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Evolution of sex-biased maternal effects in birds: I. Sex-specific resource allocation among simultaneously growing oocytes

R. L. YOUNG & A. V. BADYAEV

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA

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Abstract

Females in species that produce broods of multiple offspring need to partition resources among simultaneously growing ova, embryos or neonates. In birds, the duration of growth of a single egg exceeds the ovulation interval, and when maternal resources are limited, a temporal overlap among several developing follicles in the ovary might result in a trade-off of resources among them. We studied growth of oocytes in relation to their future ovulation order, sex, and overlap with other oocytes in a population of house finches (*Carpodacus mexicanus*) where strongly sex-biased maternal effects are favoured by natural selection. We found pronounced differences in growth patterns between oocytes that produced males and females. Male oocytes grew up to five times faster and reached their ovulation size earlier than female oocytes. Early onset and early termination of male oocytes' growth in relation to their ovulation resulted in their lesser temporal overlap with other growing ova compared with female oocytes. Consequently, ovulation mass of female but not male oocytes was strongly negatively affected by temporal overlap with other oocytes. In turn, mass of male oocytes was mostly affected by the order of ovulation and by maternal incubation strategy. These results provide a mechanism for sex-biased allocation of maternal resources during egg formation and provide insights into the timing of the sex-determining meiotic division in relation to ovulation in this species.

Introduction

In species that produce broods of multiple offspring, parents need to partition resources among simultaneously growing ova, embryos or neonates. When parental resources are limited, natural selection might favour biased allocation of resources to offspring with the greatest fitness value for the parents (Williams, 1966; Stearns, 1976) and strategies that minimize resource expenditure without sacrificing offspring number and quality (Hillström, 1999; Eising *et al.*, 2001; Williams, 2001; Heath *et al.*, 2003). Moreover, offspring often require different resources when they vary in the cost of growth (Trivers & Willard, 1973; Stamps, 1990; Leimar, 1996), or need different parental products, e.g. sex-specific hormones, for normal development (Adkins-Regan *et al.*, 1995; Schwabl *et al.*, 1997; Bowden *et al.*, 2001).

Whereas many studies have documented differential allocation of nutrients, hormones, antibodies, and parental care among offspring within a brood (Schwabl, 1993; Anderson *et al.*, 1997; Lipar & Ketterson, 2000; Eising *et al.*, 2001; Petrie *et al.*, 2001; Saino *et al.*, 2003), very little is known about mechanisms behind such biased allocation. For example, does differential allocation of nutrients and hormones among eggs of a single clutch represent a passive consequence of maternal physiology at the time of breeding (Mead & Morton, 1985; Conley, 1997; Müller *et al.*, 2002; Whittingham & Schwabl, 2002) or an active and flexible maternal strategy to influence offspring phenotype (Müller *et al.*, 2002, 2003; Lovern & Wade, 2003)? Similarly, is the frequent bias in offspring sex ratio a passive consequence of maternal reproductive constraint or is it a manifestation of maternal ability to adaptively modify the sex of

Correspondence: Alexander V. Badyaev, Ecology and Evolutionary Biology, BSW, PO Box 210088, 1041 E. Lowell, University of Arizona, Tucson, AZ 85721-0088, USA.
Tel.: +1-520-626-8830; fax: +1-520-621-9190; e-mail: abadyaev@email.arizona.edu

progeny (Dijkstra *et al.*, 1990; Ellegren *et al.*, 1996; Roosenburg, 1996; Krebs *et al.*, 2002; Badyaev *et al.*, 2003a; Lovern & Wade, 2003)? Crucial to answering these questions is knowledge of the origin and mechanisms of biased parental allocation.

