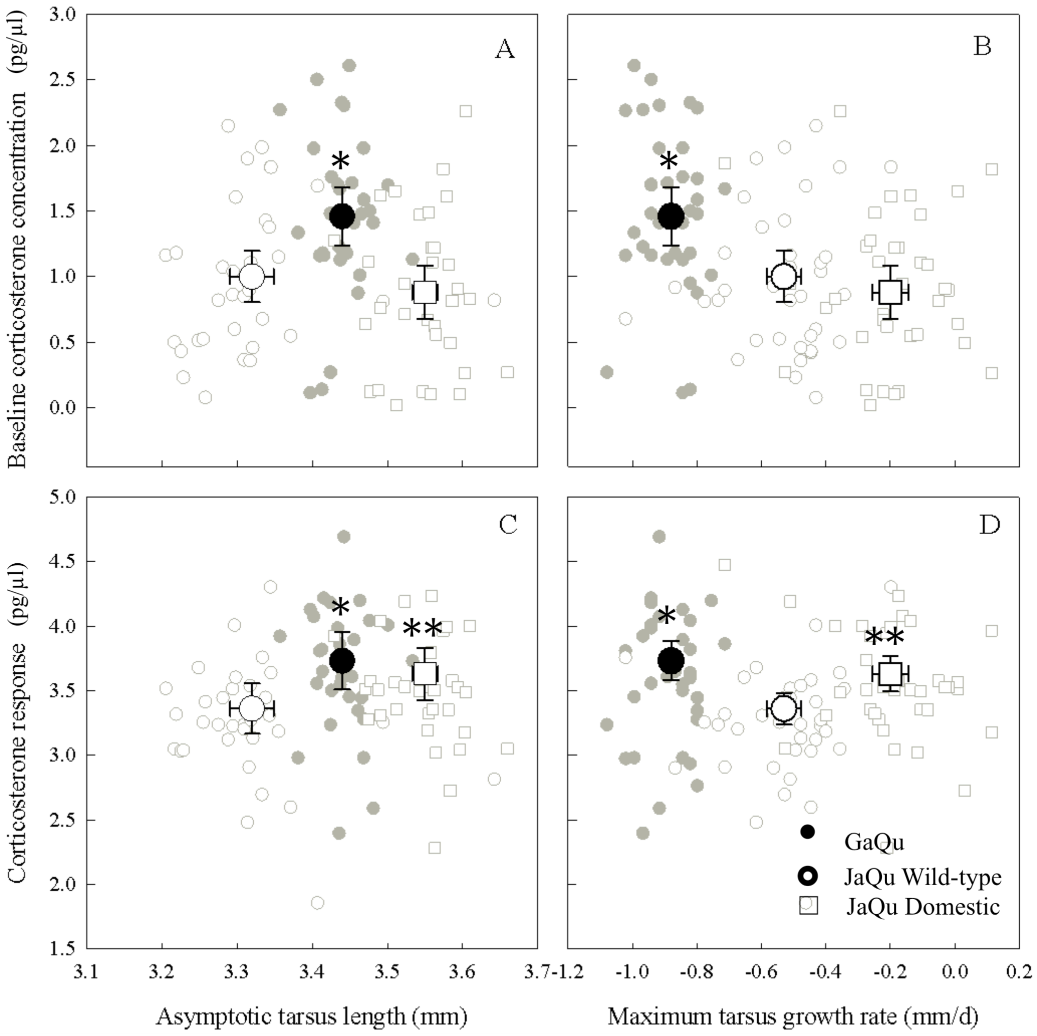

Figure 11. Corticosterone versus growth. Example of trade-offs associated with the life histories and unexpected results due to domestication observed in adults based on body size against (A) baseline CORT and (C) CORT response as well as growth rate against (B) baseline CORT and (D) CORT response. Significant differences between wild-type Japanese quail and Gambel's quail CORT concentrations are noted with an asterisk. Significant differences between wild-type and domesticated strains of Japanese quail are noted with two asterisks. All growth parameters were significantly different between species and strains within species (see Tables 3 and 5, respectively). Data are log-transformed.

