

Egg-yolk androgen and carotenoid deposition as a function of maternal social environment in barn swallows *Hirundo rustica*

Rebecca J. Safran, Kevin J. McGraw, Kevin M. Pilz and Stephanie M. Correa

R. J. Safran (rebecca.safran@colorado.edu), Dept. of Ecol. and Evol. Biol., Univ. of Colorado, Boulder, CO 80309, USA. – K. J. McGraw, School of Life Sciences, Arizona State Univ., Tempe, AZ 85287-4501, USA. – K. M. Pilz, Global Health Fellow, Public Health Inst., WA 20008, USA. – S. M. Correa, Dept of Medicine, Genetics Program, Boston Univ. School of Medicine, Boston, MA 02118, USA.

Evidence is mounting that female animals use egg-yolk compounds (e.g. steroids, antioxidants) to adaptively engineer the quality of their offspring as a function of several maternal and environmental factors. Though adjustments to yolk allocation have been well-characterized as a function of parental phenotypes, we know very little about how an individual's social environment influences yolk composition. Here, we consider how two types of yolk compounds, androgens and carotenoids, relate to the maternal social environment during the egg-laying period, controlling statistically for known correlations between various aspects of parental quality and egg yolk compounds. Barn swallows *Hirundo rustica erythrogaster* breed in groups of highly variable size and spacing, allowing us to test whether or not the social environment is correlated with these maternal effects. We found no relationship between carotenoid levels in eggs as a function of colony size, colony density, or nearest-neighbor distance. However, eggs from females in larger groups had lower concentrations and total amounts of yolk androgens than those from females in smaller, less dense social settings. Our results counter previous predictions and literature, showing that females breeding in large groups deposit more androgen in eggs, mechanistically, because they compete more with conspecifics and have higher circulating androgen levels themselves and, functionally, because it could be advantageous for their offspring to show high androgen-mediated competitive abilities early in life. Instead, because group size in this species is governed largely by site fidelity and the availability of old nests for re-use, and because reproductive output does not differ as a function of group size, it may be that competition is greater for limited nests in small groups, thus elevating androgen levels. Further, yolk androgens were previously shown to be affected by male quality, and the greater concentrations and amounts of yolk androgens in smaller sites may reflect differential allocation to darker males found at these sites.

An individual's phenotype is greatly affected early in life, by both the genetic contributions made by its parents and by features of the environment in which it is raised, including subtle maternal effects that occur at embryonic stages (Mousseau and Fox 1998). This phenomenon has been well-studied in birds which are an especially suitable model for analyzing how maternal effects shape development because avian egg yolk contains hormones (e.g. androgens: Schwabl 1993, Pilz et al. 2003, reviewed by Groothuis et al. 2005 and Gil 2008) and carotenoids (Royle et al. 1999, Bortolotti et al. 2003) of maternal origin. These molecules can have potent effects on growth, development, and behavior, and numerous factors, including the nutritional status of females and the attractiveness of their mates, can affect levels of investment of these compounds (androgens: Groothuis et al. 2005, Gil et al. 2007, Gil 2008, Kingma et al. 2009; carotenoids: Surai et al. 2001, Saino et al. 2003, McGraw et al. 2005; both: Safran et al. 2008).

The social environment that a female experiences during the egg-laying period is another likely influence on the amount of yolk compounds transferred to eggs. The proposed

adaptive explanation for greater deposition of yolk compounds in eggs laid in highly social environments is associated with the hypothesis that offspring raised in groups may need additional preparation for the stressful and competitive environments into which they are born or hatched (Schwabl 1997). Indeed, many studies have reported positive correlations between yolk androgens and extent of sociality in a wide variety of avian systems (Reed and Vleck 2001, Groothuis and Schwabl 2002, Mazuc et al. 2003, Whittingham and Schwabl 2002, Pilz and Smith 2004, reviewed by Gil et al. 2007). Moreover, a recent comparative study of birds detected a positive relationship between yolk androgens (androstenedione, hereafter A4; but not testosterone) and coloniality, such that offspring hatched into large groups had greater concentrations of A4 (Gil et al. 2007).

Less is known about the relationship between yolk carotenoids and the maternal social environment. In principle, competitive interactions could impact foraging (i.e. carotenoid intake) and numerous physiological processes (e.g. metabolism, health) related to carotenoid processing. In a study of lesser black-backed gulls *Larus fuscus* in which the

level of social interactions was experimentally manipulated, the egg yolks of females subjected to increased competitive interactions during the laying period were shown to have higher carotenoid levels (in eggs containing female offspring only) compared to those laid by females in the control group (Verboven et al. 2005). Another recent study, in which the maternal social environment was experimentally manipulated, demonstrated no effect of adult population density on yolk carotenoid deposition in collared flycatchers (Hargitai et al. 2009).