In bird species that lay clutches of several eggs, simultaneous development of offspring begins when several yolk-filled follicles enter the period of rapid yolk deposition 4–10 days before ovulation (Sturkie, 1986). During this phase, lipid-rich yolk precursors produced in the mother's liver are delivered to the membrane of developing follicles via blood, and deposited in concentric layers throughout the day (Redshaw & Follet, 1976; Astheimer & Grau, 1990; Barber *et al.*, 1991; Meathrel, 1991). The growth period of each oocyte is greater than the ovulation interval (20–30 h in most passerines, Burley & Vadehra, 1989; Zann, 1996), and multiple oocytes develop in the ovary simultaneously. The growth of oocytes is energetically expensive (Monaghan *et al.*, 1998; Nager *et al.*, 2001; Visser & Lessells, 2001; Williams, 2001; Vezina & Williams, 2002) and overlap in their development increases the demand for maternal resources (Challenger *et al.*, 2001). Therefore, selection should favour the evolution of maternal strategies that modify resource allocation among several developing ova without sacrificing offspring number and quality (Astheimer & Grau, 1990). Moreover, when offspring of different sex have different growth costs or requirements, selection should favour a sex-biased resolution of the trade-off between overlap in offspring development and parental resources (Dijkstra *et al.*, 1990; Schwabl *et al.*, 1997; Sasvári *et al.*, 1999; Anderson *et al.*, 2003).

Here, we document a sex-specific pattern of growth of preovulation oocytes in house finch (*Carpodacus mexicanus*) that resolves the trade-off between the number of simultaneously maturing ova and their temporal overlap in growth. In a recently established population of this species in Montana in north-western United States, maternal modifications of both ovulation order and growth of sons and daughters facilitated population establishment and persistence (Badyaev *et al.*, 2002). In this, and another recently established population in Alabama in south-eastern United States, the adaptive modifications of ovulation order of sons and daughters was closely associated with environmentally induced onset of incubation (Badyaev *et al.*, 2003b). The precision of sex-specific maternal effects in the house finch and their close concordance with the environment of breeding suggest a remarkable degree of maternal control over development of males and females. Here, we examine allocation of maternal resources in the earliest stages of male and female growth. We first show that the patterns of growth of male and female oocytes are highly distinct. We then document that the different growth of male and female oocytes leads to their differences in overlap with other oocytes and this provides a mechanism that enables simultaneous production of multiple offspring that differ in fitness value to parents or have different requirements for growth. We discuss the implications of these findings for understanding of the evolution of adaptive maternal effects as well as the offspring sex determination in this species.

Materials and methods

Field methods

We studied house finches in a resident population in Missoula, north-western Montana, USA. The study site has been maintained since 1995 [see Badyaev & Martin (2000) for detailed description]. All resident adults were trapped and marked with a unique combination of one aluminium and three coloured plastic rings (Badyaev *et al.*, 2003a). Data for this study were collected in February–August 2002–2003. In this population, onset of incubation is closely associated with sex-biased egg laying order (Badyaev *et al.*, 2003b). Thus we determined the onset of full incubation by daily monitoring of female presence on the nest during egg-laying and by inferring incubation patterns from thermocouples (iButton-TMEX, Dallas Semiconductor, Sunnydale, CA, USA and HOBO ProSeries; Onset Computer Corporation, Bourne, MA, USA) which were installed in each nest at the time of nest-building and were set to record egg temperature every 5 min (Badyaev *et al.*, 2003a). All females laid one egg per day between 06:30 and 11:00 until the clutch was complete and eggs were numbered sequentially on the day of laying. In nests where incubation began with the first egg, the first egg was collected 18–20 h after laying and replaced with a freshly laid house finch egg from a different nest as a part of concurrent experiment or with a dummy egg. All the subsequent eggs in these nests with full incubation were removed 18–22 h after laying and replaced with other eggs. After the female laid her last egg (fourth or fifth), the dummy eggs were either removed to enable rapid renesting or foster eggs were allowed to develop as a part of the egg-exchange experiment. In nests where incubation started with the last or penultimate egg, all eggs were removed simultaneously 18–20 h after the last egg was laid. Immediately after collection, eggs were photographed, measured and stored at −20 °C until further analysis. For this study we used data on onset of incubation, embryo sex, ovulation order and yolk data for 22 clutches containing 96 eggs.

