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Physiological flexibility in an avian range expansion

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ABSTRACT

The mechanisms that enable animals to colonize new areas are little known, but growing evidence indicates that the regulation of stress hormones is important. Stress hormones probably influence invasions because they enable organisms to adjust their phenotypes depending on environmental context. Often, studies of stress hormones are based on single or a few samples from individuals even though the flexibility in the regulation of such hormones is what enables them to achieve homeostasis and facilitate performance. Here, we asked whether flexibility in the regulation of one stress hormone, corticosterone, was related to colonization success in one of the world's most successful avian invaders, the house sparrow (*Passer domesticus*). We studied Kenyan house sparrows, as the species was recently introduced there (around 1950) and has since expanded northwestward. Previous work in this system revealed that younger populations released more corticosterone during a restraint stressor than older populations. Our first goal was to discern whether such population differences were fixed or flexible in adulthood; our second goal was to determine whether individual identity explained any variation in corticosterone regulation. As before, we found that corticosterone responses to short-term restraint (i.e., stress responses), but not baseline corticosterone, were larger in younger populations. We also found that both baseline and stress-induced corticosterone measures were flexible; both metrics became similar among sites after one week of captivity. For stress responses, we also found that individual identity was important. Altogether, the present data suggest that the colonization of Kenya by house sparrows might have been facilitated by stress hormone regulatory flexibility.

1. Introduction

Introduced species are among the greatest threats to native ecosystems, and they cost billions of dollars annually to control (Mack et al., 2000). However, introduced species also present unique opportunities for understanding important biological phenomena, such as how organisms expand their ranges and adjust to environmental novelty. Particular factors mediate organismal invasion success (Parker et al., 2013) such as reproductive life history (Sol et al., 2012), behavioral innovation (Sol et al., 2005, 2002), propagule pressure (Colautti et al., 2006), and genetic admixture (Kolbe et al., 2007, 2008). Whether variation in these and other traits arises via selection, plasticity, or both (Richards et al., 2006) remains mostly unresolved, especially for vertebrates. Selection is undoubtedly important, especially over long time scales. However, plasticity is probably impactful too because invasions commonly involve genetic bottlenecks, which can restrict responses to selection (Allendorf and Lundquist, 2003), and plasticity can match the phenotype to the environment faster than selection (Ghalambor et al., 2007).

Phenotypic plasticity is usually defined as the ability of a genotype to express different phenotypes depending on the environment (Agrawal, 2001). In plants, plasticity is related to invasion and introduction success (Davidson et al., 2011). In animals, the importance of plasticity in range expansion is less understood, especially with regards to physiology. Physiological plasticity is of likely great importance to range expansions though (Atwell et al., 2012) because of the importance of homeostasis to performance and fitness (Wingfield, 2013a). Indeed, homeostasis itself is a form of plasticity (Crespi et al., 2013; Woods, 2009); for physiological stasis to occur, one or more physiological processes must change when environments change (Sterling, 2012; Woods and Wilson, 2014). Physiological plasticity occurs on at least three time scales: during development (West-Eberhard, 2003), between seasons (Jacobs and Wingfield, 2000), and over short time frames (Piersma and Drent, 2003) such as across days or months. Epigenetic mechanisms, maternal effects, early-life experiences, and

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habituation all shape these plasticities, particularly the regulation of homeostasis mediators (Ledón-Rettig et al., 2013; Love et al., 2013; Martin et al., 2011b; Schoech et al., 2011). In the present study, we sought to determine whether plasticity occurring over short time scales, called phenotypic flexibility (Piersma and Drent, 2003) or activational plasticity (Snell-Rood, 2013), influences population differences in glucocorticoid regulation, perhaps by facilitating mosaics of adaptive traits (Woods, 2014).

To investigate the role of physiological flexibility in range expansions, we have been studying the house sparrow (*Passer domesticus*) in Kenya. House sparrows are exemplary invaders, now being one of the most broadly distributed animals in the world (Anderson, 2006). The Kenyan invasion is particularly intriguing because house sparrows were introduced there recently (~1950; Summers-Smith, 1988), and distance from the initial site of introduction (Mombasa) is a good predictor of genetic and phenotypic variation among populations (Liebl and Martin, 2012, 2013, 2014; Martin et al., 2014). Also, the observed patterns of variation (in combination with the absence of other known introductions to east Africa and the recency of the introduction to Mombasa) implicate plasticity as influential in the Kenyan range expansion (Martin et al., 2014). Indeed, microsatellite data indicate that Kenyan house sparrows are genetically less diverse than other populations (Schrey et al., 2011), and genotypes common at the site of introduction (in Mombasa) are also found at the western range edge > 850 km away (Schrey et al., 2014).