Here, in a population of barn swallows *Hirundo rustica erythrogaster*, we simultaneously examine the extent to which yolk androgens and carotenoids vary as a function of the social environment in which eggs were produced. Throughout their extensive Holarctic breeding range, barn swallows breed in solitary pairs or with groups of conspecifics; they are not obligately social breeders (Brown and Brown 1999). In North American populations, the majority of individuals typically breed either solitarily or in groups ranging from 9 to 35 pairs (Shields et al. 1988, Brown and Brown 1999, Safran 2004). As such, natural group size variation in barn swallows afforded us the opportunity to examine associations between the social environment and egg-yolk compounds. Based on previous studies of other species, we predicted to find greater levels of each yolk compound in the eggs of offspring raised in larger, dense social groups.

Because maternal and paternal phenotypic traits were previously shown to affect the amount of yolk compounds deposited in barn swallow eggs (Gil et al. 2005, Safran et al. 2008), we include these, where appropriate, as covariates in our analyses. Despite a previous study in which androgens were not shown to vary as a function of group size in barn swallows (Gil et al. 2005), further analyses are required because this study did not account for the potential confounding effect of male attractiveness, examined only one egg per clutch, and only examined group size in terms of the number of breeding pairs, whereas other measures of the social environment (e.g. nesting density) may be revealing. As such, in this study, we use the following three measures to comprehensively assess the social environment: group size, group density and the distance between active nests within a site. Collectively, these measures allow us to determine the effects of both the number of and proximity to conspecifics during egg-laying.

Methods

We studied barn swallows at 10 breeding colonies in Tompkins County, New York, USA that ranged in size from 1 to 35 breeding pairs (mean = 11.9 pairs, SD = 10.4); three of these sites contained only a solitary breeding pair. With permission from the US Fish and Wildlife Service and the New York Dept of Environmental Conservation, we collected eggs as they were laid until clutches were completed (range in clutch size = 2–6 eggs, mean = 4.91 eggs (SD = 0.65) from 23 barn swallow nests. As it was collected, each egg was replaced by a plastic egg that matched barn swallow eggs in appearance and weight. The replacement of real eggs with model eggs ensured that each clutch was not abandoned during the laying period (none were) and that a complete clutch of eggs was laid. In all cases, females continued to lay a

normal clutch size and proceeded to incubate eggs, despite the fact that her nest was full of plastic eggs by the end of the collection phase. Eggs were collected on the morning they were laid and were immediately brought to the laboratory where total egg mass (wet weight) was measured. The yolk of each egg was removed and homogenized, and aliquots of yolk were diluted and frozen at -20°C . Egg collection took place during the early part of the breeding season (in May–June 2002), when natural clutch losses commonly occur due to abandonment in cold weather or predation and are rapidly replaced with a new clutch of eggs.

Both the social mother and father at 21 of these nests were captured and individually marked. At this time, we also measured plumage color (Methods) and tail-streamer length, two traits that are associated with seasonal reproductive success in our study population (Safran and McGraw 2004, Safran et al. 2005) or in European populations of *H. rustica* (Saino et al. 1997).

Measures of maternal social environment

We used three measures to assess the social environment during egg-laying. Group size (log transformed in our analyses) is the number of breeding pairs at a single breeding location (barn or shed). Breeding density per 100 m² is the number of active breeding pairs divided by the square area of the breeding site which is then multiplied by 100. Finally, nearest neighbor distance is the distance from the focal nest to the closest active nest at the same breeding site (m). Collectively, these measures provide information about the number of and proximity to conspecifics during the breeding season.

Plumage-color scoring

Although color throughout the ventral region of barn swallows is inter-correlated, throat coloration in males and belly color in females are most strongly correlated to indicators of seasonal reproductive success (Safran and McGraw 2004), in addition to overall ventral color influencing a male's paternity (Safran et al. 2005). Because of these patterns, we analyzed yolk compound concentrations as a function of male throat and female belly coloration.