Measurement of follicle growth

During the rapid yolk deposition phase, lipids are deposited in the growing oocyte throughout the day, however, the type and density of lipids deposited differs between daytime feeding (high density lipids) and nighttime inanition (low density) (Grau, 1976; Astheimer *et al.*, 1985). The difference in lipid density creates distinct layers in the deposited yolk, each layer corresponding

to approximately 12 h of lipid deposition. Yolks of freshly laid frozen eggs were fixed and stained following protocol in Grau (1976). First, the shell and albumen were removed and intact frozen yolks were weighed to an accuracy of 1 mg on a Mettler Toledo Precision balance. A 1–2 mm slice was taken from the centre of the yolk. While still frozen, yolk slices were placed in 1.5 ml of 4% formalin and incubated in a 60–65 °C water bath for 12 h. Yolk slices were then rinsed and placed in 1.5 ml of 6% potassium dichromate and further incubated in a 60–65 °C water bath for 12 h. After staining, yolk slices were rinsed and sliced in half revealing light layers (high density lipids) and dark layers (low density lipids; Fig. 1). Yolk slices were photographed under 10× magnification and 7.2 megapixel digital images were analysed with SigmaScan software (SPSS, Inc., Chicago, IL, USA).

Duration of development was established by counting and measuring light and dark lipid layers. We used daily lipid acquisition to calculate the duration and rate of oocyte development. To measure daily lipid acquisition, we measured a distance from the centre of the yolk to the outer boundary of each layer pair (one light and one dark layer equalling 24 h of growth). Measurements were repeated three times at equally spaced rotations to each other (Fig. 1) and a mean was used in the analyses. We calculated cumulative (total hours × number of eggs) overlap among oocytes from the same clutch using a recorded time of each egg laying, and the duration of development of all eggs in the clutch. Because all eggs in a clutch were laid at similar times each morning (see above), we assumed that the duration of oviduct passage after ovulation is similar for all eggs within a clutch.

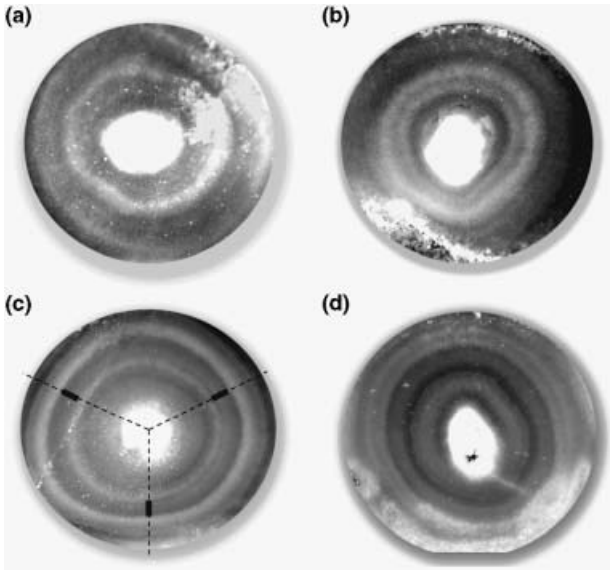

Fig. 1 Layers of high (dark) and low (light) density lipids in yolk of (a, b) male and (c, d) female house finch ova. Each pair of layers corresponds to 24 h of growth. Dashed lines in (c) indicate directions of three repeated measurement of the 12 h growth gains, which are shown in thick lines (layer widths). Length of thick line is ~0.25 mm.

