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# Evolution of sex-biased maternal effects in birds: III. Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins

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## Abstract

Overlap in growth of offspring should constrain the opportunity for sex-biased maternal effects, yet sex-specific allocation of maternal resources among simultaneously growing ova is often observed in vertebrates. In birds, such allocation can be accomplished either by temporal clustering of ova that become the same sex, resulting in sex-biased egg-laying order, or by follicle-specific delivery of maternal resources. Two house finch populations at the northern and southern boundaries of the species range have opposite ovulation sequences of male and female eggs, and thus, in the absence of sex differences in ova growth or sex-specific maternal strategies, would be expected to have opposite sex-specific accumulation of maternal products. We found that the populations had strong and similar gradients of steroid distribution in relation to ovulation order, whereas distribution of carotenoids and vitamins correlated with each follicle's accumulation of steroids. In both populations, temporal bias in production of sons and daughters within a clutch enabled strongly sex-specific acquisition of maternal products, and oocytes of the same sex were highly interdependent in their accumulation of steroids. Moreover, in nests where the sex-bias in relation to ovulation order deviated from population-specific patterns, eggs had highly distinct concentrations of steroids, carotenoids and vitamins. These results and previous findings of sex-specific yolk partitioning among oocytes suggest that oocytes that become males and females are temporally or spatially clustered during their ovarian growth. We discuss the implication of these findings for the evolution of sex-specific maternal resource allocation.

## Introduction

The ability of organisms to adapt to novel conditions is determined by both their capacity to generate variation and by the retention of adaptive directionality of this variation between generations. Because maternal strategies often accomplish both of these goals, in many species maternal effects are the most important developmental resources for offspring growth. In birds, breeding females allocate an array of maternal products into growing eggs, and such allocation has extensive, and frequently adaptive, consequences for offspring fitness (Price, 1998; Schwabl, 1999; Groothuis *et al.*, 2005).

Despite many studies of maternal effects and a wealth of empirical information, crucial questions remain unresolved. For example, most bird species have pronounced gradients of steroid allocation into eggs across the laying sequence (e.g. Schwabl *et al.*, 1997; Groothuis & Schwabl, 2002). Such gradients are often closely qualitatively concordant with the mother's own hormonal state, suggesting that maternal allocation of hormones into developing eggs is passive (Schwabl, 1996; Müller *et al.*, 2002; Badyaev *et al.*, 2005; Williams *et al.*, 2005). At the same time, adaptive effects of steroid allocation, such as increased competitive ability or growth of offspring, are often limited to only one sex or only some breeding contexts (Burke, 1992; Henry &

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Burke, 1999; Lipar & Ketterson, 2000; Eising *et al.*, 2001; von Engelhardt *et al.*, 2006), indicating that adaptive maternal allocation can be complex and needs to be precise. Similarly, it is unclear whether maternal allocation of lipids, carotenoids, and vitamins into eggs is a passive consequence of maternal diet and lipid catabolism accompanying egg production (Surai *et al.*, 1998b; Blount *et al.*, 2002; Horak *et al.*, 2002) or an active, sometimes sex-biased or breeding context-specific strategy (Verboven *et al.*, 2005) adaptively complementing the allocation of steroids (Royle *et al.*, 2001; Groothuis & Schwabl, 2002; Saino *et al.*, 2002). Finally, the simultaneous development of multiple ovarian eggs should strongly constrain sex-biased allocation of maternal products, yet sex-specific allocation of maternal resources is increasingly frequently observed (Cordero *et al.*, 2001; Petrie *et al.*, 2001; Magrath *et al.*, 2003; Rutstein *et al.*, 2005).

Comparison of the acquisition of maternal products by egg follicles in relation to the temporal sequence of their growth and overlap with other follicles can provide insight into the evolution of sex-biased maternal allocation (Fig. 1). If delivery of maternal resources is follicle-specific, then the accumulation of maternal products by oocytes is expected to reflect fluctuations in maternal plasma (Fig. 1). Under this scenario, sex-biased or breeding context-specific allocation can arise readily and is independent of overlap among simultaneously growing follicles (Salvante & Williams, 2002, Fig. 1). Alternatively, the acquisition of maternal products can be a consequence of follicle competition, trade-offs and leakage, due to temporal overlap in the growth of follicles. Under this scenario, the opportunity for sex-biased allocation depends on the congruence of the sexes among overlapping oocytes (Young & Badyaev, 2004; Badyaev *et al.*, 2006), and on the extent of the overlap and associated interactions among oocytes (Etches & Duke, 1984; Waddington & Walker, 1993; Pike, 2005). Such sex-specific 'clustering' of oocytes should be reflected in sex-specific trade-offs in their acquisition of maternal products (Fig. 1).

If temporal sequence of egg production affects oocyte exposure to maternal steroids and antioxidants and their accumulation in yolks, then adjusting the positions of males and females across the laying sequence becomes an important strategy enabling sex-specific maternal allocation despite overlap in oocyte development (Bowden *et al.*, 2000; Lovern & Wade, 2001, 2003; Badyaev *et al.*, 2006). Two recently established populations of the house finch (*Carpodacus mexicanus*) at the northwestern (Montana) and southeastern (Alabama) boundaries of the species range have the opposite sequence of within-clutch production of male and female eggs (Badyaev *et al.*, 2002). Thus, a passive reflection of maternal gradients of hormones and carotenoids would produce the opposite allocation of these substances into developing oocytes between the populations, whereas deviations from this pattern of allocation would indicate either sex-specific maternal provisioning or sex differences in development of oocytes.

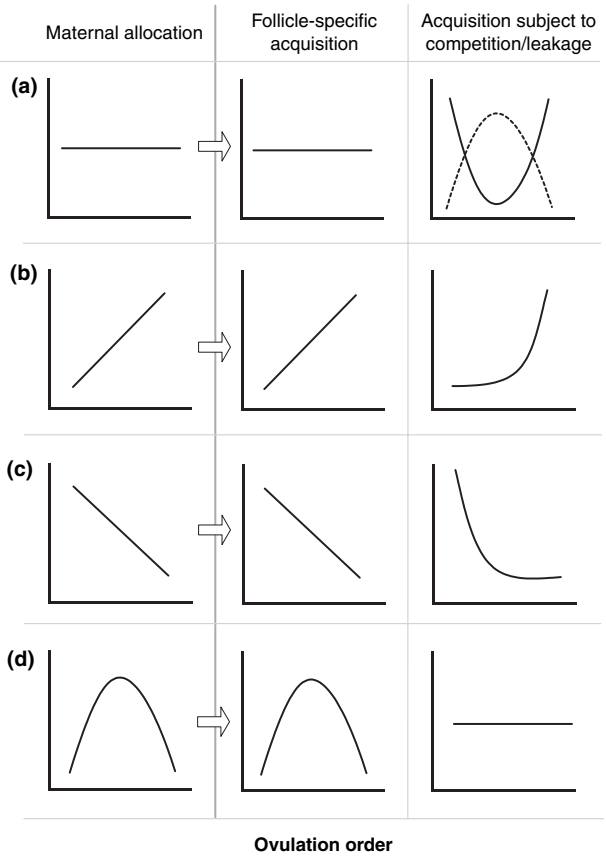


**Fig. 1** Conceptual illustration of the relationship between maternal resources allocated to follicles (left column) and each follicle's acquisition of these resources in relation to an assumed overlap with other growing follicles. Overlap among follicles is assumed to be the greatest in the middle of ovulation sequence and smaller at the onset and the commencement of ovulation sequence. The central column shows patterns expected from follicle-specific delivery of maternal resources or when the overlap among growing follicles does not affect follicle acquisition. Right column shows the following: (a) constant allocation throughout oogenesis results in the lowest acquisition of resources or the greatest leakage (dashed line) among eggs produced in the middle of the ovulation sequence, when the overlap among follicles is the greatest. (b) An increase in maternal allocation with egg production sequence produces increasingly greater acquisition because of the progressively smaller number of simultaneously growing follicles. (c) A decrease in maternal allocation with egg production leads to greater acquisition at the beginning of the ovulation sequence. (d) When maternal allocation matches follicle demand and is the highest when follicle overlap is the greatest, no relationship between ovulation order and follicle acquisition is expected. Distinct temporal patterns of resource acquisition in relation to ovulation order between male and female eggs would indicate sex-specific temporal clustering during egg production.