To analyze color, we sampled patches (3–10 feathers) of ventral plumage from the throat region of males and the belly region of females and carefully mounted these colored feathers on an index card as to re-create the natural plumage appearance of the bird. These cards were stored in the dark until plumage-color scoring. The color of feather samples was scored along three traditional axes of color (hue, saturation and brightness) using a reflectance spectrophotometer (ColortronTM; Hill 1998). This spectrophotometer does not quantify light in the ultraviolet range, but the range in which it does quantify color is sufficient for this species because the ventral plumage of barn swallows does not exhibit a unique ultraviolet reflectance peak (Safran and McGraw 2004). Each plumage patch was scored three times and we averaged these scores to determine mean hue, saturation and brightness for each region. Color scores were

significantly inter-correlated within (all $p < 0.001$, all r between -0.67 and 0.82), each ventral region (throat for males, belly for females), so a color-scoring scheme to summarize data was devised separately for each of the four plumage areas. Principal components analysis (PCA) was used to collapse hue, saturation and brightness scores within each plumage region. The first principal component (PC1) for each region explained 81–85% of the variation in the color scores of each plumage region in both sexes. Birds with lower PC1 scores have redder (lower hue values), more saturated and darker (lower brightness values) plumage.

Maternity certainty

Although female barn swallows can lay eggs in nests of other females (Brown and Brown 1999), we did not detect the presence of another female's eggs in any of the nests contained in this study and have only rarely encountered this behavior in our study area. Because the shape and color patterns of barn swallow eggs are nearly unique to a female (Brown and Sherman 1989), it is easy to discern foreign eggs in a nest using visual characteristics. Therefore, we feel confident that our assignment of the social mother and genetic mother are equivalent.

Yolk androgen analyses

The whole, fresh yolk was drained into a 1.5 ml Eppendorf tube, mixed with about 300 mg of distilled water, and homogenized by vortexing. The yolk was then frozen at -20°C . Androgens were extracted twice with four ml of petroleum ether/diethyl ether (30:70 volume). Neutral lipids were precipitated with 90% ethanol at -20°C . Extracts were then transferred to diatomaceous earth micro-columns for further purification and separation of androgens following the methods of Pilz et al. (2003) and Schwabl (1993). Radio-immunoassays were conducted for androstenedione (A4), dihydrotestosterone (DHT) and testosterone (T) following the standard methods first described by Wingfield and Farner (1975). All samples were run in a single assay for each hormone. Intra-assay variation was 7.0% for A4, 10.2% for DHT and 8.4% for T. Recoveries averaged 64% for A4, 25% for DHT and 51% for T, similar to other assays using this method (Pilz et al. 2003, Gwinner and Schwabl 2005). The rather low recoveries for DHT are not exceptional, as DHT recoveries are notoriously low across species, possibly by being strongly bound by yolk proteins or lipoproteins (H. Schwabl, unpubl.). All data used in these analyses are corrected for these recoveries.

Yolk carotenoid analyses

We analyzed yolk carotenoids via high-performance liquid chromatography (HPLC; sensu McGraw et al. 2002). We homogenized thawed egg yolks in 1 ml water and extracted lipids from 100 μl of the homogenate with 200 μl of both ethanol (containing canthaxanthin as an internal standard) and *tert*-butyl methyl ether. The solution was vortexed,

centrifuged, and the supernatant evaporated before re-dissolving the residue in 200 μl HPLC mobile phase (methanol–acetonitrile–chloroform, 46:46:8, v/v/v). We injected 50 μl into a WatersTM 717plus Autosampler HPLC fitted with a Develosil RPAqueous RP-30 HPLC column (250 \times 4.6 mm I.D.), and ran an isocratic system (HP 1050 Series Isocratic Pump) of the aforementioned mobile phase for 25 min at a constant flow rate of 1.2 ml min⁻¹. We confirmed the identity of yolk pigments by comparing retention times to those for authentic reference carotenoids, including anhydrolutein, beta-carotene, and several ketocarotenoids (e.g. astaxanthin, canthaxanthin) in addition to the three we detected in swallow yolk: lutein, zeaxanthin, and β -cryptoxanthin.

Statistical analysis

As noted in our previous study of yolk compounds in barn swallows (Safran et al. 2008), the results of statistical comparisons are not always similar when they are based on total yolk compound content (ng of androgen per whole yolk, or mg carotenoid per whole yolk) and concentration (pg androgen per mg yolk, or mg carotenoid per g yolk). Therefore, we report data for both concentrations (the more commonly used measure in the literature) and total content throughout this paper. Note that the units listed above for androgens and carotenoids are not the same, but follow the units commonly reported for these compounds in published studies (Pilz et al. 2003, Gil et al. 2005, Saino et al. 2002, 2003, McGraw et al. 2005).

Because none of the yolk compound variables (neither concentrations nor total amounts) were normally distributed, we applied transformations [\log or $\log(\text{compound} + 1)$] to meet assumptions of parametric statistics. We used smoothing splines to graphically characterize the relationships between continuous variables; these were only used to characterize the relationship between variables of interest (e.g. whether they are linear or not) so that appropriate models could be constructed (e.g. whether higher-order terms would be necessary in linear models). Using JMP 6.0 (SAS Institute), we constructed very conservative splines using a flexible tuning parameter ($\lambda = 1$) to construct pictorial relationships between variables of interest.