The sigmoid Gompertz curve best fitted the longitudinal data on the oocyte growth:

$$W_t = W_0\, e^{(K_1/K_2)(1-e^{-K2t})}$$

where W_t is the measurement at time t, W_0 is the estimated value at the onset of the rapid yolk deposition phase, K_1 the initial growth rate constant (first part of a growth curve before the inflection) and K_2 the maturation rate of the exponential rate of decay of the growth rate constant (the part of curve in which growth rate decreases until the asymptotic value is reached). The time (T_{max}, in hours) and size of oocyte at the point of inflection, where the oocyte growth is maximal (W_{max}) was calculated as:

$$T_{max} = \frac{1}{K_2}\ln\left|\frac{K_1}{K_2}\right|$$

$$W_{max} = W_0\, e^{(K_1/K_2-1)}$$

The fitted curve was accepted when absolute difference between sum of squares of the residuals of successive iterations was $<10^{-4}$, and the estimated nonlinear regression function provided the highest R^2 (SAS Institute, Cary, NC, USA, 1989). Parameters were estimated separately for each follicle using the Marquardt algorithm of PROC NLIN of SAS 8.2 (SAS Institute) that minimized the sum of squares between predicted and observed values of growth (for details of growth measurements see Badyaev *et al.*, 2001b).

Determining follicle sex

Upon thawing, blastodisc and early embryo cells were carefully separated from the surrounding tissues under 12× magnification using a Leica MZ 12.5 stereo photomicroscope and stored at −80 °C until processing. DNA was extracted using standard phenol-chloroform methods. The sex of embryos was determined by a molecular sexing technique that amplifies an intron of the CHD1 genes on the sex chromosomes of birds (Griffiths *et al.*, 1996, 1998). We used PCR primers P8 and P2 which anneal to conserved exonic regions and amplify across an intron in both CHD1-W and CHD1-Z genes (Griffiths *et al.*, 1996, 1998). PCR was carried out in a total volume of 25 μL with the following final reaction conditions: 1.5 mM $MgCl_2$, 200 mM of each dNTP, 200 ng of each primer, 0.5 U of Taq polymerase and 50–200 ng of genomic DNA. PCR amplifications were performed under the following thermal cycling conditions: an initial denaturing step at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 50 °C for 45 s and 70 °C for 30 s. The program concluded with a final cycle of 48 °C for 1 min

and 72 °C for 5 min. PCR products were electrophoresed for 90–120 min at 10 V cm^{-1} (70 V) in a 2% NuSieve 3 : 1 agarose gel stained with ethidium bromide. PCR products were visualized under UV light and scored one band as male and two bands as female. To assure accurate assignment of the sex and the lack of contamination from maternal and paternal tissues (Arnold *et al.*, 2003), the DNA extraction and PCR was carried out twice for 27 of 96 samples.

Statistical analysis

House finches typically lay clutches of either four or five eggs and clutches of more than five and less than four eggs were excluded from this study. To include both of these clutch sizes in the analysis, we recorded egg-laying sequence as the 'first', 'second', 'third' and the 'last' (the fourth or fifth) egg.

Depending on the analysis, the onset of incubation was entered as either a qualitative variable (i.e. 'from the first egg' or 'from the last egg'; Figs 2 and 3) or as the proportion of the total number of eggs that had been laid into the clutch when incubation started (Tables 1 and 2, Figs 5 and 6). Sex differences in growth parameters and overlap with other oocytes were compared with nonparametric two-tailed Kruskal–Wallis tests. The effects of sex, laying order, onset of incubation, and the interactions among these factors on duration of growth and overlap with other oocytes were evaluated with type III error general linear models.