The results from our analyses of multiple morphometric measures of growth in this study support our predictions based on demographic information gathered from literature for both species (Table 3) and strains within species (Table 5). Wild-type Japanese quail reached their asymptotic tarsus length and body mass in nearly half the time it took Gambel's quail and also experienced faster maximum tarsus and mass growth rates at earlier ages compared to Gambel's quail. The differences in growth parameters between the two strains of Japanese quail were also extensive, as domesticated had greater masses, larger tarsi, and faster growth rates. Trade-offs associated with corticosterone and growth are well documented (Siegel and Van Kampen 1984; Elsey et al. 1990; Stearns 1992; Morici et al. 1997). Because the correlation between age (hatch to sexual maturity) and stress parameters was stronger in both strains of Japanese quail than Gambel's quail (Table 2, Figure 9), the differences in growth strategies between species (Table 3) and strains within species (Table 5) could, in part, explain the observed differences in the relationship between age and stress parameters in these quail.

Corticosterone binding globulin capacity was the only stress parameter that changed (decreased) with age in Gambel's quail. According to the free hormone hypothesis (Mendel 1989), when bound to CBG, CORT is biologically inactive until it reaches the target tissue, where it is enzymatically unbound (free), becoming active and capable of being metabolically cleared from plasma (review in Malisch and Breuner 2010). Thus, binding of CORT to CBG can protect tissues from the potentially deleterious effects of CORT and reduce the clearance rate of CORT (Breuner and Orchinik 2002; Malisch and Breuner 2010). This potential buffer may be a particularly beneficial mechanism to protect vulnerable tissues that are still developing while keeping potentially biologically active CORT in plasma (Wada et al. 2007; Malisch and Breuner 2010). Because we observed a decrease in CBG with age in the slow-growing Gambel's quail but not either strain of fast-growing Japanese quail, our hypothesis is that Gambel's quail have the benefit of this mechanism to protect vulnerable tissues since they have more energy to invest outside of growth compared to Japanese quail.

Relationships between age and stress parameters were more widespread in both strains of Japanese quail than in Gambel's quail. Total CORT levels decreased in baseline measurements and at 30-minute post-ACTH challenge as individuals approached sexual maturity. In a review of multiple studies, Heidinger et al. (2008) concluded that weakening of the stress response across age may be caused by a *reduction* in any or all of the following: (i) release of hormones associated with the stress response along the HPA-axis, (ii) sensitivity of tissues along the HPA-axis associated stress response hormones, (iii) negative feedback regulation of secretion of CORT, or an *increased* (v) rate that CORT is cleared from the body. Moreover, CBG capacity did not change with age in either strain of Japanese quail. Recently, Malisch and Breuner (2010) describe the reservoir hormone hypothesis (Breuner and Boonstra, in press). Similar to the free hormone hypothesis, the reservoir hormone hypothesis states that unbound (free) CORT is biologically active and CORT bound to CBG is biologically inactive. However, the reservoir hormone hypothesis proposes that even though the portion of CORT bound to CBG is inactive, it is still biologically relevant, as it may serve as a reservoir of CORT in the blood that can be used as needed by degrading CBG or altering the binding affinity (Bruener and Boonstra, in press). Because we observed an age related decrease in total CORT but not CBG among individuals, it is possible that as both strains of Japanese quail age, they tend to lose a significant amount of reservoir CORT. Reductions in reservoir CORT could lead to an increased rate that CORT is cleared from the body and a decrease in negative feedback regulation by CORT itself, because the amount of CORT able to travel to target tissues may be reduced.