We used principal components analysis (PCA) to reduce the variables representing yolk androgens. In the two separate PCA analyses (below), the first principal component explained more than 70% of the variation among the three variables entered into each model and were therefore used this first principal component in subsequent analyses. As shown below, the eigen value associated with the first principal component (and not the remaining components) is greater than one and as such, we retained a single component from each analysis (Dearborn and Ryan 2002). We used PCA to reduce three androgen concentration measures into one component [eigen value = 2.13, total variance explained = 71.18, with the following component loadings for A4 (0.54), DHT (0.55) and T (0.63)]. We used PCA to reduce three androgen amount measures into one component [eigen value = 2.24, total variance explained = 74.85, with the following component loadings for A4 (0.54), DHT (0.56) and T (0.62)] in egg yolk. In

both PC models, our PC scores can be interpreted as representing positive amounts and concentrations of each compound, respectively, as all androgens are positively intercorrelated (Safran et al. 2008). We separated our measures of concentration and total amounts of yolk androgens because we a priori do not have predictions for whether either measure is more biologically meaningful. For both models, all of the component loadings are positive and as such, higher PC1's indicate higher concentrations/amounts of yolk androgens and thereby, results for each androgen separately follow the same patterns as the results we report for the variables derived from PCA.

Interestingly, the carotenoid variables we measured were not highly inter-correlated (Safran et al. 2008) and instead of reporting the results of each one separately, we report a composite score (total mg carotenoid content) to indicate the total amounts (mg) and concentrations (mg per g yolk) of the three carotenoids we measured in this study. We did this because we had no a priori expectation that the three types of carotenoids in barn swallow eggs would differentially relate to our measures of maternal social environment and parental quality and because our results were similar for the separate and composite measures of carotenoids.

Using PROC MIXED (SAS ver. 9.1), we applied generalized mixed linear models to analyze relationships between yolk compounds and features of the maternal social environment. Unless noted, the lack of independence of eggs within the same clutch and a given female, in addition to laying order effects, was controlled for by using 'nest' and 'egg' (note: 'egg' controls for the order in which each egg was produced) as the random effects in the model. Note also that because nests were identified with both the site name and nest number, the random effect 'nest' functionally controls for repeated measures of eggs within the same nest site.

Additional covariates

In a previous analysis (Safran et al. 2008), we found effects of plumage color (both sexes), clutch initiation date, and egg mass on either or both androgens and carotenoids in egg yolks. Although clutch initiation date was shown to affect the concentration of carotenoids detected in eggs in our previous study, this variable is highly correlated with female coloration (Safran and McGraw 2004) and was therefore excluded from our analyses. Interestingly, egg mass is highly correlated with the measures of social environment (larger eggs in more dense social groups) and as such, to avoid issues related to multi-collinearity, we do not include this variable in our models presented here. As egg weight is associated with laying order (Safran et al. 2008), using 'egg' as a random variable in all of our models, as described above, controls for potentially confounding effects of egg mass on our results. Because previous analyses found that both male and female color affected the concentrations (but not amounts) of both carotenoids (lower carotenoid concentrations deposited by darker females) and androgens (greater androgen concentration when paired to darker males) respectively (Safran

et al. 2008), we control for these variables as appropriate in this study.

Results

Interrelationships among social-environment variables

Group size and nest density were linearly related to one another (linear regression, $F_{1,8} = 4.345$, $p < 0.01$, adjusted $R^2 = 0.690$), though neither of these variables was related to nearest-neighbor distance (nearest-neighbor distance and group size: Spearman's $\rho = -0.505$, $p > 0.248$; nearest-neighbor distance and nest density: Spearman's $\rho = -0.357$, $p > 0.432$), suggesting that there is no difference in the spacing between nests as a function of group size or density. Moreover, when compared across sites of similar and varying sizes, we found no difference in the average distance between active nests at all of our 7 group study sites (ANOVA; $F_{6,17} = 2.088$, $p > 0.137$).

Are yolk androgens and carotenoids correlated?

When considered singly, none of the concentrations of androgens and carotenoids were significantly correlated with any of the carotenoid concentrations and none of the total amounts of androgens were significantly correlated with total amounts of carotenoids (all F between 0.02 and 1.52, all $p > 0.22$; 'nest' is a significant random effect in all models). Moreover, results are similar using our composite measures of androgens and carotenoids. Controlling for nest and lay order effects by using ('nest' and 'egg' as random effects) concentrations of yolk androgen (PC1) and total carotenoids are not correlated ($F_{1,97.73} = 0.04$, $p > 0.84$), and neither are total amounts of yolk androgens (PC1) and carotenoids ($F_{1,87.66} = 0.55$, $p > 0.45$); 'nest' is a significant random effect in both models.

