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Evolution of sex-biased maternal effects in birds. IV. Intra-ovarian growth dynamics can link sex determination and sex-specific acquisition of resources

A. V. BADYAEV,* R. L. YOUNG,* G. E. HILL† & R. A. DUCKWORTH‡

*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA
†Department of Biological Sciences, Auburn University, Auburn, AL, USA
‡Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

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Abstract

The evolutionary importance of maternal effects is determined by the interplay of maternal adaptations and strategies, offspring susceptibility to these strategies, and the similarity of selection pressures between the two generations. Interaction among these components, especially in species where males and females differ in the costs and requirements of growth, limits inference about the evolution of maternal strategies from their expression in the offspring phenotype alone. As an alternative approach, we examine divergence in the proximate mechanisms underlying maternal effects across three house finch populations with contrasting patterns of sex allocation: an ancestral population that shows no sex-biased ovulation, and two recently established populations at the northern and southern boundaries of the species range that have opposite sequences of ovulation of male and female eggs. For each population, we examined how oocyte acquisition of hormones, carotenoids and vitamins was affected by oocyte growth and overlap with the same and opposite sexes. Our results suggest that sex-specific acquisition of maternal resources and sex determination of oocytes are linked in this system. We report that acquisition of testosterone by oocytes that become males was not related to growth duration, but instead covaried with temporal exposure to steroids and overlap with other male oocytes. In female oocytes, testosterone acquisition increased with the duration of growth and overlap with male oocytes, but decreased with overlap with female oocytes. By contrast, acquisition of carotenoids and vitamins was mostly determined by organism-wide partitioning among oocytes and oocyte-specific patterns of testosterone accumulation, and these effects did not differ between the sexes. These results provide important insights into three unresolved phenomena in the evolution of maternal effects – (i) the evolution of sex-specific maternal allocation in species with simultaneously developing neonates of both sexes; (ii) the link between sex determination and sex-specific acquisition of maternal products; and (iii) the evolution of context-dependent modulation of maternal effects.

Introduction

Maternal effects expressed in the offspring are an outcome of both maternal and offspring strategies. Depending on the concordance of selection between the two generations and on similarity of growth requirements of male and female offspring, maternal effects can either facilitate or limit the evolutionary persistence of adaptations (Jablonka *et al.*, 1992; Cheverud & Moore, 1994; Mousseau & Fox, 1998a; Rossiter, 1998; Wolf *et al.*, 1998; McAdam *et al.*, 2002).

Because of their ubiquity and strength, maternal effects are frequently studied, and there is a wealth of

Correspondence: Alexander V. Badyaev, Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721-0088, USA.
Tel.: +1 520 626 8830; fax: +1 520 621 9190;
e-mail: abadyaev@email.arizona.edu

information on their constituents, causes and fitness consequences in many species (Mousseau & Fox, 1998b). Maternal effects in birds, in particular, have received considerable attention (e.g. Groothuis *et al.*, 2005), yet even in this group some of the most important questions on the origin and evolution of maternal effects are not answered. Most fundamentally, it is rarely known whether maternal effects represent active maternal strategies for manipulating offspring fitness or passive effects of a maternal adaptation. At the proximate level, addressing this question is complicated by the shared hormonal regulation of female reproduction and maternal effects (e.g. Johnson, 2000; Sockman *et al.*, 2006). At the ultimate level, compensatory interactions between maternal strategies and offspring counter-adaptations make it difficult to assign strategies from the degree of phenotypic expression of maternal effects in the offspring alone (Quintanilla *et al.*, 1999; Wolf & Wade, 2001; McAdam & Boutin, 2004; Wiegmann *et al.*, 2004). This is especially the case when offspring of different sex have distinct sensitivities to maternal strategies or when maternal strategies exert conflicting effects on multiple offspring traits (Rutledge *et al.*, 1972; Badyaev, 2005). Instead, studies contrasting the proximate mechanisms underlying maternal effects in both maternal and offspring generations across different selection regimes might provide greater inference into the evolution of maternal effects.

In species that produce broods of simultaneously developing male and female offspring, sex differences in the costs and requirements of growth, as well as in the expression of maternal effects, provide an opportunity to examine the evolution of adaptive maternal strategies. For example, indiscriminant maternal allocation of sex hormones to simultaneously growing neonates of both sexes could interfere with offspring sexual differentiation, and such conflicts are expected to constrain the evolution of sex-specific maternal effects (e.g. Oyhenart *et al.*, 1998; Saino *et al.*, 2006; Carere & Balthazart, 2007), favour the evolution of temporal or spatial 'clustering' of oocytes of different sexes (e.g. Uller, 2006) and produce distinct sensitivity of the sexes to maternal products (e.g. sex-difference in hormone receptor density or enzymes, Badyaev, 2002). Clustering, in particular, can be accomplished by changes in growth initiation and rate between male and female neonates and can evolve rapidly among populations and breeding contexts (Zakaria, 1999; Badyaev & Oh, 2008). Further, it is likely that such rapid evolution is facilitated by a common involvement of the same hormonal mechanisms in environmental assessment, reproduction and maternal allocation (Sockman *et al.*, 2006; Ball & Balthazart, 2008). However, no study to date has explored the consequences of variation in growth patterns of oocytes for acquisition of maternal resources (except for yolk uptake, Young & Badyaev, 2004).

Such an examination can resolve a number of unanswered questions in the evolution of maternal effects, because maternal products accumulating in avian oocytes differ in the temporal and spatial patterns of their synthesis and transport. For example, oocyte accumulation of testosterone can be due to local production by each ovarian follicle (Bahr *et al.*, 1983; Etches & Duke, 1984; Hackl *et al.*, 2003) as well as receptor-mediated uptake from maternal plasma (e.g. Yoshimura *et al.*, 1993). Importantly, however, oocyte exposure to steroids is time and stage specific (Bahr *et al.*, 1983; Etches & Duke, 1984; Schwabl, 1993; Badyaev *et al.*, 2005), resulting in temporal gradients of steroid production during oogenesis. By contrast, maternal production of carotenoids and vitamins A and E is closely linked to the diet, storage in liver and fat reserves (Surai *et al.*, 1998; Blount *et al.*, 2004) and subsequent lipoprotein-mediated transportation to the surface of each growing oocyte (Perry & Gilbert, 1979; Barber *et al.*, 1991). Thus, patterns of carotenoid and vitamin partitioning among growing oocytes might be attributed to organism-wide limitations of diet, storage or transportation.