We used path coefficients to quantify the relative strength of each proposed effect, to access the overall fit of models, and to test specific predictions of relationships among proposed factors. Path coefficients, overall fit of the model, and Akaikes's information criterion (AIC) coefficients were estimated using the covariance analysis of linear structural equations (CALIS) of SAS software (SAS Institute). Overall fit of the models (distributed as χ^2) were tested with likelihood ratio tests for the concordance between the elements in the covariance matrix computed from data and the matrix predicted by the path coefficients. Nonsignificant χ^2 values indicate

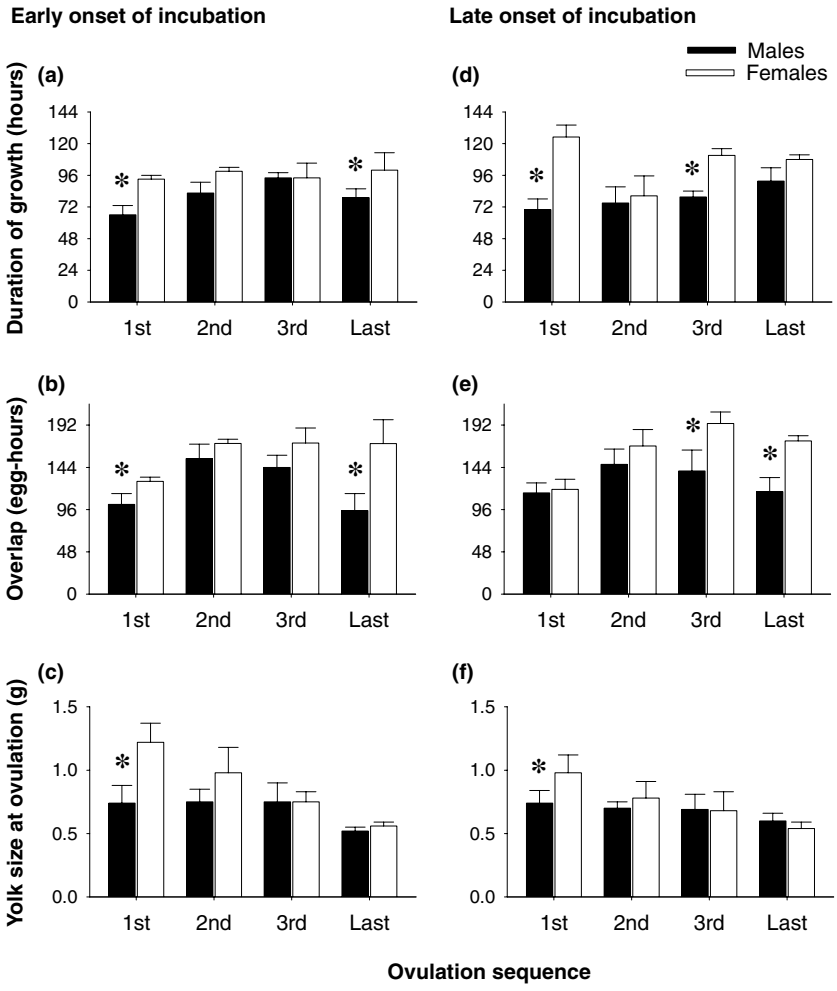

Fig. 2 Duration of growth (a), overlap with other oocytes (b) and mass at ovulation (c) in male and female oocytes in the clutches where incubation started with the first egg and the same parameters (d–f) in the nests where incubation started with the last egg. $*P < 0.05$, $**P < 0.01$ for the Kruskal–Wallis test of significant differences between the sexes. For other tests see Table 1.