In this study, we first document a strong gradient of steroid distribution across eggs within a clutch that is

similar in both populations, and a weak but distinct gradient of carotenoid and vitamin acquisition. Second, we test a novel hypothesis that if sex-specific allocation of maternal products is enabled by temporal clustering of male and female oocytes, then such clustering should be reflected in sex-specific trade-offs of maternal products among oocytes. Third, we investigate whether temporal bias in production of sons and daughters enables different acquisition of steroids, hormones and vitamins between male and female oocytes.

## Materials and methods

### Field methods

We studied house finches at two populations: at the northwestern edge of their range in northwestern Montana, where house finches started breeding in late 1970s, and at the southeastern edge of their introduced range in Alabama, where house finches started breeding in 1983 (Badyaev & Hill, 2000). The study sites in Alabama and Montana have been maintained since 1993 and 1994, respectively, and the data for this study were collected in 2002–2004. At both sites all resident adults were trapped and marked with a unique combination of one aluminium and three coloured plastic rings, and pairing associations and nest-initiation behaviour was closely followed for all breeding birds (Hill *et al.*, 1999; Badyaev & Martin, 2000b). At the time of nest-building, thermocouples (iButton-TMEX, Dallas Semiconductor) were installed at each nest to monitor the onset of incubation. All females laid one egg per day between 0630 and 1100 and eggs were numbered sequentially on the day of laying. Females never skipped days during egg-laying or laid eggs in other females' nests (Oh & Badyaev, 2006). In nests where incubation started with the last eggs, all eggs were removed at the end of the first day of full incubation. In nests where incubation began with the first egg, the first egg was collected within 24–36 h of laying and replaced with a freshly laid house finch egg from a different nest or with a dummy egg. All subsequent eggs in these nests were similarly removed within 24 h of laying and replaced with other eggs. After the female laid her last egg, the dummy eggs were either removed to enable rapid renesting or foster eggs were allowed to develop. Immediately after collection, eggs were photographed, measured and stored at −20 °C until further analysis. We obtained data on yolk steroids, carotenoids, vitamins, egg-laying order and embryo sex for 120 eggs from 29 clutches in the Montana population and 98 eggs from 24 clutches in the Alabama population.

### Hormonal, carotenoid and vitamin assays

Yolk testosterone (T), 5α-dihydrotestosterone (DHT), and 17$\beta$ estradiol (estradiol) were quantified using separation protocols (Schwabl, 1993) and competitive binding RIA described in Wingfield & Farner (1975) and Mendonça *et al.* (1996). Weighed amounts of longitudinally sliced yolks were vortexed and homogenized and 20–40 mg of the homogenate diluted in 1 mL water was used. A 1000 counts $min^{-1}$ of tritiated T and 5α-DHT (New England Nuclear, MA, USA) were added to the yolk samples for calculation of recoveries. Samples were equilibrated for 30 min and extracted twice with 3 mL diethyl ether and then dried under nitrogen. Samples were reconstituted in 10% ethyl acetate in 2,2,4-trimethylpentane and then transferred to chromatography columns containing diatomaceous earth for hormone fraction elution. 5α-DHT was eluted with a concentration of 10% ethyl acetate and *T* was eluted with a concentration of 20% ethyl acetate. *T* was measured by RIA, using a specific antibody (Endocrine Sciences, CA, USA). A 5α-DHT was assayed using a commercial 125I-labeled radioimmunoassay kit from Diagnostics Systems Laboratories (Webster, TX, USA). Average recovery efficiencies were 59% for 5α-DHT and 87% for *T*. Estradiol was eluted with a concentration of 40% ethyl acetate and was quantified by RIA using a specific antibody (Esoterix, TX, USA). Average recovery efficiency for estradiol was 91%, interassay variation was 2.46%, intra-assay variation was 6.5%, and the lower detection limit for the assay was 9.8 pg $mL^{-1}$.

Yolk carotenoids and vitamins were measured using high-performance liquid chromatography (HPLC). Weighed amounts of yolk (0.05–0.10 g) were vortexed and homogenized in 75 : 25 methanol containing pyrogallol (2% w/v)-water in a volume equalling ten times the yolk sample mass. Samples were incubated in 10% potassium hydroxide at 70 °C for 1 h, vortexing every 15 min. Triple extraction with hexane (2 mL) was followed by washing of the organic phase with water (0.5 mL). Samples were centrifuged at $1700 \times g$ for 5 min between extractions, and the combined organic phase was evaporated to dryness under vaccum at 40 °C and reconstituted in 300 $\mu$L ethyl ether and 900 $\mu$L HPLC mobile phase (methanol-acetonitrile-tetrahydrofuran, 50 : 45 : 5 v/v/v).

Carotenoids and vitamins were quantified by injecting 50 $\mu$L of yolk extract into a HPLC System (Shimadzu Corporation, Pleasanton, CA, USA) fitted with a NovaPak C18 column, 150 × 3.9 mm (Waters Corporation, Milford, MA, USA). The initial diversity and prevalence of carotenoid and vitamin compounds were assessed by Craft Technologies (Inc., NC, USA). Subsequent analyses focused on the identified compounds. Analytes were eluted isocratically at a constant flow rate of 1 mL $min^{-1}$ for 22 min using the aforementioned mobile phase. Carotenoids, retinoids and tocopherols were detected using a Shimadzu SPD-M10AVP photodiode array detector, and peak areas were integrated at 450, 325 and 294 nm, respectively. Peaks were identified and quantified ($\mu g\ g^{-1}$) using retention times and calibration curves of standards (Sigma, St. Louis, MO, USA; Supelco, Bellenote, PA,

USA; Indofine Chemical, Hillsborough, NJ, USA; CaroteNature, Lupsingen, Switzerland).

### Sex determination

Sex of nestlings and embryos was determined molecularly by amplification of an intron of the CHD1 genes on the sex chromosomes (Griffiths *et al.*, 1998). Early embryo cells of eggs that were incubated for 24–36 h were separated from the surrounding tissues of the whole yolk under 12 times magnification. DNA extraction, PCR protocol, and verification of molecular sexing of these samples are described in Young & Badyaev (2004).

### Statistical analyses

We used general linear models with nest identity as a random effect to analyse the between- and within-population effects of sex, ovulation order and the interaction between these factors on variation in maternal products. Within-sample levels of *T*, DHT and androstenedione, as well as carotenoid and tocopherol compounds strongly and linearly correlated with each other within each group (footnote of Table 2). Thus, we constructed principal components (PC) from a correlation matrix for each group of traits: testosterone, carotenoids, and vitamin E (see footnote of Table 2 for PC coefficients), whereas vitamin A and estradiol were tested individually. To examine variation in egg-laying gradients for each group of maternal products, we first constructed separate PC for testosterone, carotenoids and vitamin E for each egg-laying position. Then we calculated PC1 coefficients from a covariance matrix of PC1 scores for each group of traits across egg-laying positions (after Badyaev & Martin, 2000a). For estradiol and vitamin A, PC1 was constructed from values of individual components across egg-laying positions. High covariation among egg-laying positions (i.e. pronounced egg-laying gradient) would produce similar and monotonically increasing or decreasing PC loadings across egg-laying positions (e.g. Kirkpatrick & Lofsvold, 1992, Fig. 3). Weak or highly fluctuating PC1 loadings across egg-laying positions indicate absence of egg-laying gradient (i.e. weak interdependency among egg-laying positions in allocation of a particular maternal product). An additional advantage of this method is that it enables statistical and visual assessment of concordance in egg-laying gradients among different groups of maternal products by comparisons of vector correlations of PC1 and corresponding angles. For the PCs of two groups, the angle between them was calculated as the arc cosine of the inner product of the two vector elements. Statistical distribution of vector angles (mean and standard deviation) was obtained with resampling with replacement of each gradient sample within each population. Vector correlations were arcsin-transformed and difference between the two samples was assessed with two-tailed *t*-tests.

We used canonical discriminant analysis to derive linear combinations of individual compounds that best distinguish populations, sexes and egg-laying positions. We used correlations between canonical variables and original variables to interpret their importance in the model (Fig. 2). To examine egg-laying gradients while controlling for the effects of sex and biased sex-ratio of some egg-laying positions, we calculated least-squared means for each group of maternal products and each egg-laying position. Within each position, difference between the sexes was assessed with nonparametric two-tailed Kruskal–Wallis tests, and multiple comparisons of means were conducted with *t* (LSD) multiple range tests ($\alpha = 0.05$). Sex-ratio bias in each egg-laying position and population differences in sex-biased ovulation order were tested with the binomial tests and generalized linear mixed models and reported elsewhere (Badyaev *et al.*, 2002).