Life History Comparison

Trade-offs between survival and key life history traits are predicted to occur to different degrees among species that lie on different points along the fast-slow life history continuum (Stearns 1992). O'Neal and Ketterson (2012) noted that slow-paced species usually have lower basal metabolic rates (Wikelski et al. 2003; Wiersma et al. 2007), more robust immune responses (Martin et al. 2004; Tieleman et al. 2005; Addison et al. 2009), lower testosterone during breeding (Wingfield et al. 2001), and higher CORT levels in response to stressors (Bókony et al. 2009; Malisch and Breuner 2010; Palacios et al. 2012), than fast-paced species. Results from our analysis of differences in stress response parameters between species were consistent with these predictions (Figure 11). Gambel's quail consistently had the greater CORT levels than Japanese quail (Table 5). Furthermore, the basal metabolic rate of Gambel's quail (Weathers 1981) is lower than that quantified in wild-type Japanese quail (Prinzinger and Hänssler 1980). Additionally, free-living Japanese quail are a migratory species and face the energetically costly challenges associated with migration, while Gamble's quail are non-migratory. Though flying ability is weak in quail, they are capable of successfully migrating hundreds and, in some cases, thousands of kilometers (Chang et al. 2005). Generally, wild-type Japanese quail migrate to northwest China in March and April to breed in June and July and migrate back to coastal and southern regions of China during the winter (Change et al. 2005). Collectively, these findings suggest that Gambel's quail have the ability to direct more energy into the stress response than wild-type Japanese quail.

In addition to the list compiled by O'Neal and Ketterson (2012), life history theory also predicts that slow-paced species have higher adult survival rates compared to fast-paced species (Stearns 1992). Hau et al. (2010) found that greater levels of CORT in response to stress correlated with higher annual adult survival rates in passerine species. The authors propose that the ability to mount higher responses to stress is beneficial because it can promote the ability to escape harmful situations and suppress energetically costly activities not associated with immediate survival (e.g. reproduction), can re-establish bodily homeostasis (e.g. increase in feeding rates and energy storage), and shut down the initial stress response in a timely manner. Though we do not know the approximate adult survival rates in either species of quail used in this study, because wild-type Japanese quail exhibit more fast-paced characteristics than Gambel's quail, we can hypothesize that wild-type Japanese quail experience lower adult survival rates than Gambel's quail (Stearns 1992). It has been reported that adult survival rate is much higher than the juvenile survival rate in Gambel's quail (Gallizioli 1967), which is consistent with this hypothesis. Thus, the greater levels of CORT observed at 30-minutes after ACTH injection in Gambel's quail at both life stages could be an underlying mechanism that may help explain the evolution of the different key life history characteristics of these two species. To test this, future investigations should quantify and incorporate adult survival rates from free-living populations of these two species.

Domestication Comparison

The strain of domesticated Japanese quail used in this study require more time to reach their asymptotic mass (Figure 10) than domestic quail growth rates found in the literature (Cain and Cawley 1972). It has been demonstrated that rigorous selection for growth in the poultry industry has not only increased growth rates, but also fat deposition in quail and other poultry (Lotfi et al. 2011, Griffin 1996). Domestic Japanese quail continue to increase body mass after they reach sexual maturity (Lotfi et al. 2011), which we observed in the birds in this study. We did not perform a body composition analysis, we hypothesize that the continued increase in body mass observed in this study was due to continued fat deposition in these quail. When necropsies were performed post-euthanasia, there was more abdominal fat and fat present around the neck in the domesticated strain than the wild-type strain of Japanese quail (Gastecki, personal observations).

The selection for exaggerated breast muscle and body mass in domesticated species may also shape skeletal growth. The domesticated stain of Japanese quail had maximum tarsus and mass growth rates that were 1.5 and 2 times greater than wild-type quail, respectively (Table 5). Though wild-type Japanese quail reached their adult tarsus length at slightly earlier ages than domesticated Japanese quail, the domestic strain experienced their maximum tarsus growth rates at earlier ages than the wild-type strain, which suggests the domestic strain makes a greater investment into skeletal growth than the wild-type strain. Wild-type Japanese quail experience their maximum mass growth rates, on average, three days before they reach their adult tarsus length, alternatively, domesticated Japanese quail experience their maximum mass growth rates three days after they reach their adult tarsus length. Thus, the process of domestication in our strain of

Japanese quail has not only resulted in greater body masses and faster growth rates, but also alternative skeletal growth strategies that may be beneficial in keeping an appropriate tarsus to body mass ratio which may avoid leg problems that are traditionally associated with poultry domestication (Kestin et al. 1992; Sanotra et al. 2001; Knowles et al. 2008).

