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Phenotypic plasticity in response to breeding density in tree swallows: An adaptive maternal effect?

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ABSTRACT

Territorial animals breeding in high-density environments are more likely to engage in aggressive competition with conspecifics for resources necessary for reproduction. In many avian species, increased competition among breeding females results in increased testosterone concentrations in egg yolks. Generally, elevated yolk testosterone increases nestling growth, competitive behaviors, and bold behavioral traits. However, few studies provide an environmental context with which to examine the potential adaptive benefits of these phenotypic changes. In this study, tree swallow (*Tachycineta bicolor*) breeding density was altered to modify levels of social competition and yolk testosterone. We measured nestling growth, competitive ability, and breathing rate in response to a stressor using a partial cross-foster design. Females breeding at high-density experienced more aggressive, competitive interactions and their eggs had higher testosterone concentrations. Nestlings that hatched in high-density environments grew faster and displayed more competitive behaviors and a higher breathing rate response to a stressor regardless of post-hatching density. Our study demonstrates that phenotypic plasticity occurs in response to yolk testosterone variation resulting from different breeding densities. These findings suggest that naturally-induced maternal effects prepare offspring for competitive environments, supporting the idea that maternal effects are adaptive.

Introduction

Animals display aggressive behaviors toward conspecifics for numerous reasons, a common reason being competition for breeding resources. Territorial and monogamous female songbirds that are better at competing benefit by acquiring and maintaining breeding territories and pairing with a monogamous mate (Dunn and Hannon, 1991; Rosvall, 2008). In addition, several studies show a positive relationship between aggression among females competing for breeding resources and concentrations of androgens (e.g., testosterone) in their egg yolks (Hargitai et al., 2009; Mazuc et al., 2003; Navara et al., 2006b; Whittingham and Schwabl, 2001). Environmentally-induced variation in egg hormones is a classic example of a maternal effect, a non-genetic mechanism in which conditions females experience influence offspring phenotype (Groothuis et al., 2005; Wolf and Wade, 2009). Whether or not maternal effects are beneficial to females competing for resources or their offspring has yet to be conclusively shown.

The effects of increased yolk testosterone on offspring phenotype are best documented in terms of growth and behaviors. Artificially increased yolk testosterone increases nestling growth (Lipar and Ketterson, 2000; Muller et al., 2007, 2009; Navara et al., 2005, 2006a; Pilz et al., 2004; Schwabl, 1996; but see Andersson et al., 2004; Rubolini et al., 2006; Schwabl et al., 2012) and metabolic rate (Nilsson et al., 2011; Tobler et al., 2007). Artificially enhanced yolk testosterone also causes more competitive behaviors in nestlings, such as increased begging rate and duration (Eising and Groothuis, 2003; von Engelhardt et al., 2006) and territorial behavior (Muller et al., 2009). Furthermore, nestlings exposed to high levels of yolk testosterone display enhanced social dominance as adults (Eising et al., 2006; Partecke and Schwabl, 2008; Strasser and Schwabl, 2004). Nestlings from testosterone-injected eggs also display bolder behaviors (i.e., fewer distress vocalizations and quickly approach novel objects; Daisley et al., 2005). In general, nestlings exposed to high yolk testosterone have a more "active coping" style, which is characterized by high competitive ability, bold behaviors, and greater sympathetic reactivity as opposed to those with a more "passive coping" style defined by behavioral inhibition (Koolhaas et al., 1999, 2010). Unfortunately, few studies, if any, have examined the influence of yolk hormones on autonomic nerve responses (i.e., sympathetic and parasympathetic reactivity), despite their importance in determining how individuals cope with challenges (Chichinadze and Chichinadze, 2008; Koolhaas et al., 1999, 2010).

Environmentally-induced variation in yolk hormone provisioning likely serves an adaptive purpose. Maternal effects may be an adaptive means by which a female changes the phenotype of her offspring in

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preparation for a given environment (Carere and Balthazart, 2007; Groothuis et al., 2005; Mousseau and Fox, 1998; Schwabl, 1997). Breeding density, for example, is a prominent environmental factor that can increase levels of aggressive competition (Male et al., 2006) and circulating testosterone in females (Smith et al., 2005). A positive relationship between density and yolk testosterone is seen in European starlings (*Sturnus vulgaris*; Pilz and Smith, 2004), tree swallows (*Tachycineta bicolor*; Whittingham and Schwabl, 2001), house sparrows (*Passer domesticus*; Mazuc et al., 2003; Schwabl, 1997), American coots (*Fulica americana*; Reed and Vleck, 2001), and collard flycatchers (*Ficedula albicollis*; Hargitai et al., 2009). The phenotypic changes associated with high yolk testosterone (i.e., increased growth and a more active coping style) would certainly appear to be beneficial in high breeding densities. However, to date, most studies have investigated the influence of maternal hormones on offspring by artificially increasing yolk testosterone, which lacks the ability to show that specific phenotypes arise in response to an environment. Thus, the adaptive value of phenotypes derived from this maternal effect can only be assumed in the absence of an environmental context.