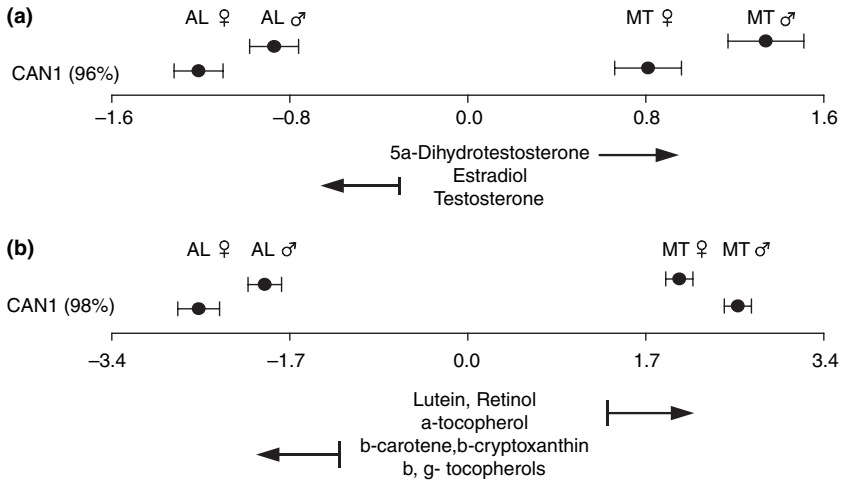


**Fig. 2** Differences in (a) yolk steroids and (b) yolk carotenoids and vitamins A and E between Montana (MT) and Alabama (AL) populations of house finches. Shown are means ± SE of the first canonical discriminant axes. The (96%) difference in steroids between the populations and sexes was along canonical axis = 6.50*DHT*-3.29*Estradiol*-0.98*T*, while 98% of difference in antioxidants was along canonical axis = 2.88*Lutein*+2.01$\alpha$-*tocopherol*+1.17*Retinol*-1.28$\beta$-*Carotene*-0.93$\beta$-*Cryptoxanthin*-0.61$\beta$-*tocopherol*-0.48$\gamma$-*tocopherol*.

## Results

### Population differences in yolk steroids, carotenoids and vitamins

Populations differed in concentrations of all compounds examined in this study, with exception of $\beta$-tocopherol (Table 1, Fig. 2, Appendices S1 and S2). In Montana, eggs had lower levels of DHT, but higher estradiol and *T* (Fig. 2; Wilks' $\lambda = 0.41$, $F_{9,389} = 18.92$, $P < 0.001$), as well as more diverse array and greater concentrations of carotenoids and higher concentration of vitamins A and E compared to eggs of Alabama finches (Fig. 2; Wilks' $\lambda = 0.10$, $F_{24,450} = 22.55$, $P < 0.001$). Oocytes that became males and females (male and female oocytes hereafter) differed in the amount of steroids (Fig. 2; Waller-Duncan K-ratio $t = 2.97$, $P < 0.05$; Table 1), and some individual carotenoid compounds and vitamin A (Table 1), but were similar in the composite measures of carotenoids and vitamin E (Fig. 2; Waller-Duncan $t = 1.78$, $P > 0.05$).

In both populations, a composite measure of testosterone (footnote in Table 2) and estradiol formed strong egg-laying gradients (i.e. high and consistent eigenvector correlations in Fig. 3a and c, as well as large eigenvalues for testosterone and estradiol in Fig. 4). However, the gradients were distinct between testosterone and estradiol: testosterone consistently increased, while the levels of estradiol either remained relatively constant with egg-laying order (Montana, solid line in Fig. 5a, b) or peaked at the middle of egg-laying sequence (Alabama, solid line in Fig. 5f, g). In both populations, the allocation of testosterone to last-laid eggs was most variable and least dependent on testosterone allocation to other eggs within a clutch (Fig. 3a, c). In both populations, oocyte acquisition of carotenoids and vitamin E (both composite measures, footnote in Table 2) and of vitamin A was highly variable and formed no consistent egg-laying gradients (i.e. highly fluctuating eigenvector correlations in Fig. 3b, d, as well as small eigenvalues for carotenoids and vitamins in Fig. 4).

### Concordance of steroid and antioxidant acquisition

In Montana, oocytes' accumulation of steroids and vitamins was highly concordant (Fig. 4, above diagonal). Within clutches, testosterone levels closely covaried with levels of vitamin E in all but the last-laid eggs, and with levels of vitamin A and carotenoids in most egg-laying positions (Tables 2 and 3). In Alabama, the concordance was weaker, but overall, the oocyte acquisition of vitamins A and E and carotenoids closely covaried with the levels of steroids (Fig. 4, below diagonal), especially for the first three egg-laying positions (Tables 2 and 3). When the effects of egg-laying order and nest identity were statistically controlled, in both populations, a higher concentration of testosterone was associated with greater acquisition of vitamin A (Montana: standardized regression coefficient $b_{ST} = 0.25$, $t = 2.39$, $P = 0.02$; Alabama; $b_{ST} = 0.19$, $t = 1.99$, $P = 0.05$; Fig. 6c, g), higher levels of estradiol (Montana: $b_{ST} = 0.46$, $t = 4.69$, $P < 0.001$; Alabama: $b_{ST} = 0.38$, $t = 4.23$, $P < 0.001$; Fig. 6a, b), lower acquisition of carotenoids (Montana: $b_{ST} = -0.35$, $t = -3.40$, $P = 0.001$; Alabama: $b_{ST} = -0.20$, $t = -2.06$, $P = 0.04$; Fig. 6b, f), and did not vary with vitamin E (Montana: $b_{ST} = 0.06$, $t = 0.66$, $P = 0.55$; Alabama: $b_{ST} = 0.11$, $t = 1.07$, $P = 0.29$; Fig. 6d, h).

**Table 1** Between-population variation in individual compounds of yolk hormones, carotenoids and vitamins in relation to ovulation order (OV) and sex of the embryo in recently established Montana ($n = 120$ eggs and 29 nests) and Alabama ($n = 98$ eggs and 24 nests) populations of house finches.

| Substance | Population | | Sex | | Population × sex | | OV | | Population × OV | | Sex × OV | | Population × Sex × OV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hormones | $F_{1,218}$ | $P$ | $F_{1,218}$ | $P$ | $F_{1,218}$ | $P$ | $F_{4,218}$ | $P$ | $F_{4,218}$ | $P$ | $F_{4,218}$ | $P$ | $F_{4,218}$ | $P$ |
| T | **5.86** | **0.01** | 2.87 | 0.09 | 0.72 | 0.39 | **5.64** | **<0.01** | 1.72 | 0.14 | **2.68** | **0.03** | **4.65** | **0.01** |
| DHT | **52.4** | **<0.01** | **5.53** | **0.02** | **4.89** | **0.02** | 0.92 | 0.45 | 0.79 | 0.53 | 0.34 | 0.85 | **3.49** | **0.02** |
| E | **8.2** | **0.03** | **3.48** | **0.04** | 0.26 | 0.61 | 0.53 | 0.71 | 0.22 | 0.93 | **3.11** | **0.02** | **4.46** | **0.01** |
| Carotenoids | | | | | | | | | | | | | | |
| Lutein | **77.4** | **<0.01** | 1.86 | 0.17 | 0.40 | 0.53 | 0.62 | 0.65 | 0.84 | 0.50 | 0.61 | 0.65 | 2.08 | 0.10 |
| $\beta$-Cryptoxanthin | **30.8** | **<0.01** | **4.71** | **0.03** | 1.77 | 0.18 | 0.68 | 0.60 | 0.66 | 0.62 | **2.94** | **0.04** | **3.07** | **0.03** |
| $\beta$-Carotene | **28.1** | **<0.01** | **4.32** | **0.04** | 2.84 | 0.10 | 1.27 | 0.28 | 0.97 | 0.42 | **2.44** | **0.05** | **3.38** | **0.02** |
| Vitamin A | | | | | | | | | | | | | | |
| Retinol | **55.4** | **<0.01** | **4.07** | **0.05** | 0.13 | 0.72 | 0.34 | 0.85 | 0.34 | 0.85 | 0.56 | 0.69 | **3.20** | **0.03** |
| Vitamin E | | | | | | | | | | | | | | |
| $\delta$-tocopherol | **5.69** | **0.01** | 0.39 | 0.53 | 0.80 | 0.37 | 0.05 | 0.99 | 0.74 | 0.56 | **3.27** | **0.02** | 0.15 | 0.93 |
| $\gamma$-tocopherol | **70.4** | **<0.01** | 0.08 | 0.78 | 0.33 | 0.57 | 0.81 | 0.52 | 1.19 | 0.32 | 0.99 | 0.42 | 1.45 | 0.23 |
| $\beta$-tocopherol | 1.69 | 0.20 | 1.69 | 0.20 | 2.48 | 0.11 | 0.56 | 0.69 | 1.45 | 0.22 | 0.77 | 0.54 | **3.27** | **0.02** |
| $\alpha$-tocopherol | **44.57** | **<0.01** | 0.79 | 0.38 | 1.01 | 0.31 | 0.23 | 0.92 | 0.97 | 0.43 | **4.31** | **0.01** | **2.53** | **0.04** |

Bold values show $P < 0.05$.