Thus, as in a previous study, we found no statistical association between yolk carotenoids and androgens (Safran et al. 2008), and therefore, treat these as independent variables in this study.

Do yolk androgens vary with features of the maternal social environment?

Our results differed slightly based on whether the response variable was total amount or total concentration of androgen found in barn swallow egg yolk, with group size affecting both variables in similar ways and group density demonstrating nearly the same result (Table 1, Fig. 1): we found greater concentrations and amounts of androgens in smaller and less dense breeding groups. As was previously revealed (Safran et al. 2008), male color had an effect on the concentrations of yolk androgens, with greater amounts found in association with darker males. In this data set, we found no statistically significant relationships between male color and measures of the social environment, although in a previous analysis of a larger sample of birds, darker males were found more often in solitary and small breeding groups (Safran 2007). We found no effect of nearest-neighbor

Table 1. Results from a mixed linear model (with nest and egg as random effects) testing the effects of various social environment variables on androgen amounts and concentrations deposited in barn swallow egg yolks. NND = nearest distance to active nest within the breeding site. Results for total androgen mounts are based on a sample of 23 nests and a total of 111 eggs; results for androgen concentrations are based on a sample of 21 nests and a total of 105 eggs. Note that we used PC scores for androgens in which positive scores are indicative of greater amounts or concentrations of these compounds. We also used PC scores for male plumage color, in which case darker males are associated with lower/negative PC scores.

Androgen response variable	Variable(s) in model	Parameter estimate	F	p	Variation explained in model (%)	Significant random effects
Total amounts	Group size	-0.70	7.29	<0.05	74.3	nest
	Group density	-0.09	3.44	<0.08		nest
	NND	0.07	0.08	0.78		nest
Concentrations	Male color	-0.05	4.65	<0.05	63.9	nest
	Group size	-0.48	5.14	<0.01		nest
	Male color	-0.07	8.90	<0.01	64.1	nest
	Group density	-0.08	9.05	<0.01		nest
	Male color	-0.05	4.97	<0.05	14.7	nest
	NND	0.02	0.03	0.80		nest

distance on either yolk androgen amount or concentration (Table 1).

Note that although we previously reported slight differences in male color across group sizes (darker males tending to be found in solitary and smaller group sites; Safran 2007), such an effect was not discernible in this smaller sub-set of our study population for either males or females (male color as a function of group size, regression: $F_{1,17} = 0.01$, $p > 0.92$) and female (female color as a function of group size, regression: $F_{1,20} = 0.34$, $p > 0.27$) color.

Do yolk carotenoids vary with features of the maternal social environment?

None of the features of the maternal social environment we analyzed explained variation in either total carotenoid amounts or concentrations found in barn swallow egg yolks (Table 2, Fig. 2). As was previously documented (Safran et al. 2005), female color affected the concentrations of yolk carotenoids; females that were darker in color deposited lower carotenoid concentrations in their eggs (Table 2).

Discussion

Previous studies of avian maternal effects have separately explored the effects of parental quality (e.g. indicators of maternal fecundity, features of mate attractiveness) or the maternal social environment on yolk compounds, including carotenoids and androgens. Here, we consider several measures of the adult and offspring social environment, while controlling for known parental features previously shown to affect concentrations and amounts of yolk androgens and carotenoids found in barn swallow eggs (Safran et al. 2008). In our study area, sites with larger groups have a higher nest density, yet the distance between active nests across all of our sites of varying size and density were roughly equal. That neither group size nor density was correlated with the average distance between active nests makes sense in light of previous studies, showing that barn swallows do not actively establish nest sites near one another (Brown and Brown 1996); our results suggest that, while sites with a greater number of breeding pairs have more active nests per square area, these nests are not spatially clumped. The lack of a difference in the spacing patterns between active nests among sites is apparent in our results; this variable is not at all associated with differences in the amounts of yolk compound found across sites, as are the other two variables used to represent the maternal social environment.