With this background information in mind, we consider three general scenarios of oocyte acquisition of resources that can be tested by examining the patterns of oocyte growth and overlap (Fig. 1) – local 'active growth-related follicular production', 'active receptor-mediated uptake' from maternal plasma of distantly produced products and 'passive diffusion'. Further, we assign the synthesis, delivery and availability of maternal resources to three general categories – 'time- or stage-specific delivery' of resources in relation to the stage of oogenesis or reproduction and in response to external cues, 'limited delivery' of resources constrained by diet, transportation or oocyte competition and 'unlimited delivery' of resources that are abundant and non-time specific in synthesis or delivery to oocyte (Fig. 1).

When substances accumulated by oocytes are locally produced by their follicles, are abundant, or when the transport of these substances is not limited to a particular time and stage, then faster and longer growing oocytes should accumulate more of such substances (Fig. 1a,b, left column). Age- or time- specific production or delivery to the oocyte should result in the lack of a relationship between substance accumulation and growth rate and duration (Fig. 1a,b, center column), whereas accumulation by passive diffusion should result in a positive relationship with growth duration, but no relationship with growth rate (Fig. 1a,b, right column). Further, we consider three hypothetical scenarios for how a temporal hierarchy of oocytes in the ovary should affect trade-offs in acquisition of resources among oocytes (Fig. 2). First, a temporal hierarchy with single-sex clusters of oocytes should result in stronger trade-offs in the accumulation of maternal resources among oocyte clusters of the same vs. the opposite sex (Fig. 2a). Second, a temporal hierarchy with no single-sex clusters

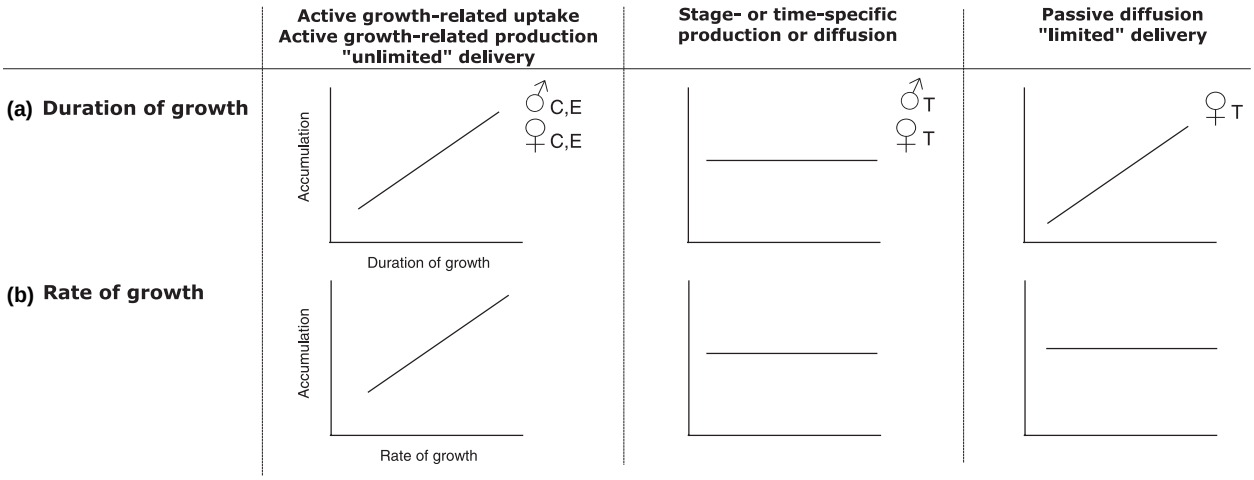

Fig. 1 Conceptual illustration of the relationship between oocyte accumulation of maternal resources in relation to (a) duration of growth and (b) rate of growth. Predicted relationship between growth and accumulation of resources expected under three general scenarios: (left column) oocyte acquisition of substances is related to follicle's own production of these substances or to acquisition of consistently supplied maternal resources; (central column): oocyte acquisition is related to age- or time-specific exposure or age-related production; (right column): oocyte acquisition is related to passive, non-age- or time-specific exposure to substances. Symbols in the upper right corner show scenarios most consistent with empirical data reported in this study: T = accumulation or partitioning of testosterone by male (♂) or female (♀) oocytes; C, E = accumulation or partitioning carotenoids (C) and vitamin E (E).