**Table 2** Analysis of variance in yolk levels of testosterone (T, composite measure, see note below) and estradiol (Est) in relation to sex, carotenoid (Car, composite measure), vitamin A (VitA) and vitamin E (VitE, composite measure) concentrations, as well as sex of the preceding egg (PrSex) and the level of testosterone (PrT) or estradiol (PREst) in the preceding egg. OV – ovulation order. Shown are *F* values, bold values indicate significance ($P < 0.05$), d.f. indicates degrees of freedom.

| Substance | Montana | | | | | | | | | Alabama | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T*† | | Sex | Car‡ | VitA | VitE§ | Est | PrSex | PrT | PrSex*PrT | | Sex | Car | VitA | VitE | E | PrSex | PrT | PrSex*PrT |
| OV | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| 1 | 1.20 | 1.12 | 0.38 | **5.22** | **3.07** | **13.78** | – | – | – | 1.24 | **7.07** | **4.50** | 1.81 | 0.34 | 0.06 | – | – | – |
| 2 | 1.23 | **3.68** | **8.94** | 3.62 | **7.03** | 0.02 | 1.47 | **7.06** | **3.85** | 1.21 | 6.45 | **10.13** | 0.01 | 1.11 | 0.11 | 9.84 | 0.31 | **5.53** |
| 3 | 1.23 | 1.29 | **5.06** | 3.03 | **3.45** | **7.83** | 0.75 | **4.09** | 0.95 | 1.20 | **12.86** | **4.66** | **12.06** | 0.01 | 2.91 | 0.31 | **3.65** | 0.98 |
| 4 | 1.21 | **6.67** | 2.30 | **27.34** | **28.30** | **10.50** | **13.10** | **4.91** | **8.96** | 1.17 | 1.31 | 0.40 | 1.00 | 0.55 | 1.56 | 0.03 | 1.68 | 0.01 |
| 5 | 1.14 | **8.91** | **6.37** | 0.11 | 0.98 | 1.15 | **21.22** | **7.19** | **6.45** | 1.13 | 0.43 | 0.38 | **3.25** | 0.37 | 10.5 | 2.10 | **18.54** | 0.91 |
| *Est* | | Sex | *T* | Car | VitA | VitE | PrSex | PrEst | PrSex*PrEst | | Sex | T | Car | VitA | VitE | PrSex | PrEst | PrSex*PrEst |
| OV | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| 1 | 1.20 | 0.02 | **21.47** | 0.30 | **9.78** | 0.01 | – | – | – | 1.24 | **4.47** | 0.06 | 0.77 | 0.99 | **4.71** | – | – | – |
| 2 | 1.23 | **16.99** | **9.50** | 1.02 | 1.08 | 2.39 | **5.08** | 3.04 | **13.01** | 1.21 | 1.32 | 0.72 | 2.19 | 1.02 | 0.04 | 0.22 | 1.33 | 0.53 |
| 3 | 1.23 | 0.29 | **5.37** | 4.06 | 1.61 | 0.92 | 1.46 | **5.81** | **12.47** | 1.21 | 1.16 | 1.84 | 0.13 | 0.23 | 0.34 | 1.31 | 0.08 | **10.72** |
| 4 | 1.21 | **5.33** | **10.80** | 4.45 | 2.36 | **6.90** | 0.36 | **6.09** | 1.07 | 1.18 | 0.02 | 1.45 | 0.03 | 0.29 | 0.76 | 0.01 | 0.61 | 2.75 |
| 5 | 1.13 | 0.36 | 0.12 | 2.83 | 0.06 | 1.61 | 1.06 | 1.30 | **6.30** | 1.14 | 3.00 | 0.54 | 0.81 | 0.14 | 0.35 | 3.11 | **6.59** | **5.88** |

†Montana: ***T*** is principal component 1 (PC1) = 0.71*T*+0.71*DHT*; Alabama: PC1 = 0.36*Androstenedione* (*A4*)+0.69*T*+0.58*DHT*.
‡Montana: ***Car*** is PC1 = 0.68*Lutein*+0.35*Zeaxanthin*+0.23α-Cryptoxanthin+0.49*β-Cryptoxanthin*+0.32*α-Carotene*+0.53 trans-*β*-Carotene+0.37 *13-cis-β-Carotene*+0.34 *15-cis-β-Carotene*+0.47 *other cis-β-Carotenes*; Alabama: PC1 = 0.58*Lutein*+0.55*β-Cryptoxanthin*+0.60*β-Carotene*.
§Montana ***Vitamin E*** is PC1 = 0.48*δ-tocopherol*+0.57 *γ-tocopherol*+0.48 *β-tocopherol*+0.49 *α-tocopherol*; Alabama PC1 = 0.54*δ-tocopherol*+0.52*γ-tocopherol*+0.37*β-tocopherol*+0.55*α-tocopherol*. Analysis is based on residuals from the model including the nest identity.

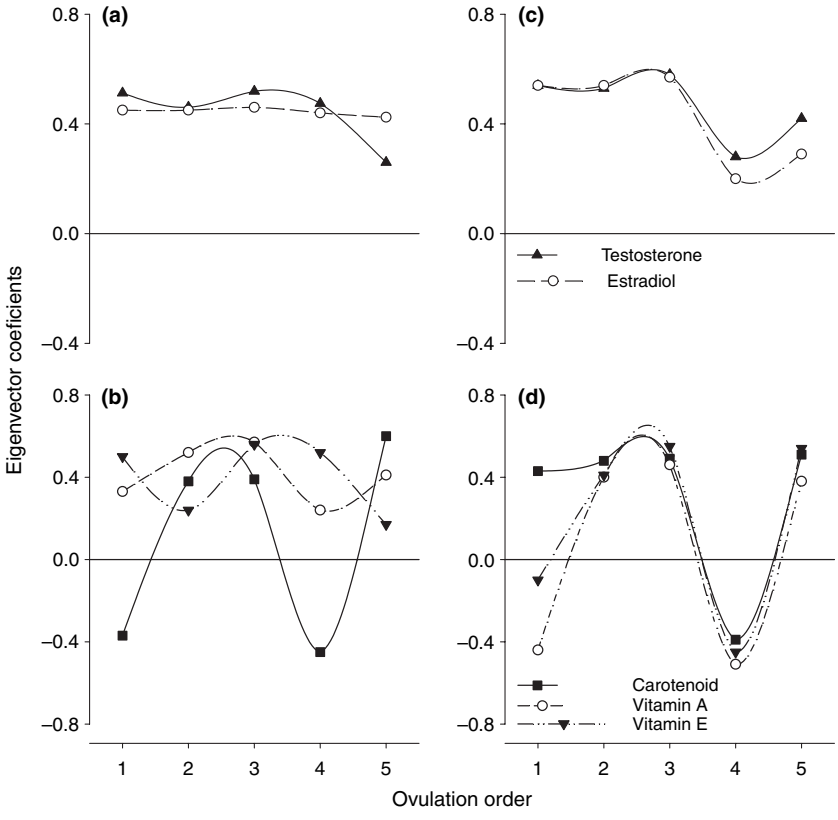


**Fig. 3** Gradients of steroids, carotenoids, and vitamins in relation to ovulation order in Montana (a, b) and Alabama (c, d) populations. Shown are correlations of the first eigenvector (PC1) of testosterone change with each ovulation position for testosterone and estradiol (a, c), and for carotenoids and vitamins A and E (b, d). Fluctuating correlations (b, d) indicate a lack of consistent ovulation gradient and high variability in vitamin and carotenoid acquisition across nests. Eigenvalues associated with each PC1 are shown in Fig. 4.