Here, we tested for correlations between breeding density and yolk testosterone as well as between yolk testosterone and offspring growth. Following this, we then experimentally manipulated breeding density and measured yolk testosterone concentrations. Yolk testosterone was expected to positively correlate with density because of increased numbers of competitive interactions (Whittingham and Schwabl, 2001). We then measured subsequent phenotypic differences in growth, competitive ability in the nest, and breathing rate in response to handling stress in the altricial offspring of tree swallows. Breathing rate is commonly used in avian studies to measure responsiveness to handling stress and has been linked to coping style (Carere and van Oers, 2004; Fucikova et al., 2009; van den Brink et al., 2012). Furthermore, by incorporating a cross-foster design, we tested the relative importance of pre-natal breeding density versus post-natal environment in influencing offspring phenotype. Therefore, this study tests whether a naturally-induced maternal effect can act as a proximate mechanism influencing the development of offspring phenotype in anticipation of a high-density environment. If higher yolk testosterone in eggs laid at high-density cause nestling phenotypes to be characterized by faster growth and a more active coping style, then this maternal effect is likely an adaptive mechanism to prepare offspring for competitive environments.

Methods

Study species

We studied a natural population of tree swallows, a socially-monogamous passerine, in Watauga County, North Carolina (36°12′41″N, 81°40′7″W) between April and July in 2009 and 2011. Our study sites are characterized by hayfields and pastures and are no more than 5 km apart. Tree swallows readily accept nest boxes (Robertson et al., 1992) and are particularly appropriate for testosterone-related research because females aggressively compete for their cavities (Rosvall, 2008). This species breeds in a variety of nesting densities and the number of agonistic interactions between females is positively related to nesting density (Male et al., 2006). Furthermore, tree swallows do not vary yolk androgen concentrations within a clutch, but rather between clutches in response to environmental factors, such as breeding density (Whittingham and Schwabl, 2001). All animals were treated in accordance with the Appalachian State University Institutional Animal Care and Use Committee (IACUC).

Correlational study

In 2009, we placed nest boxes randomly throughout our study sites to determine natural breeding densities and the potential for the influence of breeding density on offspring traits. We recorded breeding density by delineating a 300 m radius around each nest box in ArcGIS v. 10 (ESRI, Redlands, CA) and recorded the proportion of active nests during each pair's breeding cycle. Tree swallows typically remain within 100–300 m of the breeding site when foraging, so a 300 m radius should encompass the area within which a female could contact a conspecific (McCarty and Winkler, 1999). Yolk testosterone concentrations were determined for each clutch by collecting the 3rd egg; eggs were collected prior to incubation to ensure that hormones measured were maternally-derived (Elf and Fivizzani, 2002). Eggs were then frozen at −20 °C and yolk testosterone was measured via radioimmunoassay (see detailed methods below). Nestling growth rate was determined by measuring mass (±0.1 g) on days 2, 5, 8, 11, and 14 post-hatch. Growth follows a sigmoidal pattern and normally begins to plateau after day 11. Therefore, growth rates were derived from the slope of a linear regression of nestling mass on days 2–11 post-hatch (Hinde et al., 2009) and averaged for each brood.

Density manipulation

In 2011, we manipulated breeding density creating high- and low-density sites with the intention of altering aggressive interactions among female tree swallows. Breeding densities were measured the same way as the 2009 correlational study (see detailed methods above). In the high-density site, 53 nest boxes were placed so that no box was >12 m from the nearest nest box. All nest boxes (53) fit within each individual box's 300 m radius when measuring breeding density, allowing each box at a site to have the same potential density. In the low-density site, 50 nest boxes were placed so that no box was <36 m apart and, on average, 10 nest boxes fit within the 300 m radius of each pair's nest box.

To confirm that our nest box manipulation affected the number of social interactions tree swallows experienced, we observed randomly selected pairs for vocal and physical interactions for 20 min once the pair had successfully built a nest. To determine total number of interactions, all physical and vocal interactions were combined (Male et al., 2006).

Parental measurements

In 2011, at the high- and low-density sites, we measured female aggression levels to determine if the number of aggressive interactions or yolk testosterone concentrations at the sites were a product of breeding density or inherent aggression of females that choose these sites. During the incubation stage, we presented pairs with a conspecific model and a playback of tree swallow chatter at their nest box for 5 min (i.e., simulated territorial intrusion) and counted how often pairs aggressively flew by, hovered, or attacked the model (Duckworth, 2010). Adults were given unique bands to aid in their identification. Parental feeding rates were measured during the competition trials for nestlings (see methods below) as the number of feeding visits per minute. We also captured each female and measured mass (±0.1 g) and wing chord (±0.1 mm) to create an index of female body condition using residuals of a regression of mass on wing length ($R^2 = 0.11$, $F_{1,51} = 6.28$, $p = 0.016$; Brown, 1996). Female age was estimated as second year (SY) or after-second year (ASY) using plumage coloration (Hussell, 1983).

To determine yolk hormone concentrations, we again collected the 3rd egg from each clutch prior to incubation and froze them at −20 °C, and yolk testosterone and corticosterone were measured via radioimmunoassay (see detailed methods below). Yolk corticosterone was measured because previous studies link yolk corticosterone with breeding density (Love et al., 2008) and because corticosterone can negatively influence testosterone concentrations (Henriksen et al., 2011). The percentage of mass the yolk comprised in each egg was