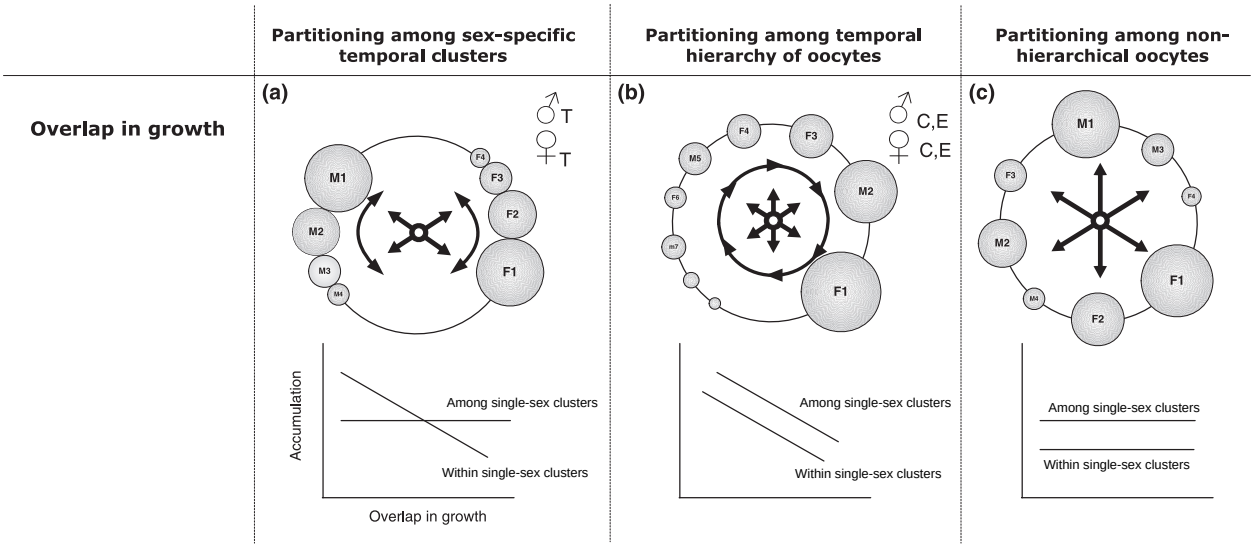

Fig. 2 Conceptual illustration of the relationship between oocyte accumulation of maternal resources in relation to overlap with oocytes of the same and opposite sexes. Products are delivered centrally to the ovary through female's vitelline artery (central black circle) and distributed to each follicle (black arrows and circles) (a,b) in a proportion to its maturation stage or (c) directly to individual oocytes. (a) Temporal or spatial sex-specific clusters of oocytes result in greater correlation between accumulation of resources and overlap within single-sex clusters compared with among single-sex clusters. (b) Lack of single-sex clusters, but sequential hierarchical arrangement of oocytes results in similar relationships between oocyte accumulation of resources and oocyte overlap within and among single-sex clusters. (c) Lack of temporal and spatial clustering of follicles, including those of different sexes (e.g. oocytes at the similar maturation stage are distributed throughout the ovary), of follicle-specific delivery of resources (as shown by individual arrows) results in the lack of relationship between accumulation of resources and oocyte overlap both within and among single-sex clusters. The effect of oocyte overlap on trade-off in accumulation is expected to be stronger for distantly (e.g. carotenoids and vitamins) vs. locally produced maternal products (e.g. follicular steroids). Symbols in the upper right corner show scenarios most consistent with empirical data reported in this study (see Fig. 1).

should produce a similar negative correlation between overlap in oocyte growth and accumulation of maternal resources and this correlation should not differ within and between single-sex clusters (Fig. 2b). Third, the lack of a hierarchical arrangement or follicle-specific delivery of resources would result in the absence of a relationship between oocyte growth overlap and accumulation of resources (Fig. 2c). Further, the acquisition trade-off should be greater for distantly (e.g. carotenoids) vs. locally (e.g. androgens) produced maternal products and should increase with overlap in follicle development.

Here, we take advantage of extensive variation in oocyte growth rate, duration and overlap among an ancestral and two recently established house finch (*Carpodacus mexicanus*) populations to examine the effect of this variation on sex-specific acquisition of hormones, carotenoids and vitamins. The three study populations show contrasting patterns of sex-biased ovulation order: in the ancestral population, females do not have sex-biased ovulation, except for facultative adjustment during mite-infestation season, whereas, in the two newly established populations at the north-western (NW Montana) and south-eastern (SE Alabama) boundaries of the species range, females have opposite sequences of ovulation of male and female eggs (Badyaev *et al.*, 2002, 2006b).

Here, we first show that oocyte growth dynamics have distinct effects on the accumulation of testosterone, carotenoids and vitamins, corroborating reports of temporal and spatial differences in the synthesis and transport of these maternal substances to growing oocytes. Second, we document that oocyte growth dynamics and overlap differentially affect the accumulation of maternal substances in oocytes that subsequently become males and females. Third, we report extensive population divergence in the effect of oocyte growth and overlap on sex-specific accumulation of maternal products. We discuss these results in the light of three unresolved phenomena in the evolution of maternal effects – (i) the evolution of sex-specific maternal allocation in species with simultaneously developing neonates of both sexes; (ii) the link between sex determination and sex-specific acquisition of maternal products; and (iii) the evolution of context-dependent modulation of maternal effects.

Materials and methods

Field methods

We studied house finches at three populations: in the ancestral population in south-western Arizona and in two recently established populations – at the northern part of the species' range in north-western Montana, where this species started breeding in the late 1970s, and at the south-eastern edge of their introduced range in Alabama, where house finches started breeding in 1983 (Badyaev & Hill, 2000). The study sites in Arizona (AZ), Montana (MT) and Alabama (AL) have been maintained since 2002, 1994 and 1993, respectively, and the data for this study were collected in 2004–2005. In all three study populations, resident birds were marked with a unique combination of four rings, and age category and prior breeding experience were known for the birds included in this study. The main focus of this study was an accurate assessment of oocyte growth in relation to accumulation of maternal substances and, thus, special care was taken to closely monitor egg laying and to collect eggs within 16–20 h after laying and incubation. This helped us to assure both the intact yolk lipids layers for measurements of oocyte growth (see below) and accurate measurements of yolk hormones, carotenoids and vitamins. At the time of nest building, thermocouples (iButton-TMEX; Maxim Integrated Products, Sunnyvale, CA, USA) were installed at nests to monitor the onset of incubation; all eggs were numbered sequentially on the morning of laying and were collected after being incubated for 16–20 h, replaced with a fresh house finch egg from a different nest or with a dummy egg, and stored at −20 °C (details of protocols in Badyaev *et al.*, 2006a). In all populations, we collected eggs from first breeding attempts and, in the AZ population, before nest mite infestation season.

Oocyte sex, growth and overlap

At the time of collection (i.e., after 16–20 h of incubation), embryos were 36–40 h old, enabling reliable separation of blastodisks from surrounding membranes under 12× magnification needed for molecular sexing of the embryo. Details of molecular analysis, PCR condition, yolk fixing and staining protocol and the description of the lipid accumulation method for calibrating and measuring oocyte development are described in Young & Badyaev (2004). Briefly, to assess daily lipid acquisition, we measured the distance from the center of the yolk to the outer boundary of each layer pair (one light and one dark layer equals 24 h of growth). Measurements were repeated three times at 120° rotations to each other and a mean was used in the analyses. Duration of oocyte growth, in hours, was the mean number of lipid layers multiplied by 12 h. Rate of oocyte growth was assessed from the fully grown oocytes as g^{-3} of yolk accumulation per hour of growth and broadly corresponds to the late growth constant reported in Young & Badyaev (2004). Cumulative (total hours × number of oocytes) overlap among oocytes from the same clutch was then calculated, for each sex separately, using the recorded time of egg laying and the duration of development of all oocytes in the clutch.