Because our previous analysis of yolk carotenoids and androgens in barn swallow eggs showed that these yolk compounds do not co-vary (Safran et al. 2008), it is not surprising that the relationships between the social environment and patterns of yolk androgens and carotenoids found

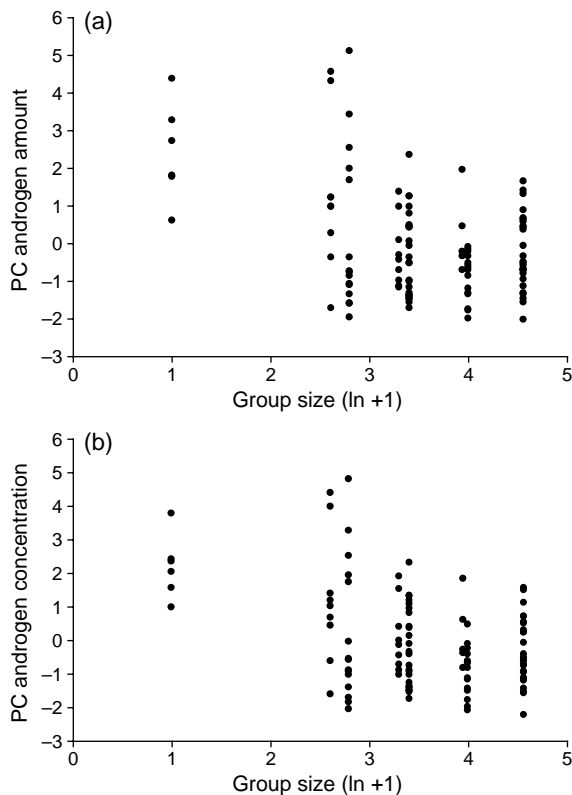


Figure 1. Relationship between yolk androgen (a) amounts and (b) concentrations as a function of group size across 10 breeding sites ranging from 1 to 35 breeding pairs of barn swallows.

Table 2. Results from a mixed linear model (with nest and egg as random effects) testing the effects of various social environment variables on carotenoid amounts and concentrations deposited in barn swallow egg yolks. NND = nearest distance to active nest within the breeding site. Results for total amounts are based on a sample of 23 nests and a total of 111 eggs; results for concentrations are based on a sample of 21 nests and a total of 105 eggs. Note that we used PC scores for androgens in which positive scores are indicative of greater amounts or concentrations of these compounds. We also used PC scores for female color in which case darker females are associated with lower/negative PC scores.

Carotenoid response variable	Variable(s) in model	Parameter estimate	F	p	Variation explained in model (%)	Significant random effects
Total amounts	Group size	-0.001	0.44	0.50	N/A	nest, egg
	Group density	-0.002	0.65	0.40	N/A	nest, egg
	NND	-0.008	0.46	0.50	N/A	nest, egg
Concentrations	Female color	0.01	5.47	<0.05	7.3	nest, egg
	Group size	0.004	0.16	0.65		
	Female color	0.01	5.86	<0.02	8.9	nest, egg
	Group density	-0.001	0.75	0.35		
	Female color	0.02	6.50	<0.02	8.4	nest, egg
	NND	-0.001		0.80		

in barn swallow eggs were quite different. Whereas both compounds have previously been shown to vary as a function of maternal and paternal phenotype in barn swallows (these effects are controlled for in this current study; Safran et al. 2008), here we found that yolk androgens, but not carotenoids, co-varied significantly with features of the social environment during breeding.

Yolk androgens

Our results were slightly different for the two different measures of yolk androgens: whereas group size (the number of breeding pairs at a site) was a significant negative predictor of both concentrations and total amounts of androgens found in barn swallow egg-yolks, nest density

was important only in our analyses of yolk androgen concentrations. It is important to note that, in the analyses where male color was included because it was previously shown to affect concentrations of maternally-derived yolk androgens, we found no statistically significant of male color and features of the social environment (group size, nest density, distance to nearest active nest) although previous analyses in a larger sample size from the same study area demonstrated that darker birds are more often found at solitary and small breeding sites (Safran 2007). Aside from demonstrating that yolk androgens vary as a function of the social environment, what is noteworthy is the amount of explained variation (ranging from ~64–74%) in the models containing total amounts/concentrations of yolk androgens and group size/group density (Table 1), which suggests that group size in particular is important for predicting total amounts of yolk androgens. Of course, these correlational results do not indicate a causal relationship between group size and total amounts of androgens in barn swallow egg yolk; the strength of the relationship is fascinating however and merits further exploration.

Interestingly, our results are distinct from many previous studies in which androgen levels in egg yolks were analyzed as a function of the maternal social environment. Rather than detecting higher levels of yolk androgens in larger groups (recently summarized in Gil et al. 2007, Gil 2008), we found the opposite: lower levels of yolk androgens (both in terms of total amounts and concentrations) were found in eggs produced in large and dense social environments. A previous investigation of yolk androgens in a different population of barn swallows yielded different findings: Gil et al. (2005) analyzed the yolk androgens of one egg per clutch across a very similar social spectrum (a total of 12 sites ranging in size from 2–14 pairs) and this research group found no differences in yolk androgens (A4 only) across different social environments. Aside from conducting their study on a different sub-species of barn swallow *H. rustica rustica*, another difference between Gil et al.'s (2005) study and ours is that, although the effects of male phenotype were shown to affect the concentration of this yolk compound in eggs, potential confounding covariates (e.g. male streamer length) were not included in their models.