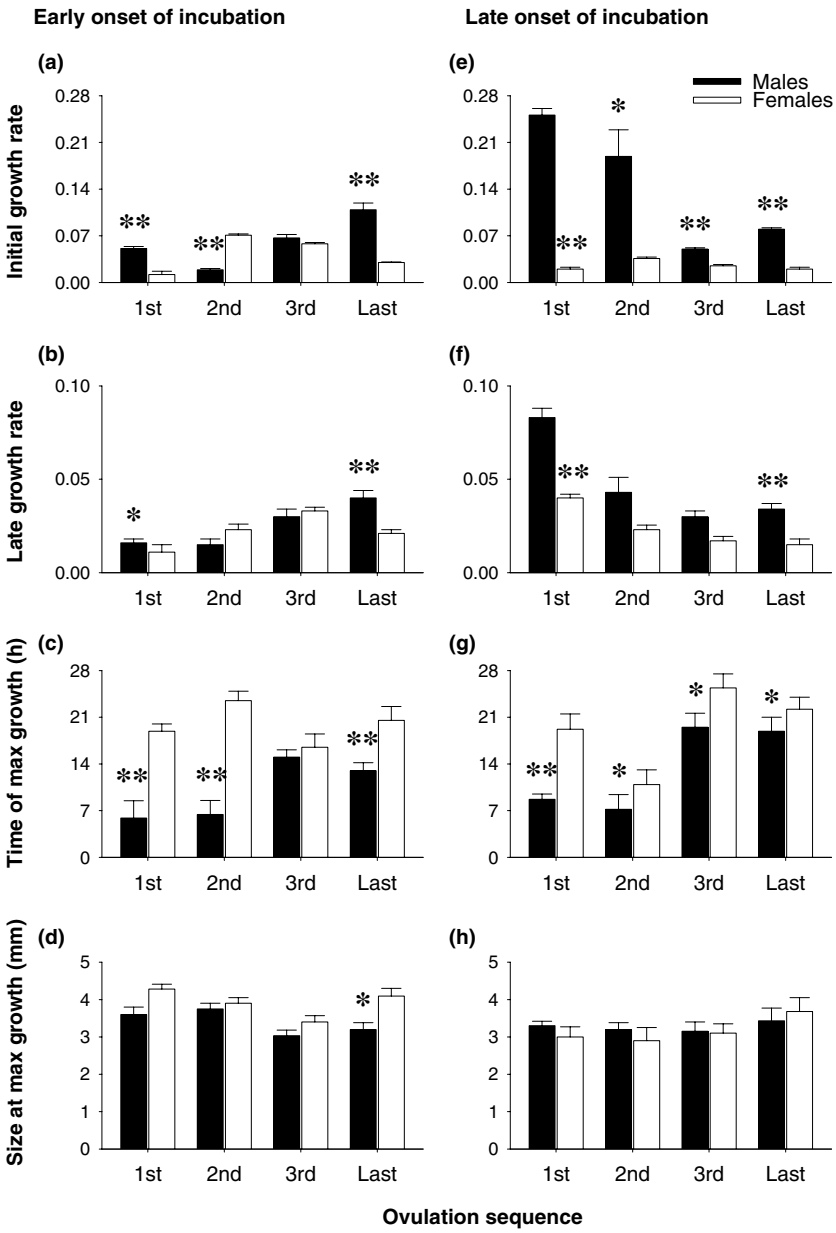

Fig. 3 Growth of male and female oocytes in the house finch. Shown are initial growth rate (a), late growth rate (b), age of oocyte at the time of maximum growth (c), size of oocyte at the time of maximum growth (d) in the nests where incubation started with the first egg and the same parameters (e–h) in the nests where incubation started with the last egg. $*P < 0.05$, $**P < 0.01$ for the Kruskal–Wallis test of significant differences between the sexes. For other tests see Table 2.

that the model predicts a pattern of correlation among the variables that does not differ from the observed correlation and, therefore, constitutes a 'good fit' of data to the model (SAS Institute). The distribution of the differences between χ^2 values has a χ^2 distribution and thus allows for direct comparisons among the models. We examined residual plots and residual statistics to ensure validity of normality and variance homogeneity assumptions in regression analyses. The sequential Bonferroni tests were used to evaluate individual coefficient significance in ANCOVA and multiple regressions. The total effects on mass of oocyte were calculated from the path diagram by combining the direct and indirect effects and excluding the effects of unmeasured variables.