Hormonal, carotenoid and vitamin assays

Whole yolks were weighed, thawed and a yolk sample was added to 1 mL of ultrapure water. Steroids were

extracted by adding 3 mL of diethyl ether to the sample and vortexing for 30 s and left for 5 min. The samples were then placed in a bath of alcohol and dry ice to freeze the lipid portion and the ether portion was decanted into a fresh tube and evaporated in a warm bath at 30 °C. This process was repeated twice for each sample. We then added 1 mL of 100% ethanol to the dried extract and vortexed for 30 s. These samples were placed at −20 °C overnight before centrifuging for 5 min at 4 °C at 3400 × ***g***. The ethyl alcohol was decanted into a fresh tube and this was evaporated to dryness under vacuum at 50 °C. The dried extract was redissolved in 300 uL of EIA buffer. Yolk androgens were quantified using a commercially available enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI, USA), at 100%, 27.4% and 18.9% for testosterone, 5α-dihydo-testosterone and for 5β-dihydo-testosterone, respectively, with a lower detection limit of 6 pg mL^{-1}. The intra-assay variation, calculated as mean coefficient of variance of the duplicate samples, was 8.88. The inter-assay variation, calculated as the mean coefficient of variation from a pooled yolk sample that was analyzed in all three assays, was 3.01.

Yolk carotenoids and vitamins were extracted using high-performance liquid chromatography (HPLC, details of protocol in Badyaev *et al.*, 2006a). 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 10 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 for 5 min between extractions, and the combined organic phase was evaporated to dryness under vacuum at 40 °C and reconstituted in 300 uL of ethyl ether and 900 uL of HPLC mobile phase (methanol–acetonitrile–tetrahydrofuran, 50 : 45 : 5 v/v/v). Carotenoids and vitamins were quantified by injecting 50 uL of yolk extract into a HPLC (Shimadzu Corporation, Pleasanton, CA, USA) fitted with a NovaPak C18 column, 150 × 3.9 mm (Waters Corporation, Milford, MA, USA). 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). For growth measures, concentrations of all compounds were calculated as the total amount per entire yolk.

Statistical analyses

Within-sample amounts of lutein, β-cryptoxanthin, β-carotenes (*trans*- and several *cis*-compounds) and retinol (vitamin A) closely and linearly correlated with each other, and thus we constructed a linear principal component ('Carotenoids and vitamin A', footnote of Table 1) from a correlation matrix of these elements standardized to mean = 0 and SD = 1. Similarly, within-sample amounts of δ-, γ-, β- and α-tocopherols were highly intercorrelated and thus the standardized values of these compounds were converted to linear principal component ('Vitamin E'; footnote of Table 1). Raw data for each of the individual compounds are given in Online Appendices S1 and S2 in Badyaev *et al.* (2006a). To achieve normal distributions, values of all individual components were log-transformed with the exception of β-cryptoxanthin, β-carotene values which were arcsin transformed. Raw values of oocyte growth rate, duration

Table 1 Analysis of variance of yolk concentration of testosterone (*T*), carotenoids and vitamin A (*Carot*) and vitamin E (*VitE*) in relation to population (*Pop*), ovulation order (*OV*), growth overlap with male oocytes (*mover*), growth overlap with female oocytes (*fover*), growth duration (*Dur*), growth rate (*Rate*), other compounds (T, Carot and Vit E) as well as statistical interactions between population, growth and overlap parameters.

	Pop	OV	mover	fover	Dur	Rate	T	Carot	VitE	Pop × mover	Pop × fover	Pop × Dur	Pop × Rate
Males (n = 125)													
T[1]	**24.68**	**13.53**	**12.11**	0.06	1.74	**4.97**	–	5.24	3.25	**5.29**	0.28	0.08	**6.96**
Carot[2]	**6.77**	**14.94**	**6.11**	3.97	**10.24**	0.18	**13.22**	–	**8.22**	0.22	0.34	**5.01**	1.93
VitE[3]	**13.09**	1.15	0.04	4.57	**8.92**	2.82	2.40	**16.12**	–	0.57	0.52	3.23	0.24
Females (n = 67)													
T	**5.84**	**5.51**	0.25	**4.35**	**6.52**	0.09	–	2.10	3.20	0.93	**4.49**	**6.06**	0.03
Carot	**4.45**	3.74	**7.12**	**11.16**	**7.52**	2.28	**7.71**	–	**5.26**	0.98	**7.79**	0.02	0.27
Vit E	1.31	2.14	3.17	0.27	**5.82**	**4.14**	**6.62**	2.93	–	**10.79**	1.48	0.31	1.37

Shown are *F*-values, bold values indicate significance ($P < 0.05$). Analysis controls for nest identity.

[1]T combines testosterone and 5α-dihydrotestosterone.

[2]Carot is PC1 = 0.53 *Lutein* + 0.49 *β-Cryptoxanthin* + 0.49 *β-Carotene* + 0.52 *Retinol* (eigenvalue $\lambda = 2.59$, 67% of variation).

[3]VitE is PC1 = 0.61 *δ-tocopherol* + 0.20 *γ-tocopherol* + 0.45 *β-tocopherol* + 0.62 *α-tocopherol* ($\lambda = 2.25$, 57% variation).

and overlap with other follicles were log-transformed. We used type III sum of squares from general linear models with nest identity as a random effect to analyze the extent to which variation in androgens, carotenoids and vitamins in male and female oocytes is attributed to population, ovulation order, overlap with oocytes of the same and opposite sexes, growth duration and rate, as well as interactions between these factors. Multiple linear regressions were used to calculate slopes of relationships between growth components and residual levels of maternal resources across and within populations. Significance of the partial regression slopes was assessed with a *t*-test, and the slopes among the populations were compared with either non-parametric two-tailed Kruskal–Wallis tests or ANCOVA.