Not only has domestication affected multiple growth parameters of Japanese quail, it has also clearly affected the reproductive effort of Japanese quail. In a recent study that compared multiple behavioral differences between domesticated and wild-type Japanese quail, female domestic quail produced nearly 10-fold more eggs per year than wild-type Japanese quail, domestic Japanese quail mated more frequently than wild-type Japanese quail, and domestic Japanese quail had much higher fertility and hatching rates than wild-type Japanese quail (Chang et al. 2009). It is well documented that CORT suppresses the reproduction system, as has inhibitory effects on the hypothalamo-pituitary-gonadal axis (Maeda and Tsukamura 2006). Life history theory predicts that as individuals age, hormone responses that can interfere with reproduction may decline (O'Neal and Ketterson 2012). Our data corroborate Malisch and Bruener's (2010) suggestion that stress reactivity is minimized by reducing the total CORT circulating in the plasma in response to a stressor. Therefore, the observed decrease in total CORT response with age observed only in the domesticated strain could be a consequence of the selection for the dramatic increased reproductive effort. Additionally, the decrease in total CORT measured at baseline and 30-minutes post-ACTH injection with age in both strains of Japanese quail follows some predictions from the life history theory.

The results from our analysis of effect of strain of Japanese quail on stress response parameters, however, were not consistent with our prediction that the domesticated strain would have lower stress response parameters than the wild-type strain because the domesticated strain devote more energy to growth (Figure 11). When both life stages (juvenile and adult) were considered together and separately, the domesticated strain had greater levels of total CORT at 30-minutes and in the response to an ACTH challenge. It is well documented that artificial selection on physical traits can rapidly alter behavioral traits as well (reviewed in Muir and Craig 1998). For example, physical traits (rate of lay and longevity) were selected for in laying hens, which resulted in significant behavioral modifications (less feather pecking and cannibalism) by the sixth generation (reviewed in Muir and Craig 1998). In a recent study, male domestic Japanese quail showed more aggression towards each other than males from the wild-type strain (Chang 2009). In addition to rapid behavioral modifications observed in domestication studies, changes in physiological responses can also occur quickly (Gross and Siegel 1985; Siegel 1993). For example, by the sixth generation in a study that selected chickens for high and low response to social stress, there was no overlap in distribution of plasma CORT response between individuals of both lines (Gross and Siegel 1985; Siegel 1993). Because physiological responses and behavior are not mutually exclusive (Siegel 1993), the differences in behavior and CORT parameters between the two strains could indicate that domestication and selection for large body size has resulted in correlated selection on hormone and receptor dynamics of the stress response (e.g. decreased sensitivity of negative feedback regulation of CORT secretion) resulting in an exaggerated response to stress.

Conclusions

Our data suggest trade-offs between growth and stress response in wild-type Japanese quail and Gambel's quail. For example, Gambel's quail are larger, they grow at a slower pace, and have a more robust stress response than wild-type Japanese quail. This pattern is consistent with the predictions from life-history theory suggesting that energy is allocated preferentially to mechanisms that promote survival. In contrast, changes in body size and growth due to domestication do not result in the same changes in stress response. The domesticated strain of Japanese quail are also larger than the wild-type strain, but they experience faster growth rates, and are able to mount a response to stress at greater magnitudes than the wild-type strain.

Domestication of Japanese quail has clearly led to differences in growth patterns compared to the wild-type strain, but our data do not strongly indicate that this selection for increased size and growth rate has led to a traditional trade-off between growth and the stress response in the domesticated strain of Japanese quail. Instead, it seems that the domesticated line used in the study has *increased* the stress response, but possibly at the cost of being able to maintain the same magnitude of response to stress over the individual's lifetime, as we observed a decrease in stress response across age only in this strain of Japanese quail.