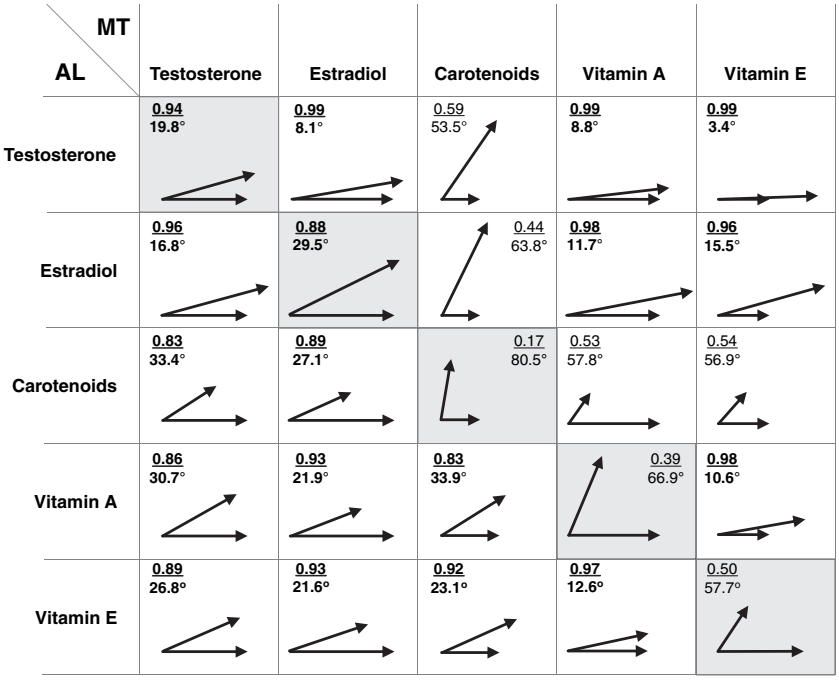


**Fig. 4** Concordance of egg-laying gradients in steroids, carotenoids, and vitamins between Montana (MT) and Alabama (AL) populations of the house finch (diagonal and grey background) and within-populations (Montana – above the diagonal, Alabama – below). Drawn are the first eigenvectors (PC1) of the egg-laying gradients and the corresponding correlations and angles (in degrees) between them (in the upper corner of each cell). The length of each vector is proportional to its eigenvalue (for reference, the width of each cell is 4 units). Longer vectors indicate stronger egg-laying gradient of a particular substance (upper row – lower vector, left column – upper vector) and smaller angles (greater correlations) between two vectors indicate greater concordance in the two egg-laying gradients. Vector correlations that are not significantly different between the samples, i.e. substances with identical egg-laying gradients, are shown in bold.

### Sex-specific allocation of steroids and antioxidants in relation to ovulation sequence

Overall, testosterone levels were gradually increasing with egg-laying order in both populations, carotenoid and vitamin A levels were gradually increasing with egg-laying in Montana and decreasing in Alabama, while estradiol levels and vitamin E either remained constant or showed a slight peak in the middle of egg-laying sequence (Fig. 5, solid least-squared-means line). In both populations male and female oocytes commonly accumulated highly distinct levels of steroids, carotenoids, and vitamins (i.e. least-squared-means line vs. histograms of means for each sex in Fig. 5). For example, despite a gradual increase in testosterone levels throughout egg-laying in both populations, the first-, second- and last-laid eggs in Montana (Fig. 5a; Kruskal–Wallis $\chi^2_1 = 3.68$, $P = 0.05$; $\chi^2_1 = 4.73$, $P = 0.04$; and $\chi^2_1 = 4.98$, $P = 0.02$, correspondingly) and the third- and last-laid eggs in Alabama (Fig. 5f; both $\chi^2_1 > 3.80$, $P < 0.05$) had strongly distinct levels of testosterone between male and female eggs.

Gradients of steroids, carotenoids and vitamins were distinct between the sexes (solid vs. dashed regression lines in Fig. 5, Table S3). In Montana, female oocytes showed concave gradients with the highest levels of steroids and antioxidants in the middle of the egg-laying (Fig. 5a–e, Table S3), whereas male oocytes showed either a linear increase (testosterone, Fig. 5a) or convex gradient with lower levels in the middle of egg-laying (estradiol, carotenoids, vitamins, Fig. 5b–e). Thus, in Montana female oocytes, the pattern was qualitatively similar to that expected under constant maternal allocation and higher oocyte leakage with greater overlap (Fig. 1a right column), while in males, the pattern was similar to either constant maternal allocation and greater overlap of the middle egg-laying positions, or to increased maternal allocation with egg-laying (Fig. 1a, b). In Montana, the egg-laying gradients differed between the sexes for testosterone (Sex × ovulation order interaction: $F_{4,91} = 2.72$, $P = 0.05$), estradiol ($F_{4,89} = 3.40$, $P = 0.01$), carotenoids ($F_{4,90} = 2.94$, $P = 0.04$) and approached significance for vitamin A ($F_{4,90} = 2.34$, $P = 0.07$), but not for vitamin E ($F_{4,90} = 1.37$, $P = 0.26$).

Interestingly, in Alabama, the pattern was the opposite: with exception of egg-laying gradients in vitamin E, female oocytes showed pronounced linear patterns – increase in testosterone and estradiol (Fig. 5f, g, Table S3) or decrease in carotenoids and vitamin A, (Fig. 5h, i). Male oocytes showed concave gradients (steroids, carotenoids, vitamin A, Fig. 5f–i; Table S3) or linear increase with ovulation sequence in vitamin E levels (Fig. 5j). Thus, in Alabama female oocytes the pattern was qualitatively similar to that expected under changing maternal allocation and follicle-specific or overlap-insensitive pattern of allocation among oocytes (Fig. 1b, c, middle column), while in males, the pattern was similar to constant maternal allocation and greater leakage among follicles with greater overlap in the middle of egg-laying sequence (Fig. 1a right column). In Alabama, the ovulation gradients were statistically distinct between the sexes for all components, but estradiol and vitamin A (Sex × ovulation order interaction; testosterone: $F_{4,98} = 2.43$, $P = 0.05$, estradiol: $F_{4,98} = 1.81$, $P = 0.33$, carotenoids: $F_{4,88} = 3.26$, $P = 0.05$, vitamin A: $F_{4,88} = 2.67$, $P = 0.08$, and vitamin E:

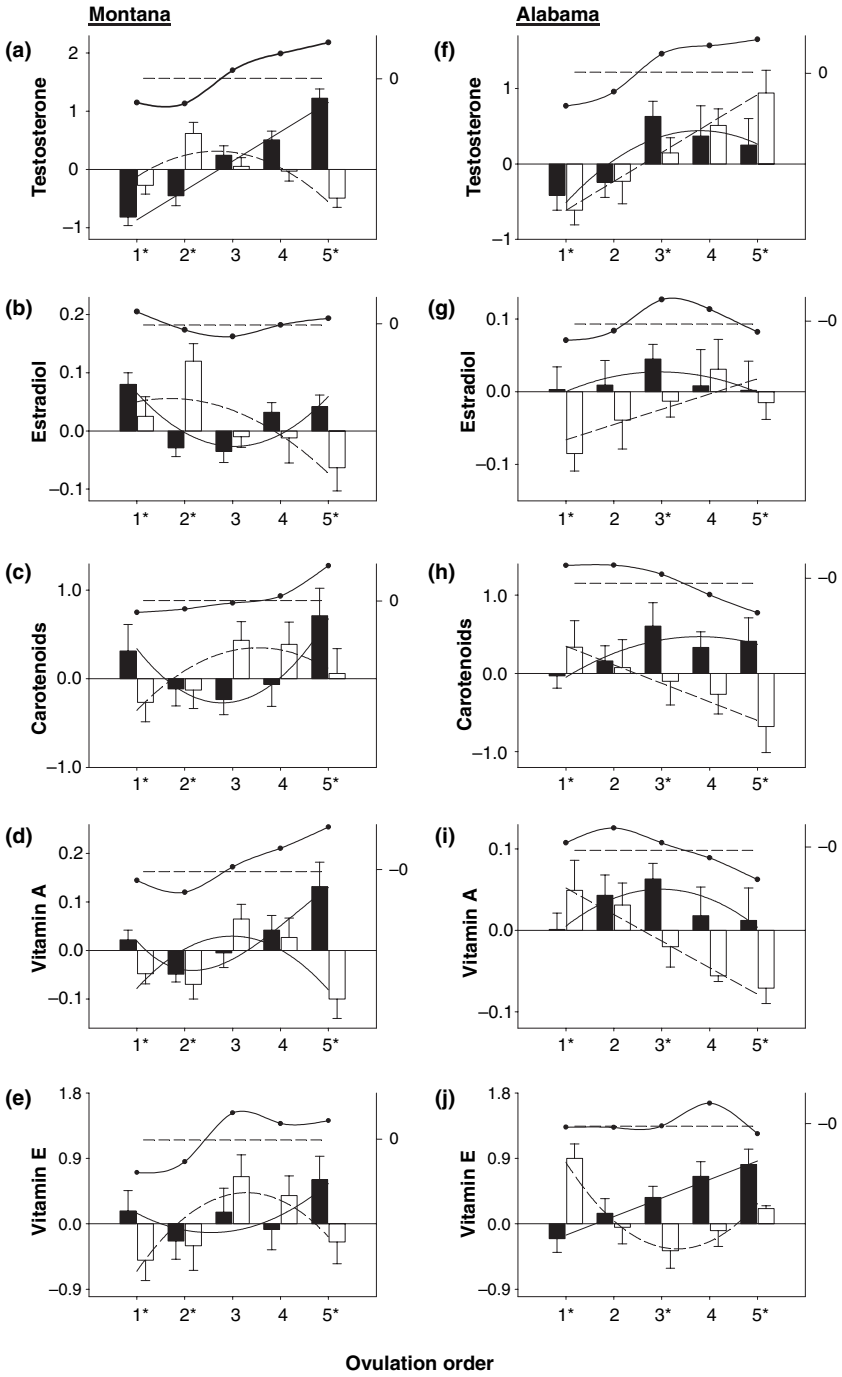


**Fig. 5** Patterns of acquisition of steroids, carotenoids, and vitamins in relation to oocyte sex and ovulation order in Montana (a–e) and Alabama (f–j) populations. Bars and left ordinate axis show mean ± SE for male (black) and female (white) oocytes after statistically controlling the effect of nest identity. Solid (males) and dashed (females) lines show linear or quadratic regressions best describing the within-sex patterns of acquisition in relation to ovulation order (Online Table S3). Fitted line above the bars and the right ordinate axis show egg-laying gradient (least squared means for each egg-laying position) of resource acquisition when the effects of follicle sex and biased sex-ratio are controlled statistically. Asterisks indicate significantly sex-biased ovulation orders for each population.

$F_{4,88} = 3.47$, $P = 0.05$). Populations differed in sex-specific ovulation gradients of all individual compounds (Table 1, Population × sex × ovulation order interaction) with exception of $\delta$- and $\gamma$-tocopherols.

Distinct sex-specific gradients, especially in steroid deposition, were also evident in strong interdependency between steroids deposited in sequentially ovulating oocytes [Table 2: significance of previous egg's testosterone (PrT) and previous egg's estradiol (PrEst) terms for most ovulating positions in Montana, and third and fifth ovulating position in Alabama]. Most importantly, the interdependence in steroid deposition was influenced by the concordance of sexes in sequentially ovulating oocytes [Table 2: significance of interaction of previous egg's sex and previous egg's testosterone (PrSex × PrT) and estradiol (PrSex × PrEst) terms for most ovulating positions in Montana and some ovulating positions in Alabama].

**Table 3** Analysis of variance in yolk levels of carotenoids (Car), vitamin A (VitA), and vitamin E (VitE) in relation to sex, testosterone (T) and estradiol (Est) concentrations, as well as sex of the preceding egg (PrSex) and the level of carotenoids (PrCar), vitamin A(PrVitA) and vitamin E (PrVitE) in the preceding egg. OV is ovulation order. Shown are *F* values, bold values indicate significance ($P < 0.05$).

| Substance | Montana | | | | | | | | | Alabama | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Car* | | Sex | *T* | VitA | VitE | Est | PrSex | PrCar | PrSex*PrCar | | Sex | T | VitA | VitE | Est | PrSex | PrCAR | PrSex*PrCar |
| OV | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| 1 | 1.21 | **3.81** | 0.01 | 0.02 | **4.55** | 0.36 | – | – | – | 1.24 | 2.11 | **4.96** | **12.54** | 0.07 | 0.77 | – | – | – |
| 2 | 1.23 | 2.09 | 2.92 | **7.01** | 2.94 | 0.01 | 1.55 | 1.88 | 0.07 | 1.20 | 0.85 | 0.08 | 0.31 | 0.11 | 0.32 | 0.15 | 0.14 | 0.48 |
| 3 | 1.21 | 2.07 | 1.15 | **5.66** | 1.53 | 2.82 | 3.66 | **5.35** | 0.09 | 1.21 | 1.86 | **9.00** | 4.09 | 1.62 | 5.23 | 1.98 | 1.89 | **8.84** |
| 4 | 1.20 | 0.82 | 2.30 | **5.03** | **7.64** | **9.24** | 0.34 | 0.24 | 0.84 | 1.17 | 0.66 | 0.13 | **27.18** | 1.52 | 0.26 | 1.45 | 0.29 | 0.67 |
| 5 | 1.13 | **9.72** | 1.24 | 1.54 | 0.08 | 2.90 | 0.89 | 0.09 | 0.37 | 1.13 | **11.93** | 0.65 | **6.28** | 4.59 | 1.96 | 0.00 | 2.65 | 1.32 |
| Vitamin A | | | | | | | | | | | | | | | | | | |
| | | Sex | T | Car | VitE | Est | PrSex | PrVitA | PrSex*PrVitA | | | T | Car | VitE | Est | PrSex | PrVitA | PrSex*PrVitA |
| OV | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| 1 | 1.21 | 0.36 | 5.16 | 0.01 | **4.36** | 9.78 | – | – | – | 1.24 | 0.03 | 1.81 | 3.01 | **19.11** | 0.99 | – | – | – |
| 2 | 1.23 | 0.31 | 0.01 | 0.32 | **7.91** | 0.38 | 0.29 | 0.04 | 1.70 | 1.21 | 0.01 | **6.02** | 3.13 | 0.04 | 0.91 | 0.13 | 1.65 | 0.32 |
| 3 | 1.21 | 0.50 | 4.46 | **7.01** | **14.80** | 1.64 | 1.53 | 0.01 | 0.34 | 1.21 | **8.27** | **14.17** | **6.37** | 0.57 | 0.63 | 1.06 | 6.19 | 1.65 |
| 4 | 1.20 | 0.54 | **7.33** | 4.80 | **21.70** | 0.75 | 0.56 | 3.06 | 1.64 | 1.18 | 0.03 | **10.84** | **12.90** | 2.09 | 0.48 | 2.29 | 1.01 | 0.11 |
| 5 | 1.13 | **6.03** | 4.49 | 0.43 | **6.84** | 0.06 | 0.67 | 0.03 | 0.03 | 1.14 | **5.25** | 0.41 | **6.21** | **7.50** | 0.80 | 0.09 | 1.89 | 1.28 |
| Vitamin E | | | | | | | | | | | | | | | | | | |
| | | Sex | T | Car | VitA | Est | PrSex | PrVitE | PrSex*PrVitE | | Sex | T | Car | VitA | Est | PrSex | PrVitE | PrSex*PrVitE |
| OV | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | d.f. | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| 1 | 1.19 | 0.05 | 0.30 | **5.28** | **6.36** | 0.01 | – | – | – | 1.19 | 1.63 | 0.34 | **6.07** | **19.11** | **4.31** | – | – | – |
| 2 | 1.21 | 0.03 | 1.92 | 2.09 | **8.87** | 2.47 | 0.08 | 0.67 | 0.00 | 1.17 | 1.43 | **8.54** | 0.26 | 0.29 | 0.07 | 2.53 | 3.34 | 1.80 |
| 3 | 1.20 | 0.78 | 4.28 | 2.85 | **18.06** | 1.31 | 1.27 | 0.06 | 0.06 | 1.16 | **9.84** | **12.29** | 2.73 | 0.27 | 2.65 | 2.97 | 0.21 | 1.75 |
| 4 | 1.19 | **5.06** | **14.48** | 0.61 | **17.69** | **10.68** | 0.54 | **8.72** | **7.17** | 1.17 | 0.52 | 0.64 | 0.63 | **5.27** | 0.17 | 0.45 | 1.10 | 0.10 |
| 5 | 1.12 | **4.31** | 0.78 | 0.40 | **8.40** | 1.61 | 1.76 | 1.72 | 0.12 | 1.11 | 0.04 | 0.39 | 1.57 | **8.96** | 0.20 | 0.31 | 4.36 | 2.65 |

### Sex-bias in ovulation sequence and steroid and antioxidant acquisition

In both populations, oocytes in sex-biased ovulation orders had highly distinct levels of steroids, carotenoids, and vitamins between the sexes. For example, in the Montana population, where the first, second, and last eggs are highly sex-biased, the last-laid male and female eggs had different levels of all measured substances except estradiol (Tables 2 and 3; Fig. 5), the second-laid male and female eggs had different levels of testosterone and estradiol, and the first-laid male and female differed in the levels of carotenoids (Tables 2 and 3; Fig. 5a–e). In the Alabama population, where the first, third and last eggs are highly sex-biased, the third-laid male and female eggs differed in testosterone, vitamin A and vitamin E, the first-laid male and female eggs had different levels of testosterone and estradiol, and the last-laid male and female eggs differed in the levels of carotenoids and vitamin A (Tables 2 and 3; Fig. 5f–j).