Results

Oocyte overlap and resource acquisition

Acquisition of testosterone in relation to overlap with the same and opposite sex differed between male and female oocytes (Fig. 3a,d; difference in slopes, same sex: $F_{1,117} = 4.15$, $P = 0.04$, opposite sex $F_{1,63} = 17.28$, $P < 0.001$; interaction: Sex × Overlap: $F = 15.37$, $P = 0.0002$). In males, accumulation of testosterone increased with greater overlap with male oocytes, but did not vary with overlap with female oocytes (Tables 1 and 2, Fig. 3a). In females, accumulation of testosterone decreased with greater overlap with female oocytes, but increased with greater overlap with male oocytes (Tables 1 and 2, Fig. 3d). Only MT and AL populations that have consistent sex-biased ovulation showed a significant effect of the interaction between oocyte sex and overlap on testosterone acquisition (Fig. 3d), and both populations differed from the patterns observed in the AZ population (Table 1, type III errors, interaction: Population × Sex × Overlap, males: $F_{6,119} = 3.18$, $P = 0.0002$; females: $F_{6,65} = 2.41$, $P = 0.001$).

Acquisition of carotenoids in relation to overlap of oocytes was similar between the sexes, although accumulation of carotenoids was more affected by overlap with oocytes of the same vs. opposite sex (Fig. 3b,e; Tables 1 and 2) and populations were similar in these patterns (interaction: Population × Sex × Overlap, males: $F_{6,118} = 1.16$, $P = 0.11$; females: $F_{6,63} = 0.42$, $P = 0.83$). Accumulation of vitamin E did not vary with growth overlap and sexes and populations were similar in these patterns (Fig. 3c,f; Tables 1 and 2; interaction: Population × Sex × Overlap, both sexes $F < 0.90$, $P > 0.53$).

Oocyte growth patterns and resource acquisition

In male oocytes, accumulation of testosterone did not vary with the duration of growth, but increased with growth rate (Fig. 4a, Tables 1 and 2). By contrast, in female oocytes, acquisition of testosterone increased with growth duration, but did not vary with growth rate (Fig. 4d, Tables 1 and 2). The accumulation of testosterone differed between the sexes for growth duration ($F_{1,117} = 10.07$, $P = 0.002$; Fig. 4a), but not for growth rate ($F_{1,117} = 1.65$, $P = 0.20$; Fig. 4d). The sex-specific effect of oocyte growth on acquisition of testosterone differed among populations (Fig. 4a,d; Table 1; Interaction; Population × Sex × Growth rate $F_{6,124} = 2.88$, $P < 0.001$; Population × Sex × Growth duration $F_{6,124} = 2.66$, $P = 0.002$). Overall, accumulation of carotenoids and vitamins increased with growth duration (Table 2, Fig. 4), and did not vary with growth rate in male oocytes, but increased with both growth rate and duration in females (Table 2, Fig. 4). Accumulation of carotenoids in oocytes of both sexes, but especially in males, was most closely linked to oocyte accumulation of testosterone (Table 1), whereas accumulation of vitamin E was most strongly related to oocyte-specific accumulation of carotenoids in male oocytes and with oocyte-specific accumulation of testosterone in female oocytes (Table 1). With the exception of the MT population, the relationship between oocyte growth and carotenoid and vitamin E accumulation was similar among the populations (carotenoids and vitamin A: Population × Sex × Growth rate $F_{7,124} = 1.35$, $P = 0.24$; Population × Sex × Growth duration $F_{7,124} = 0.75$, $P = 0.63$. Vitamins E: Population × Sex × Growth rate $F_{7,124} = 1.54$, $P = 0.15$; Population × Sex × Growth duration $F_{7,124} = 0.92$, $P = 0.49$). In MT, female oocytes with a faster growth rate accumulated less vitamin E (Fig. 4e,f), possibly as a consequence of the unusually rapid growth of female oocytes in some ovulation positions in this population (see below).

Discussion

In species that produce broods of offspring with distinct growth requirements, such as in mixed sex broods, simultaneous development of offspring favors the evolution of mechanisms that enable sex-specific parental manipulations of offspring growth, resource partitioning among offspring and maintenance of sex-specific development of male and female offspring (Uller, 2006; Carere & Balthazart, 2007). One such mechanism is the temporal or spatial 'clustering' of male and female offspring as found in the spatial segregation of male and female embryos in the mammalian uterus (Clark & Galef, 1990; Ryan & Vandenbergh, 2002) the sex-specific ovulation order of large avian raptors (Bortolotti, 1986; Byholm *et al.*, 2002) and seasonal and life-history changes in sex ratios of avian broods (Dijkstra *et al.*, 1990; Cordero *et al.*, 2001; Andersson *et al.*, 2003). In many birds and reptiles, the conflict between simultaneous development of male and female eggs and sex-specific maternal allocation to offspring seems particularly difficult to resolve not only because female reproduction and sex-specific