In conclusion, the findings in this study contribute to the understanding of differences in the stress response between species that exhibit different life history strategies. Furthermore, we have explored possible consequences of domestication on the stress response in a species of quail. These findings should be further integrated with current and future analyses that examine other life history characteristics (e.g. immune system and reproductive system dynamics) in these species to build a more comprehensive foundation for life history analysis.

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CONCLUSIONS AND SUGGESTED FUTURE RESEARCH

Our data suggest trade-offs between growth and stress response in wild-type Japanese quail and Gambel's quail. For example, Gambel's quail are larger, they grow at a slower pace, and have a more robust stress response than wild-type Japanese quail. This pattern is consistent with the predictions from life-history theory suggesting that energy is allocated preferentially to mechanisms that promote survival. In contrast, changes in body size and growth due to domestication do not result in the same changes in stress response. The domesticated strain of Japanese quail are larger, they experience faster growth rates, and, surprisingly, they are able to mount a response to stress at greater magnitudes than the wild-type strain.

Domestication of Japanese quail has clearly led to differences in growth patterns compared to the wild-type strain, but our data do not strongly indicate that this selection for increased size and growth rate has led to a traditional trade-off between growth and the stress response in the domesticated strain of Japanese quail. Instead, it seems that the domesticated line used in the study has *increased* the stress response, but possibly at the cost of being able to maintain the same magnitude of response to stress over the individual's lifetime, as we observed a decrease in stress response across age only in this strain of Japanese quail.

Though we did not analyze inter-individual variation in stress response parameters within and among species/strains within species in this study, these data are important and should be explored in the future. Because individual variability is important in optimizing life histories, the high degree of inter-individual variation in all parameters of CORT among the species and strains observed in this study may provide valuable insight for the field of evolutionary biology (Williams 2008). To date, evolutionary modifications in hormone regulation are thought to be an important mechanism underlying the evolution of entire suites of traits in a coordinated manner in response to environmental change by either selection on heritable, fitness-related, individual variation or through adaptive phenotypic plasticity (reviewed in Dufty et al. 2002; Zera et al. 2007; Williams 2008). For example, in nesting mourning doves (*Zenaida macroura*) individual variation in baseline CORT was positively associated with parental care (Miller et al 2009). Additionally, individual variation in stress induced CORT response was related to parental effort. Future research should focus on explaining inter-individual variation and the costs or benefits it contributes to the individual's fitness. In agreement with Zera et al. (2007), we argue that continued progress in the explanation of inter-individual variation requires greater integration among (a) traditional comparative endocrine approaches (e.g. analysis of titer measures, such as the current study); (b) studies that focus on hormone receptor dynamics and intracellular signaling pathways (see Appendix A); (c) studies that focus on fitness of genetically variable endocrine traits in appropriate ecological settings.

In conclusion, the findings in this study contribute to the understanding of differences in the stress response between species that exhibit different life history strategies. Furthermore, we have explored possible consequences of domestication on the stress response in a species of quail. These findings should be further integrated with current and future analyses that examine other life history characteristics (e.g. immune system and reproductive system dynamics) in these species to build a more comprehensive foundation for life history analysis.

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APPENDIX. MODIFIED PERFUSION PROTOCOL

Collection of Avian Brain Tissue for Receptor Analysis

Purpose

This protocol should be used to collect avian brain tissue for use in analyses of receptors associated with the stress response (e.g. glucocorticoid receptor and mineralocorticoid receptor). The specific brain regions collected in this protocol are the hippocampus, hypothalamus, and pituitary. Prior to collection, these brain tissues must be perfused with physiological saline to remove corticosterone binding globulin and endogenous hormones that bind with high affinity to these receptors. Usually, the heart is used to pump the saline through the bird as it is anesthetized during this procedure. We found that we could successfully perfuse brains by euthanizing the bird and physically pushing the saline mixture with a large syringe through the body. However, this modification should be used with caution, as we used quail species (Gambel's quail and Japanese quail) and are unsure if smaller bird circulatory systems can handle the pressure of the forced saline.