Eggs in the nests where sex-bias in relation to ovulation order strongly deviated from population-specific patterns (e.g. males in the first- and females in the last-laid eggs in Montana, and females in the first and the last laid-eggs in Alabama) had significantly distinct concentration of steroids, carotenoids and vitamins [Fig. 7; Montana: Canonical axis 1 (Can1) $F_{9,93} = 3.59$, $P = 0.001$, Can2: $F_{8,93} = 2.19$, $P = 0.03$; Alabama: Can1 $F_{9,89} = 3.62$, $P = 0.001$, Can2: $F_{9,89} = 1.24$, $P = 0.28$]. For example, in Montana, first-laid male and last-laid female eggs had unusually low levels of testosterone and carotenoids and high levels of estradiol (males only) compared to first-laid female eggs and last-laid male eggs (LSD test, d.f. = 92, critical $t = 1.99$; M1 vs. F1: difference between means – $\epsilon$Can1 = 0.97, $P < 0.05$; $\Delta$Can2 = 1.26, $P < 0.05$; M5 vs. F5: $\Delta$Can1 = 3.88, $P < 0.05$; $\Delta$Can2 = 0.10, $P > 0.05$; Fig. 7a). Females produced in second-laid eggs (a strongly male-biased position) had much higher levels of estradiol and testosterone than males in second-laid eggs (M2 vs. F2: $\Delta$Can1 = 1.74, $P < 0.05$; $\Delta$Can2 = 0.45, $P < 0.10$; Fig. 7a). Similarly, in Alabama, females in first-laid eggs (male-biased position) had unusually low levels of estradiol and testosterone compared to other oocytes, including first-laid male eggs (M2 vs. F2: $\Delta$Can1 = 1.01, $P < 0.05$; $\Delta$Can2 = 1.41, $P < 0.05$; Fig. 7b). Females in last-laid eggs (male-biased position) had the highest levels of testosterone of all oocytes, including last-laid male eggs (M2 vs. F2: $\Delta$Can1 = 1.32, $P < 0.05$; $\Delta$Can2 = 0.21, $P > 0.05$; Fig. 7b).

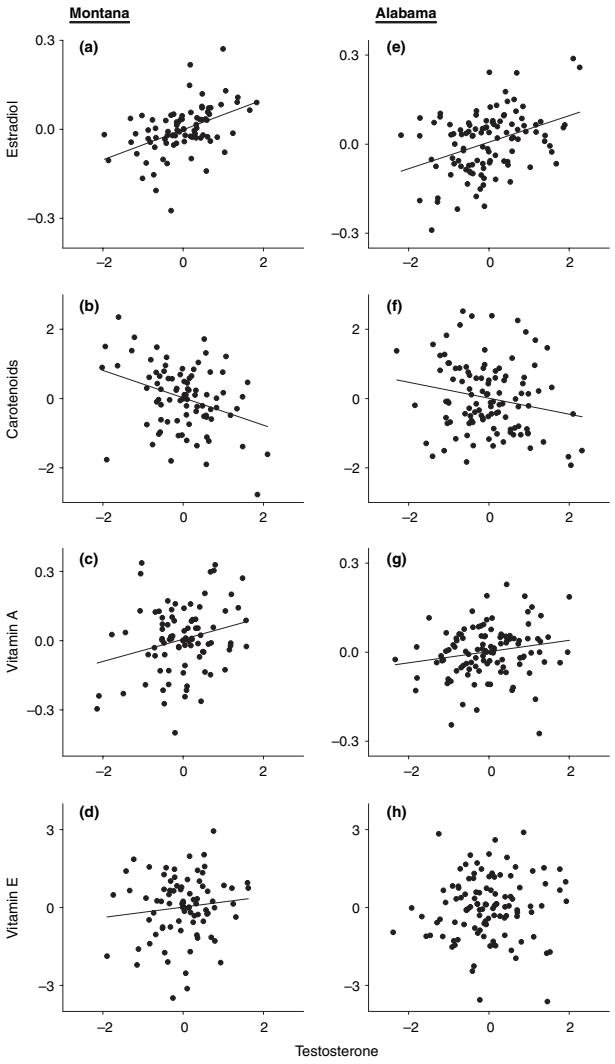


**Fig. 6** Acquisition of estradiol, carotenoids, and vitamins A and E in relation to oocytes' levels of testosterone in Montana (a–d) and Alabama (e–h) populations. Shown are partial regression plots of residuals where the effects of nest identity and ovulation order are statistically controlled. Testosterone, carotenoids and vitamin E are composite variables as shown in footnote of Table 2.

## Discussion

The evolutionary importance of maternal effects is determined by the interplay of maternal adaptations and strategies, the sensitivity of offspring to such strategies, and the similarity of selection pressures acting on maternal and offspring generations (Kirkpatrick & Lande, 1989; Rossiter, 1996; Ginzburg, 1998; Badyaev, 2005). Despite a considerable empirical effort, disentangling maternal and offspring strategies has been difficult (Schwabl, 1996; Whittingham & Schwabl, 2002; Gil *et al.*, 2004; Michl *et al.*, 2005), especially in the studies of sex-specific maternal effects (Bowden *et al.*, 2000; Lovern & Wade, 2001; Müller *et al.*, 2002; Saino *et al.*, 2003; Veiga *et al.*, 2004; Verboven *et al.*, 2005; Uller, 2006). In particular, the extent to which overlap in the development of male and female eggs – a common occurrence in birds – limits maternal ability to provide sex-specific resources is not known.

Our study of sex-specific allocation of steroids, carotenoids, and vitamins in two populations of house finches with opposite, but equally sex-biased, maternal effects produced four principal results. First, we found that despite large population differences in amounts of steroids, carotenoids and vitamins allocated to growing eggs, the laying order gradients in such allocation were often similar between the populations, suggesting that allocation of steroids is likely a reflection of female's own hormonal profile. Second, we found sex-specific trade-offs in the follicle partitioning of steroids and carotenoids, corroborating documentation of sex-specific temporal clustering of male and female oocytes in this species (Badyaev *et al.*, 2006). Third, our results suggest that the strong sex-bias in egg-laying order in these populations enables pronounced sex-specific accumulation even under conditions of nonsex-specific maternal allocation. Fourth, we show that eggs in the nests where sex-bias in relation to ovulation order deviated from population-specific patterns had highly distinct and unusual concentrations of steroids, carotenoids, and vitamins compared to the eggs of the opposite sex in these positions. These results support the finding of suboptimal growth of nestlings that hatch from the eggs of the rare sex (Badyaev *et al.*, 2003) and provide further evidence for sex-specific temporal clustering of oocytes in newly established house finch populations. We discuss the implications of these findings for understanding the evolution of sex-specific maternal effects in birds.

Our finding of population similarity in egg-laying gradients of steroids despite strong differences in levels of steroid, carotenoid, and vitamin accumulation provides insight into the mechanism of maternal allocation in relation to oocyte growth. Maternal allocation of carotenoids and vitamins A and E to eggs is thought to be determined by availability of antioxidant precursors in mothers' diets (Surai *et al.*, 1998b). Because these compounds are not stored by breeding females for a prolonged time (Surai *et al.*, 1998b; Surai, 2000), their allocation often differs among habitats, nesting attempts, and with temporal changes in female health (Surai *et al.*, 1998a; Horak *et al.*, 2002; Saino *et al.*, 2002). Correspondingly, the findings of egg-laying gradients or sex-specific allocation of carotenoids and vitamins are often attributed to the passive effects of depletion of some vitamins with egg-laying, to sex-biased egg-laying order, or to biochemical interactions among maternal carotenoids and vitamins during catabolism and deposition (Royle *et al.*, 1999; Surai & Sparks, 2001; Blount *et al.*, 2002; Koutsos *et al.*, 2003; Verboven *et al.*, 2005). Strong differences between the sexes in accumulation of