Glucocorticoids are integral mediators of organismal performance (Crespi et al., 2013; Love et al., 2013), serving basal metabolic functions but also enabling individuals to endure or recover from stressors (Boonstra, 2013; Wingfield, 2013b). In most studies, glucocorticoid actions are inferred from just a few samples from individuals, but whether so few measures capture the complexity and dynamism of glucocorticoid regulation is questionable. Indeed, a major part of what enables glucocorticoids to facilitate homeostasis and coping with various stressors is that their regulation is quite flexible. Subsequently, it might be informative to consider how critical aspects of glucocorticoid regulation (i.e., their baseline levels and responses to stressors) change with environments. By using a classic reaction norm approach (i.e. measuring glucocorticoid traits several times across an environmental gradient), one might better capture the regulatory flexibility that allows these hormones to achieve their diverse functions. At the same time, one might determine whether regulatory flexibility is consistent within-individuals, which is often assumed but rarely tested in field studies of glucocorticoids.

Here, we used a reaction norm study design to ask whether the glucocorticoid profiles of birds from the oldest Kenyan population (Mombasa) would come to resemble birds from some of the newer populations (Nairobi and Nakuru) when birds were held in captivity for one week. We focused on glucocorticoids because we found previously that distance from the site of introduction predicted the magnitude of corticosterone (the main avian glucocorticoid) stress responses (i.e. corticosterone in response to restraint) (Liebl and Martin, 2012): the nearer birds occurred to range edges, the stronger their stress responses. We used captivity duration as an environmental gradient because we found previously that keeping house sparrows captive for short periods alters corticosterone regulation (Martin et al., 2012, 2011a); in that study, baselines were elevated and stress responses damped after a period of captivity. By measuring corticosterone repeatedly in captivity, we could assess whether populations would come to resemble each other if all individuals experienced similar conditions. A second motivation of our study was to discern whether individual regulatory personalities (Dingemanse et al., 2010; Nussey et al., 2007; Williams, 2008) occurred and/or influenced plasticity across populations, and we used random regression to test these expectations (Dingemanse and Dochtermann, 2012).

2. Methods

2.1. Bird capture, care and morphometrics

Wild, adult Kenyan house sparrows (n = 29; Mombasa = 8 (6M, 2F), Nairobi = 14 (6M, 8F), Nakuru = 7 (4M, 3F)) were captured in mist nets in May–July 2012; the oldest site was Mombasa where birds were introduced ~1950, whereas the newest was presumed to be Nakuru, with Nairobi intermediate in age. 50 μL of blood was collected within 3 min of capture from the brachial vein, and sex was recorded for each individual. Birds were then held in cloth bags in the shade for 30 min when a second blood sample (50 μL) was taken; the difference between this value and the initial baseline sample was considered the stress response. Birds were then returned to bags where they were held singly (2.5 h maximum) until they were transferred to and housed singly in conventional songbird cages (35 cm wide × 27.5 cm deep × 47.5 cm high) for 7 days. Each cage included two perches, *ad libitum* access to mixed seeds (red and white millet, rice, and sorghum) and water treated with anti-coccidian medication (to prevent mortality due to naturally occurring infections). For the duration of captivity, birds remained isolated from human disturbance except a <10 min period daily when food and water were checked and replaced as needed. However, all birds were allowed to see and hear each other throughout the study. Photoperiod and climate conditions were maintained at ambient levels for the study duration. Three days post capture (around 0800 h), each bird was caught from its cage, and 50 μL of blood was taken from the brachial vein within 3 min of researcher entry into the room; birds were then placed individually in cloth bags, and 30 min later, another 50 μL blood sample was taken. Immediately afterwards, birds were returned to their cages and maintained as above. At 7 days post-capture, birds were bled at 3 min and 30 min similarly to day 3; after the last blood sample, birds were released near their site of capture. To ensure samples were collected within 3 min in captive birds, multiple researchers entered the housing room simultaneously. Blood was centrifuged (<2 h post-collection) and plasma was removed from samples and stored in liquid nitrogen until return to the USA, where they were stored at −40 °C. All procedures met guidelines for the use of animals in research and were approved by the USF IACUC (#W3202) and the Kenya Ministry of Science and Technology (NCST/RRI/12/1/MAS/15).

2.2. Corticosterone assay

An EIA kit (Enzo Life Sciences, Ann Arbor, MI; cat# 900-097) was used to measure total plasma corticosterone (CORT) (Breuner et al., 2006; Liebl and Martin, 2012; Martin et al., 2012). Briefly, 10% steroid displacement reagent (5 μl) was added to 5 μl of plasma and 5 min later, assay buffer (240 μl) was added to each sample, vortexed, and aliquoted in duplicate (100 μl per well) to plates. In addition, a standard curve (ranging from 200,000 to 32 pg) was measured in duplicate on each plate. Samples and standards were then incubated with conjugated CORT and antibody for 2 h at room temperature while being shaken. Wells were emptied and washed 3 times before substrate was added to all wells; plates were incubated an additional 1 h at room temperature without shaking. Stop solution was then added, and each plate was read at 405 nm (corrected at 590 nm to minimize background absorbance). Average intra-assay variation was 7.8% and inter-assay variation was 7.0%.