Materials Needed

- Halothane
- Physiological saline with heparin (1000 usp U L^{-1})
- Butterfly needle (~20 gauge) connected to a large (~500cc) syringe
- Probe
- Surgical scissors
- Forceps
- Ethanol
- Dry ice in a cooler
- Aluminum foil

Perfusion of Brain Tissues

1. During euthanasia with halothane, secure the bird to a surface that allows fluids to pass through freely yet stay contained (Figure A1).

Figure A1. Animal set-up.

2. Once the bird has been euthanized, immediately cut the chest cavity open to expose the heart. A probe should be used to slightly readjust the heart to make it more accessible (Figure A2).

Figure A2. Exposed heart.

3. Sever both jugular veins to prevent fluid from returning to the heart and insert a butterfly needle that is connected to a syringe filled with the saline mixture into the left ventricle (Figure A3).

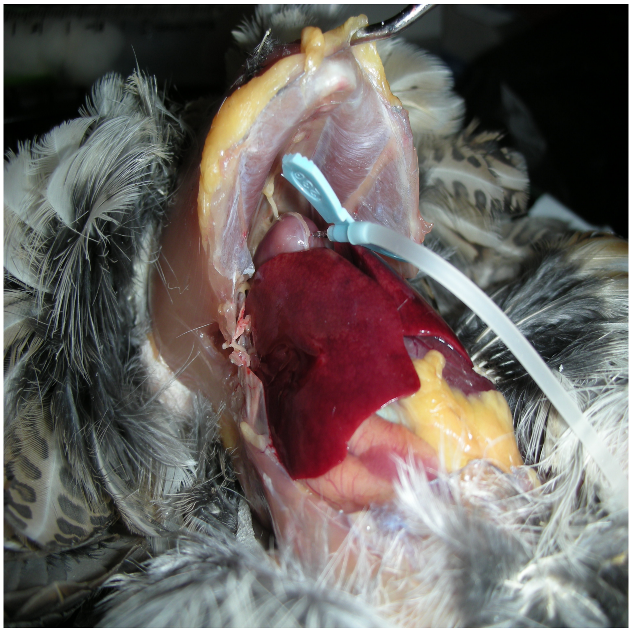

Figure A3. Butterfly needle inserted in left ventricle.

4. Push approximately 150 mL of saline mixture through the left ventricle for about 10 minutes (Figure A4). If the needle is positioned correctly, the saline will travel through the carotid artery into the brain and out the severed jugular veins. There should be a continuous drip of fluid exiting the cut jugular veins.

Figure A4. Pushing of saline mixture with syringe.

5. If the liver is becoming blanched, that is a good indicator that the perfusion is going well (Figure A5). When the fluid dripping from the jugular veins becomes *completely* clear, the brain tissues should be properly perfused.

Figure A5. Blanching of liver.

Collection of Brain Tissues

The hippocampus, hypothalamus, and pituitary must be immediately collected once perfusion is successfully completed. Collection of tissues should be done with forceps that have been sterilized with ethanol before collection of each tissue. When each tissue is removed, it should be tightly closed in aluminum foil and immediately put on dry ice. When collection of all tissues is completed, the samples should be stored at -40ºC until the assay. For use in optimizing the intracellular assay, the remaining portions of the brain (thalamus, midbrain, hindbrain and cerebrum) should also be collected and stored. Since the pituitary is so small, the pituitaries from at least three individuals will most likely be pooled in the assay.

1. To begin tissue collection, cut the head from the body so it is easier to manipulate. Then cut or pull the skin and muscles from the skull to expose the brain case (Figure A6).

Figure A6. Exposed brain case.

2. Remove a large enough portion of the skull to expose the cerebrum (Figure A7 A). The region containing the hippocampus (approximately 0.014 g) should be collected first (Figure A7 B).