Previous studies of barn swallows have revealed few benefits associated with group breeding in this species

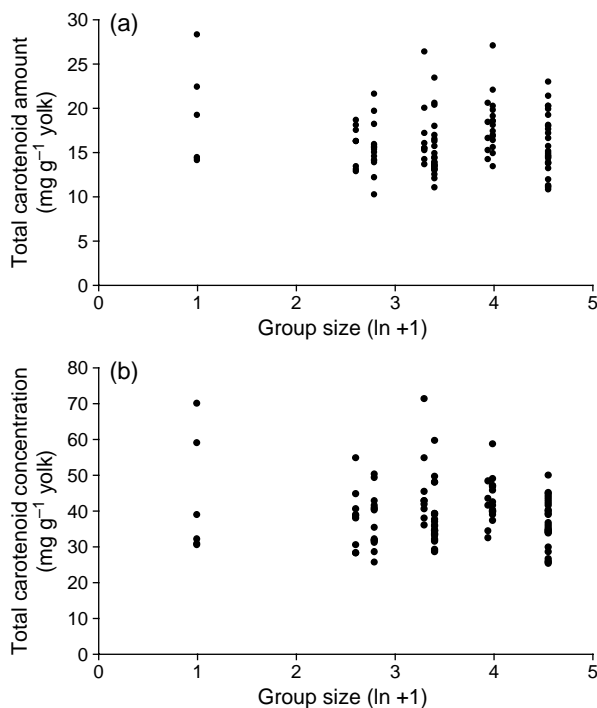


Figure 2. Relationship between yolk carotenoid (a) amounts and (b) concentrations as a function of group size across 10 breeding sites ranging from 1 to 35 breeding pairs of barn swallows.

(Snapp 1976, Møller 1987, Shields and Crook 1987, Shields et al. 1988). Further, research on barn swallow sociality has shown either no relationship between average reproductive success and group size (Snapp 1976, Safran 2004) or a negative relationship between average reproductive success and group size (Shields and Crook 1987), suggesting that individuals do not breed in groups to derive direct benefits from their neighbors (Safran 2004, 2007, Safran et al. 2007). Our data on the lack of a clumped spatial pattern between nests, a pattern seen in highly cooperative breeding groups like cliff swallows (Brown and Brown 1996), is further evidence that barn swallow groups are not formed because of the benefits of company, *per se* (Snapp 1976, Shields et al. 1988, Safran 2007, Safran et al. 2007). Moreover, previous studies of the settlement patterns of barn swallows demonstrate that colonies are comprised of individuals settling as far apart as possible from other active nests (Brown and Brown 1996, Fujita and Higuchi, 2007, Stanback unpubl.). Accordingly, the positive correspondences between group size and yolk androgens often found in highly colonial birds may not be predicted for the relatively neutral social groups of the barn swallow, because the adaptive benefits of increased androgens in a highly competitive environment may not apply to these loose groups. While the number of social interactions in larger barn swallow groups should be greater than the number of social interactions that occur at smaller sites, the stress of these interactions may be minimized by the fact that barn swallows do not defend feeding territories, for example, and rely on ephemeral aerial insect populations that are not a defensible resource. Still, these patterns in the social lives of barn swallows do not explain the higher yolk androgens in eggs produced in solitary and small breeding sites.

One possible explanation for greater amounts and concentrations of androgens in the eggs of parents breeding solitarily could be a by-product of slight differences in male – but not female – quality across the social spectrum. Whereas this pattern was not found in this current data set of 23 breeding pairs, in a larger, long-term data set from the same study area, males with dark feather coloration – one indicator of parental quality that affects yolk androgen levels (Safran et al. 2008) that was statistically controlled for in this study – were found to settle with a greater probability at smaller sites compared to larger group settings (Safran 2007). Because females were previously shown to deposit greater concentrations of yolk androgens in their eggs when paired to dark-colored males (Safran et al. 2008), the relationship between androgens and the maternal social environment revealed by this current study may be, in small part, a function of patterns of deposition according to mate quality between solitary and group sites. Still, that our models indicate both measures of the maternal social environment and potentially confounding effects of male quality explain variation in the androgens deposited in barn swallow egg yolks suggests that features of social mates and the social environment in which these eggs are produced are important.