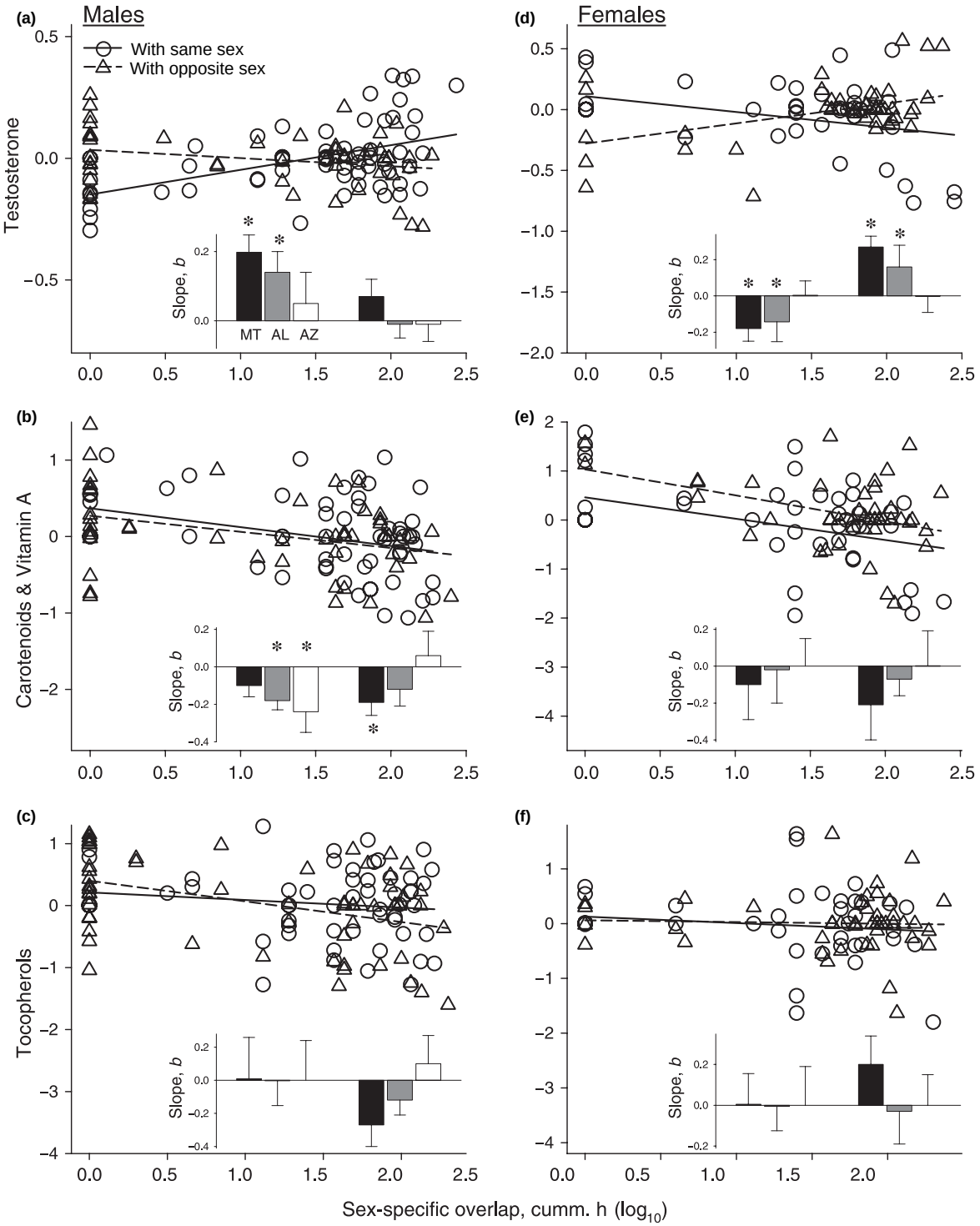

Fig. 3 Effects of growth and overlap between oocytes of the same and opposite sexes on whole yolk accumulation of (a) testosterone, (b) carotenoids and vitamin A and (c) tocopherols (vitamin E) in male oocytes and (d–f) these substances in female oocytes. Lines show partial regressions on residuals controlling for population, nest identity, ovulation order, all other growth parameters and other oocyte substances (Table 1). Circles and solid lines indicate growth overlap with same sex oocytes; triangles and dashed lines indicate growth overlap with oocytes of the opposite sex. Bars (mean ± SEM) show population-specific (MT, AL and AZ) slope of the partial regression for growth overlap with oocytes of the same sex (first three bars) and the opposite sex (last three bars). Asterisks indicate that population-specific regression slope is significantly different from zero.

Table 2 Relationship between concentration of testosterone, carotenoids and vitamin A, and vitamin E and oocyte overlap during growth with oocytes of the same vs. opposite sex (mover – overlap with males, fover – overlap with females), as well as growth duration (Dur) and rate (Rate).

	Testosterone			Carotenoids and vitamin A			Vitamin E		
	$b \pm SE$	b_{ST}	t	$b \pm SE$	b_{ST}	t	$b \pm SE$	b_{ST}	t
Males									
mover	0.10 ± 0.02	0.49	**4.37**	−0.24 ± 0.09	−0.35	**−2.74**	−0.12 ± 0.12	−0.14	−1.06
fover	−0.03 ± 0.18	−0.25	−1.86	−0.21 ± 0.08	−0.35	**−2.68**	−0.33 ± 0.10	−0.41	**−3.49**
Dur	0.26 ± 0.13	0.24	1.93	3.00 ± 0.56	0.56	**5.41**	1.83 ± 0.61	0.36	**2.98**
Rate	0.65 ± 0.12	0.57	**5.42**	0.73 ± 0.58	0.17	1.25	1.23 ± 0.52	0.30	2.38
Females									
mover	0.16 ± 0.04	0.45	**3.42**	−0.53 ± 0.18	−0.41	**−2.94**	−0.03 ± 0.12	−0.04	−0.26
fover	−0.13 ± 0.04	−0.39	**−2.88**	−0.43 ± 0.13	−0.43	**−3.28**	−0.11 ± 0.11	−0.16	0.29
Dur	1.12 ± 0.18	0.66	**6.12**	2.61 ± 0.86	0.40	**3.02**	2.65 ± 0.92	0.38	**2.88**
Rate	−0.01 ± 0.20	−0.02	−0.09	2.59 ± 0.68	0.49	**3.80**	2.15 ± 0.61	0.47	**3.56**

Shown are partial multiple regression coefficients ± SEM, standardized regression coefficients (b_{ST}) and associated t-statistics. Bold t-values indicate significance ($P < 0.05$).

development of offspring are often regulated by the same hormones (Chaudhuri & Maiti, 1998; Sockman & Schwabl, 1999), but also because the allocation of resource into oocytes is thought to precede sex determination (Harper, 1904; Warren & Scott, 1935; Olsen, 1942). A potential resolution of this conflict – the temporal ordering of oocytes that become male and female – is proximately enabled by three interconnected mechanisms: growth differences among oocytes, associated differences in oocyte acquisition of maternal products and oocyte sex determination. The extent and directionality of causation among these processes is a debated issue (Alonso-Alvarez, 2006; Badyaev *et al.*, 2006a; Radder, 2007; see also Janzen & Phillips, 2006), but, importantly, the variation in time and rate of oocyte growth and associated accumulation of maternal resources is a crucial, but unstudied, link among these processes.