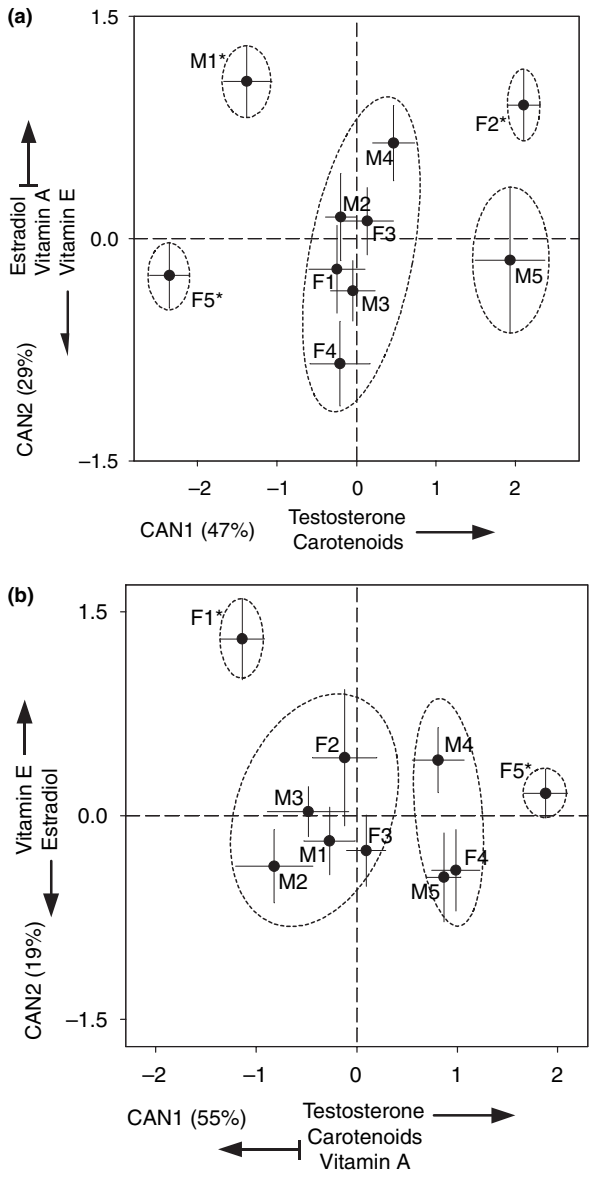


**Fig. 7** Yolk testosterone, estradiol, carotenoids, and vitamins A and E in oocytes of different sex and ovulation order in (a) Montana and (b) Alabama populations. Shown are the means (±bivariate SE) of the first two canonical discriminant axes and percent of associated variance. Letters associated with numbers indicate sex (male or female) and ovulation order (1–5). Dotted ellipsoids enclose groups of follicles not statistically different from each other. Asterisks indicate male oocytes produced in strongly female-biased position and female oocytes produced in strongly male-biased positions in each population. Canonical axes are as follows: Montana: Can 1 (eigenvalue $\lambda = 0.40$, 47% of variation) = 0.8*Testosterone*-1.9*Estradiol*+0.5*Carotenoids*+0.07*Vitamin A*-0.31*Vitamin E;* Can 2 ($\lambda = 0.25$, 29%) = 8.7*Estradiol*-0.5*Testosterone*+0.3*Carotenoids*+5.91*Vitamin A*-0.89*Vitamin E*, Alabama: Can 1 ($\lambda = 0.47$, 55%) = 0.6*Testosterone*-0.1*Estradiol*+0.6*Carotenoids*-14.1*Vitamin A*-0.3*Vitamin E*; Can 2 ($\lambda = 0.16$, 19%) = 0.3*Testosterone*-3.4*Estradiol*-0.2*Carotenoids*+6.8*Vitamin A*-1.1*Vitamin E*.

carotenoids and vitamins and sex-specific gradients of allocation of these compounds (Fig. 5) might be caused by sex differences in either the timing of oocyte growth or carotenoid and vitamin acquisition. However, in both sexes, antioxidant acquisition was closely concordant with the levels of steroids in the oocyte's yolk (Fig. 6, Tables 2 and 3). Moreover, the egg-laying gradients of antioxidants were weak and differed between populations (Figs 3 and 4). Thus, our results are most easily explained by temporal differences in growth of male and female oocytes (Fig. 1) and by the close link of steroid and carotenoid acquisition at the level of each follicle (e.g. Royle *et al.*, 2001; Groothuis *et al.*, 2005).

Changes in maternal testosterone and estradiol throughout egg-laying are well documented in birds (Johnson, 2000), and close qualitative correspondence between maternal plasma hormones and hormones acquired by oocytes growing at the same time is thought to reflect passive exposure of growing eggs to maternal steroids (Schwabl, 1996; Müller *et al.*, 2002; Williams *et al.*, 2004, 2005). Lack of such correspondence can be due to active maternal adjustment of allocation to each follicle, the follicle's own differential production or transport of steroids, or maternal manipulation of timing of oocyte production, including in relation to the sex of the oocyte (Etches & Duke, 1984; Porter *et al.*, 1989; Lovern & Wade, 2001; Lovern & Passek, 2002; Badyaev *et al.*, 2005). In particular, the production of estradiol increases during early stages of oocyte growth, reaching its peak at the time of maximum overlap among oocytes and then decreasing with the onset of egg-laying (Williams *et al.*, 2004) and incubation (Sockman & Schwabl, 1999). In contrast, plasma testosterone typically shows linear and gradual change with egg-laying (e.g. Groothuis & Schwabl, 2002). Thus, in our study, simple control of the levels of estradiol and testosterone in growing oocytes by the levels of these hormones in the mother's plasma should produce strong differences between early and late-laid eggs but no differences between the sexes within a given position in the clutch. Instead, we observed highly distinct levels of accumulation of steroids between male and female oocytes within an egg-laying position, that were consistent with sex-specific gradients of steroid accumulation. Moreover, when the effects of sex are controlled statistically, both populations have similar gradients of oocyte acquisition despite the opposite sequence of production of sons and daughters. These findings corroborate previous documentation of sex-specific trade-offs among oocytes in yolk partitioning and suggest that male and female oocytes differ either in their recruitment sequence or in timing of their growth in relation to maternal hormonal profile (Young & Badyaev, 2004; Badyaev *et al.*, 2005, 2006). Alternatively, exposure to different maternal steroid profiles throughout oogenesis might bias sex determination of maturing oocytes if products accumulating in oocyte yolks affect sex-determination or subsequent

sex-biased viability of oocytes (Veiga *et al.*, 2004; von Engelhardt *et al.*, 2004, 2006). In this case, sex and yolk resources will be linked regardless of changes in resource allocation gradients or laying positions of the sexes.

Interestingly, earlier studies suggested that accumulation of steroids by growing oocytes indicates their ovarian hierarchy better than does their relative size (Etches & Duke, 1984), such that groups of follicles recruited at the same time can form distinct clusters, with ovulation alternating between such clusters (Waddington & Walker, 1993). Similarly, several recent studies documented distinct gradients of steroids and lipids in male and female eggs (e.g. Blanco *et al.*, 2003; Rutstein *et al.*, 2005) – the pattern most consistent with sex differences in oocyte growth or sex-specific temporal clustering. Our comparisons of native and recently established populations of the house finch suggested that sex-specific temporal clustering of oocytes can evolve rapidly, most likely as a result of maternal adaptation to breeding in novel environments (Badyaev *et al.*, 2005, 2006). This study extends these findings by suggesting that the strong sex-specific maternal effects on growth of offspring in these newly established populations can be due to distinct accumulation of maternal products between male and female oocytes (Badyaev *et al.*, 2003, Fig. 5).

In summary, whereas modification of sex-biased egg-laying order, often in close concordance with environmental conditions, is common in birds (reviewed in Pike & Petrie, 2003), the mechanisms for such adjustment and its consequence for sex-specific allocation of maternal resources are not known. This study suggests that temporal sex-specific clustering of oocytes can not only be one such mechanism, but also could enable sex-specific maternal allocation of resources despite overlap in offspring development.

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## Supplementary material

The following supplementary material is available for this article online:

**Appendix S1.** Concentrations (mean ± SEM) of hormones, carotenoids and vitamins in yolks of house finch eggs in Montana population.

**Appendix S2.** Concentrations (mean ± SEM) of hormones, carotenoids and vitamins in yolks of house finch eggs in Alabama population.

**Table S1.** Best fit simple regressions and associated statistics (slope $b$, $r^2$) for the patterns of acquisition of steroids, carotenoids and vitamins in relation to ovulation order shown in Fig. 5. Most common pattern is shown in parenthesis.

This material is availble as part of the online article from http://www.blackwell-synergy.com

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