What proximate mechanisms might explain the strong relationship between various aspects of the maternal social environment and yolk androgens? One possibility is that the amounts and concentrations of yolk androgens reflect

ovarian hormone levels during the egg-laying period and that maternal androgens vary across different group sizes. Though it is logical to assume that the amounts of yolk compounds are a function of the circulating levels of these compounds in females during the egg-laying period, there are very little data to support this hypothesis. For example, in canaries, Schwabl (1996) found that circulating androgens in egg-laying females corresponded positively to the amount of androgens found in her eggs and others have reported similar patterns (Adkins-Regan et al. 1995, Williams 2005). However, a correspondence between maternal plasma androgens and those found in the eggs she produces has not always been detected (Mazuk et al. 2003, Navara et al. 2006) and such a relationship obviously requires further testing in barn swallows. There is no *a priori* logical reason why the circulating levels of androgens in females should be higher at solitary sites, unless, for example, competition among other females for access to nest sites and their territory-holders in small groups is somehow greater than the competition for sites and mates in larger, group settings, which may be a possibility (Safran 2007). Although the reproductive consequences for pairs breeding solitarily are not greater than for those breeding in large, dense aggregations, once settled, solitary pairs are likely to overall encounter a lot less aggression and competition among neighbors than are group-breeding conspecifics.

While not a causal factor associated with lower yolk androgens in larger breeding groups, one fascinating pattern that is perhaps a consequence of differences in yolk androgens across different social environments is an association we detected between nestling mass and group size. Although not recorded from the same individuals that were a part of this study (because eggs in this study were collected for yolk compound analyses), the mass of nestlings at many of the same breeding sites (but not always from the same breeding pairs) used in this study were found to be highly associated with group size, such that nestling mass was lower at larger sites where average androgen concentrations were lower relative to smaller sites (Safran unpubl.). This pattern is concordant with the idea that higher-quality pairs, and thus, greater amounts of parental investment, occur at solitary and small sites. Note, however, that reproductive performance does not vary across the social spectrum in barn swallows (Safran 2004, 2007) and that while these differences in parental care may differ across group sizes, they do not appear to effect patterns of reproductive success across different sites on the whole.

Yolk carotenoids

Because we did not find a correspondence between yolk androgens and carotenoids in prior analyses (Safran et al. 2008), we did not expect to find similar patterns in their association with the maternal social environment. However, theoretical predictions suggest that it would be adaptive for females to allocate greater amounts of carotenoids to their offspring born into potentially stressful situations, like large, complex breeding groups. To the best of our knowledge, this is the first study to directly analyze yolk carotenoids deposited in eggs produced in across a range of social

environments. An interesting study conducted by Verboven et al. (2005) explored the relationship between yolk carotenoids and the maternal social environment in gulls by manipulating the number of intraspecific interactions a female experienced during the egg-laying phase. Verboven et al. (2005) found that females experimentally encountering greater intraspecific interactions during egg-laying produced female eggs with higher carotenoid levels compared to females in control groups. However, this study is fairly different from ours due to a number of factors, not the least differences in the natural social environment in which these two species lay their eggs (individuals breeding in gull colonies are notoriously competitive). Similar to our results, although obtained in a species of bird where breeding density is a function of nest box locations, a recent experimental study by Hargitai et al. (2009) on collared flycatchers found no differences in the amounts of yolk carotenoids found in eggs produced by females exposed to increased social challenges during the egg-laying period and control pairs. As there are no additional studies, that we are aware of, that have looked directly at similar features of a female's social environment during the egg-laying phase, we have no comparisons for the lack of a statistical association between yolk carotenoids and various aspects of the maternal social environment, including group size, density or distance to closest active nest. Additional correlational studies from a diversity of social systems would be informative for making finer-scale predictions for future experimental work on this topic.

Summary

We analyzed associations between measures of sociality and yolk androgens and carotenoids in breeding barn swallows. Controlling for aspects of parental phenotypes that contribute to variation in yolk compounds, we found no association between three measures of the maternal social environment and yolk carotenoids, which are often associated with offspring quality. Counter to the prevailing pattern of greater androgen amounts and concentrations found in the yolks of offspring from dense breeding aggregations, we found that young raised in larger groups had lower concentrations and total amounts of yolk androgens than those from females in smaller, less dense social settings. That we detected a trend for the eggs of parents breeding solitarily to contain greater amounts and concentrations of yolk androgens, might reflect previously recorded relationships between greater allocation of yolk androgens from females paired to darker males (Safran et al. 2008) and a tendency for darker males to be found breeding at solitary and small sites (Safran 2007), suggesting competition for these breeding locations.

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