Our study of sex-specific oocyte acquisition of maternal products in relation to oocyte growth and temporal overlap with other oocytes produced three principal findings. First, patterns of testosterone accumulation were most consistent with the prediction of time- or stage-specific production (Fig. 1a,b, center), whereas the accumulation of carotenoids and vitamins was most consistent with the predictions of oocyte-specific growth patterns (Fig. 1a,b, left) and closely associated with accumulation of testosterone by oocytes when growth dynamics was controlled statistically (Table 1). Second, the accumulation of testosterone in relation to oocyte overlap depended on the sex of overlapping oocytes (Fig. 2a,d, Tables 1 and 2), such that several simultaneously developing oocytes that shared a steroid environment were also likely to become the same sex. By contrast, acquisition of carotenoids and vitamins in relation to oocyte overlap did not differ between the sexes and decreased with the number of simultaneously growing oocytes (Fig. 2b). These results corroborate previous findings that sex-biased growth can accomplish sex-specific acquisition of products by oocytes even when the timing of maternal production and allocation of steroids is similar across populations (Badyaev *et al.*, 2006a). These results are also consistent with a greater trade-off in the uptake of steroids and yolk among oocytes of the same vs. the opposite sex as is expected when male and female oocytes form distinct temporal clusters (Fig 2a, Badyaev *et al.*, 2006c). Third, the effect of growth and overlap dynamics on the acquisition of resources varied among the study populations (Table 1, Figs 3 and 4).

The observed patterns raise several questions. First, what are the mechanisms behind the association between sex-specific resource acquisition and sex determination of oocytes? Second, what are the proximate mechanisms behind the close coordination of oocyte-specific uptake of androgens, carotenoids and vitamins observed in this (Table 1) and other studies (Royle *et al.*, 2001; Badyaev *et al.*, 2006a; Groothuis *et al.*, 2006). And what are the mechanisms enabling rapid divergence in oocyte growth, resource acquisition and sex-biased ovulation observed among these populations?

A recent review of molecular and cytological aspects of avian meiosis II (Rutkowska & Badyaev, 2008) revealed several compensatory mechanisms necessary for the maintenance of unbiased segregation of sex chromosomes despite their pronounced differences in size, shape, alignment at the meiotic plate and microtubule attachment (e.g. Panov & Bulatova, 1972; Belterman & Deboer, 1984; Solari, 1993; Krasikova *et al.*, 2005). We suggested that epigenetic effects linked proximately to oocyte growth and accumulation of maternal products modify such compensatory mechanisms and enable context-dependent adjustment of the primary sex ratio (Rutkowska & Badyaev, 2008). In turn, close integration