Results

Growth and overlap among oocytes

Duration of growth and temporal overlap with other oocytes differed between sexes, and to a lesser extent, among oocytes of different ovulation order (Table 1; Fig. 2). Oocytes producing males grew for a shorter period and overlapped with other oocytes less than oocytes producing females, especially in the first and last ovulation orders (Fig. 2a–e). Male and female oocytes were similar in size at ovulation (with the exception of the first ovulation order where males were smaller,

Table 1 Duration of oocyte growth and overlap among oocytes in relation to the future sex of the oocyte (sex), order of ovulation (order), the onset of full incubation (onset), and the interactions among these factors in female house finches. Shown are *F*-values.

	Source of variation						
Trait	Sex	Order	Onset	Sex × order	Order × onset	Sex × onset	Sex × order × onset
Duration of growth	**18.66****	**4.72***	0.09	0.82	3.04	0.53	1.06
Overlap with other eggs	**19.94****	**4.61***	0.21	4.11	0.72	0.03	0.63

Bold values indicate significance after the within-model Bonferonni adjustment ($n = 7$). *$P < 0.05$, **$P < 0.01$.

Table 2 Estimated parameters of oocyte growth in relation to temporal overlap with other oocytes (over), duration of growth (duration), order of ovulation (order), future sex of the oocyte (sex), onset of full incubation (onset) and the interactions among these factors in female house finches. Shown are *F*-values.

	Sources of variation										
Trait	Over	Duration	Order	Sex	Onset	Over × sex	Over × order	Over × onset	Duration × sex	Duration × order	Duration × onset
W_0	0.38	3.92	**8.21****	**9.45****	1.13	**5.58****	4.37*	**7.96****	0.68	**7.74****	2.16
K_1	3.55	7.30*	**6.59****	**9.95****	**18.37****	**12.26****	**9.76****	2.00	**17.62****	2.15	**25.69****
K_2	4.59*	9.92*	**17.00****	1.19	**44.36****	6.62*	**15.56****	2.41	**12.26****	3.76	**51.35****
T_{max}	1.25	4.25	7.73*	**18.95****	2.13	3.47	9.57*	**21.57****	1.74	7.78*	**16.54****
W_{max}	2.47	1.47	0.57	1.47	1.87	**20.25****	5.24*	**17.54****	**18.54****	**10.28***	**15.24****

Bold values indicate significance after the within-model Bonferonni adjustment ($n = 11$). *$P < 0.05$, **$P < 0.01$.

Fig. 2c,f) and during the period of maximum growth (Fig. 3e,h; Table 2).

The sigmoid Gompertz curve best described the longitudinal data on ova growth, and male and female oocytes differed strongly in growth patterns (Table 2; Fig. 3). Male oocytes grew faster throughout the entire growth period, especially in nests with late onset of incubation (Table 2, Fig. 3a–f). The only exception was faster initial growth of female oocytes of the second ovulation order in the nests with early onset of incubation (Fig. 3a; Table 2). Male and female oocytes differed strongly in their temporal patterns of growth. Male oocytes accumulated most of their mass sooner than female oocytes, and growth of male, but not female oocytes was slower during the late part of the growth (Fig. 3c,g; Table 2).

Maternal strategies and oocyte growth

Effects of overlap with other oocytes and duration of rapid yolk deposition on the growth parameters were distinct between the sexes (Table 2, significant overlap × sex and duration × sex interactions; Fig. 4). Mass of female, but not male oocytes at ovulation was closely associated with temporal overlap with other oocytes (Fig. 4; simple regression: females, $b_{ST} = -0.56$, $t = -4.34$, $P < 0.01$; males, $b_{ST} = 0.18$, $t = 1.04$, $P = 0.11$). Growth of male and female oocytes was affected by different factors [full path model statistics – males (Fig. 5a): $\chi^2 = 1.44$, n.s., AIC = −8.52; females (Fig. 5a): $\chi^2 = 0.98$, n.s., AIC = −10.52]. Larger male oocytes grew faster at the early stages of development (early growth: $b_{ST} = 0.34$, $P < 0.05$, Fig. 5a), grew longer ($b_{ST} = 0.18$, $P = 0.06$; Figs 5a and 6), and were ovulated earlier in the sequence ($b_{ST} = -0.40$; $P < 0.01$). When other factors were considered simultaneously, mass of male oocytes was only weakly negatively affected by the overlap with other oocytes (path model, Fig. 5a: $b_{ST} = -0.19$, $P = 0.05$). In turn, the early growth rate of male oocytes was influenced by onset of incubation ($b_{ST} = 0.45$, $P < 0.01$) and duration of growth varied with the order of ovulation (Fig. 5a: $b_{ST} = 0.58$, $P < 0.01$).