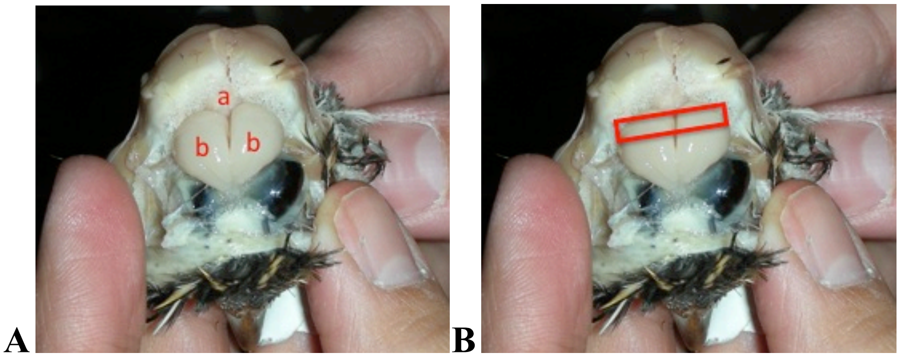

Figure A7. Collection of hippocampus. A: A dorsal view of a perfused domestic Japanese quail cerebellum (a) and cerebrum (b). B: The red box indicates the region collected for the hippocampus (~1 mm deep).

3. Remove the remainder of the cerebrum to expose the hypothalamus and thalamus lobes (Figure A8 A). Collect the hypothalamus (Figure A8 B; approximately 0.043g).

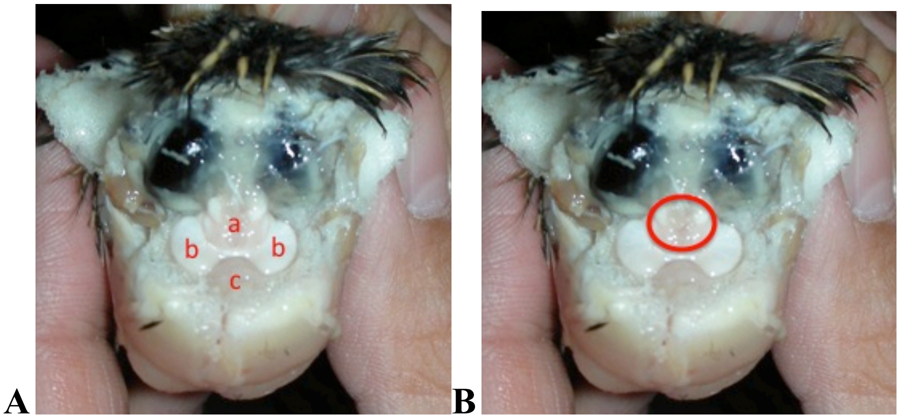

Figure A8. Collection of hypothalamus. A: A dorsal view of the hypothalamus (a), thalamus lobes (b), and cerebellum (c) after the cerebrum was removed. B: The red circle indicates the region collected for the hypothalamus.

4. Remove the thalamus lobes and optic nerves to expose the pituitary. To extract the pituitary more easily from the sella turcica, remove the rest of the skull from the posterior sphenoid bone (Figure A9 A). Collect the pituitary (Figure A9 B; approximately 0.0016g)

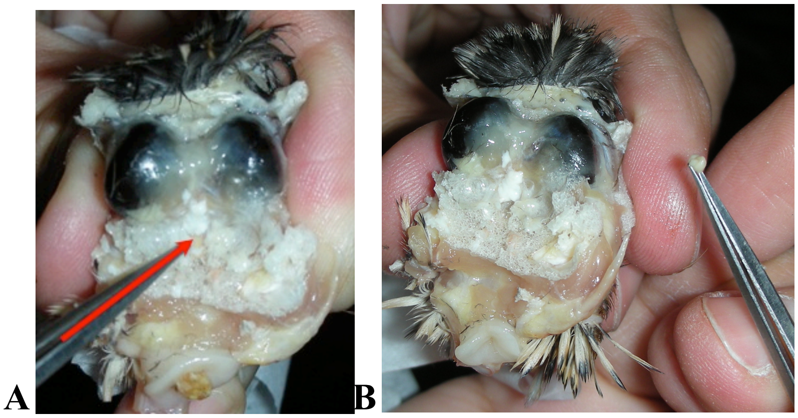

Figure A9. Collection of pituitary. A: A view of the pituitary (at the end of the red arrow) resting in the sella turcica after part of the skull was removed. B: The pituitary removed and on the end of the forceps.