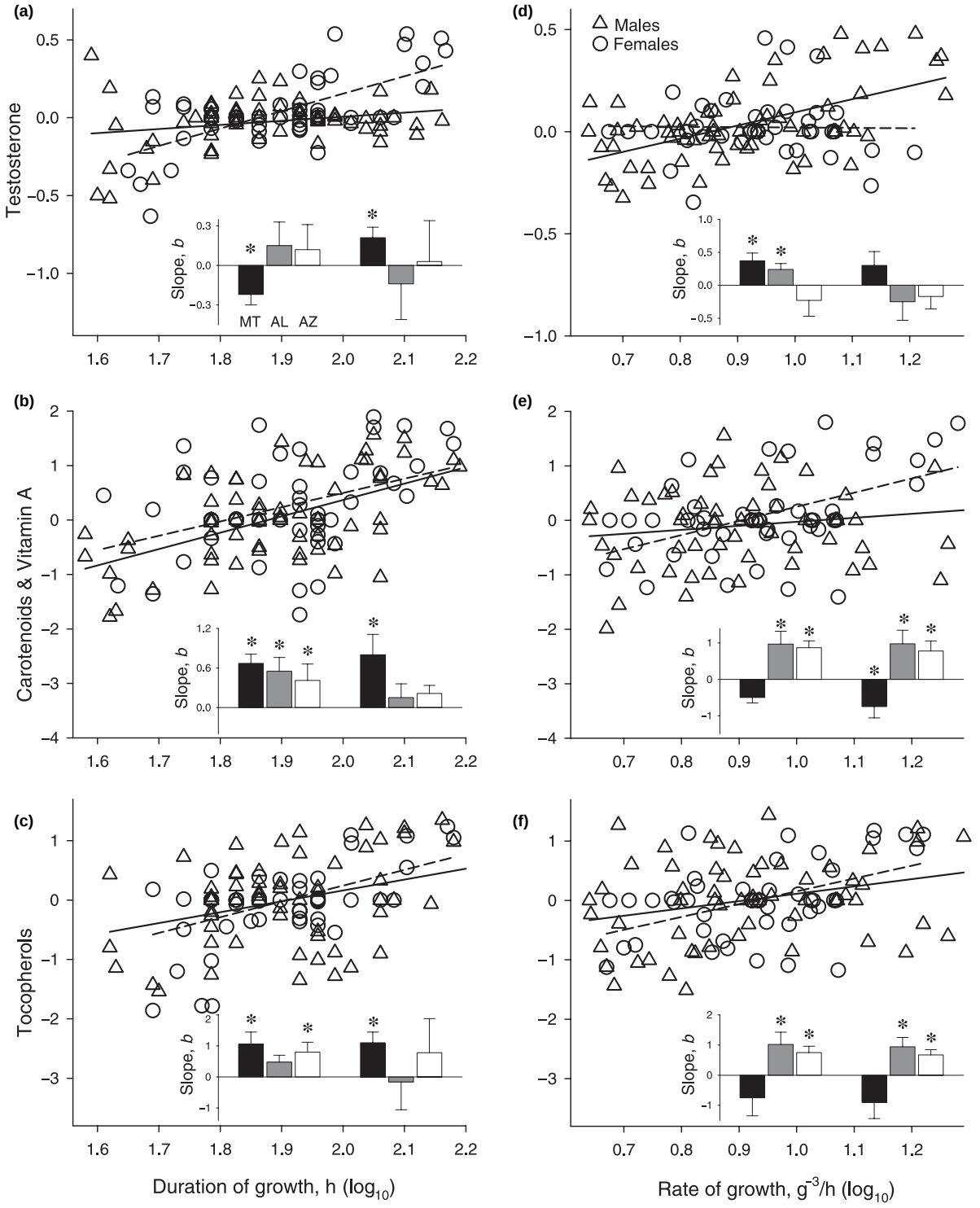

Fig. 4 Effect oocyte growth duration (a–c) and growth rate (d–f) on the whole yolk accumulation of (a,d) testosterone, (b,e) carotenoids and vitamin A and (c,f) tocopherols (vitamin E) in male (triangles and solid line) and female (circles and dashed line) oocytes. Lines show partial regressions on residuals controlling for population, nest identity, ovulation order, all other growth parameters and other oocyte substances (Table 1). Bars (mean ± SEM) show population-specific (MT, AL and AZ) slope of the partial regression for growth parameters for male oocytes (first three bars) and female oocytes (last three bars). Asterisks indicate that population-specific regression slope is significantly different from zero.

of epigenetic mechanisms influencing sex determination and the mechanisms enabling sex-specific accumulation of hormones should significantly facilitate both evolutionary retention of maternal adaptations (Badyaev, 2005) and evolution of sex-specific maternal effects (e.g. Legge *et al.*, 2001; Rutstein *et al.*, 2005). In this study, we found an association between oocyte sex and growth dynamics (see also Young & Badyaev, 2004) and raise three arguments in support of the proximate link between sex determination of oocytes and oocyte accumulation of maternal products in this species. First, oocytes sequestered at different times during oogenesis accumulated distinct concentrations of steroids, probably as a consequence (direct or indirect) of their growth in different hormonal milieus (Badyaev *et al.*, 2005). Second, temporal variability in recruiting groups of oocytes to the rapid yolk deposition stage (Young & Badyaev, 2004) and growth inhibiting interactions among maturing oocytes (Wang & Johnson, 1993; Chen & Johnson, 1996; Yang *et al.*, 2001) appear to be crucial for the formation of groups of oocytes of single sex under some breeding contexts in this species (Badyaev *et al.*, 2006c). Such single-sex temporal clusters can be induced by a shared involvement of the same hormone – pituitary prolactin – in both the regulation of maternal reproductive decisions in response to environmental cues (e.g. incubation in relation to ambient temperature) and the regulation of oocyte proliferation and maturation (Badyaev *et al.*, 2005; reviewed in Sockman *et al.*, 2006). Third, close integration of sex-specific allocation of hormones and sex determination has significant fitness consequences in this system; in the recently established populations, males produced in female-biased positions and females produced in male-biased positions accumulated hormones incompatible with their normal development and had reduced growth (Badyaev *et al.*, 2003, 2006a). Taken together, these observations suggest a proximate link between sex determination and accumulation of maternal products in this species. Overall, such integration can strongly facilitate evolutionary maintenance of adaptive maternal effects, making the examination of molecular and cellular mechanisms that accomplish such integration an important task for future studies.

Close association between oocyte acquisition of steroids, carotenoids and vitamins is one of the most ubiquitous patterns in studies of avian maternal effects. Such association is commonly interpreted as an active maternal strategy of greater antioxidant allocation to compensate for the fast embryo development facilitated by greater allocation of steroids (e.g. Groothuis *et al.*, 2005). However, at the proximate level, the association can be due to shared elements of production or delivery. For example, during oogenesis, the production of androgens is regulated by lipid-based enzymes that share precursors with lipoproteins involved in the delivery of carotenoids to the oocytes (e.g. Hertelendy & Asem, 1984). Similarly, the relationship between accumulation of steroids and carotenoids might be proximately regulated by oestrogen regulation of vitallogenesis and associated transport of carotenoids and vitamins to growing oocytes (e.g. Williams *et al.*, 2005). At a more general level, the link can be produced by shared lipid-based precursors of both oocyte membranes and hormone synthesis. Our results show that accumulation of carotenoids and vitamins is a product of both organism-wide processes of production and delivery (as evidenced by the dependency of accumulation on oocyte growth duration and a trade-off among simultaneously growing oocytes, Fig. 4) and oocyte-specific acquisition in relation to steroid uptake (Table 1), pointing to shared elements of passive uptake by the oocyte membrane as a likely proximate mechanism.

What are the mechanisms that enable divergence in oocyte growth, resource acquisition and sex-biased ovulation that are observed among the ancestral and recently established populations? In particular, the effect of oocyte growth on acquisition of androgens varies widely between the populations that show distinct sex bias in ovulation order (e.g. Fig. 4a,d). Two phenomena might account for such rapid adaptive divergence. First, sex-biased ovulation in this species is accompanied by a greater within-sex similarity in the uptake of steroids and lipids – a pattern that is expected when the timing of oocyte sequestration and their subsequent sex determination are linked (see above). Second, such temporal clustering can be environmentally induced within a generation (Badyaev & Oh, 2008), potentially through shared hormonal regulation of environmental assessment, modification and reproduction (Graham & Desjardins, 1980; Pfaff *et al.*, 2004; Sockman *et al.*, 2006; Ball & Balthazart, 2008). Finally, across populations of this species, sex-biased ovulation sequence and sex-specific allocation of maternal resources seem to be produced by rearrangement of the same proximate mechanism – temporal clustering of male and female oocytes (Badyaev & Oh, 2008). Such reuse of the same mechanism for context-specific inputs, through a shared hormonal coordination of female reproduction and maternal effects, might enable rapid population divergence in oocyte growth and corresponding accumulation of maternal products.

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