When all factors were considered simultaneously, mass of female oocytes at ovulation was most strongly influenced by overlap with other oocytes (path model, Fig. 5a: $b_{ST} = -0.44$, $P < 0.05$, see also Fig. 4a) and by the duration and rate of growth ($b_{ST} = 0.58$, $P < 0.01$, and $b_{ST} = 0.30$, $P < 0.05$; Fig. 5b). In turn, duration of growth varied with the timing of incubation onset ($b_{ST} = -0.68$, $P < 0.01$; Fig. 5b). In males, the deletion of the direct causal path between overlap with other oocytes and yolk ovulation mass (but retaining the overlap variable in the model, Fig. 5a) did not result in significant changes in the model fit ($\chi^2_1 = 1.96$, $P = 0.08$). In females, the model without the direct path from the overlap to the yolk mass performed significantly worse than the full model ($\chi^2_1 = 5.02$, $P < 0.01$). Overall, mass of male oocytes was affected by maternal strategies (onset of incubation and order of ovulation) more (59.7%) and by the growth patterns of the oocytes (duration, overlap and rate) less (39.2%), than the mass of female oocytes (20.8 and 79.7%, respectively; Fig. 6).

Discussion

When several offspring are produced simultaneously, parents need to partition resources and care to enable

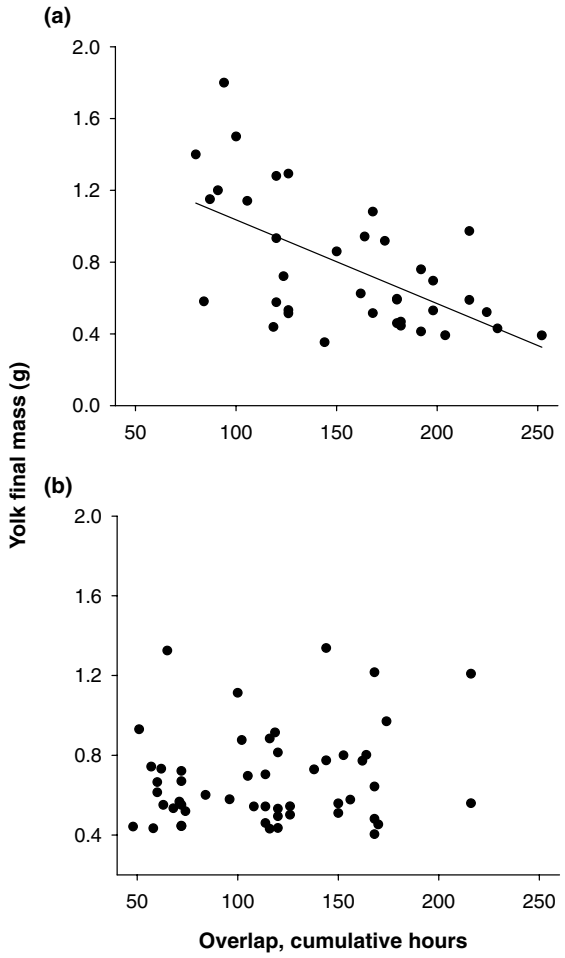

Fig. 4 Simple regression of overlap among simultaneously growing oocytes (cumulative egg-hours) on the mass of ovulating oocytes (g) in (a) females, and (b) males.

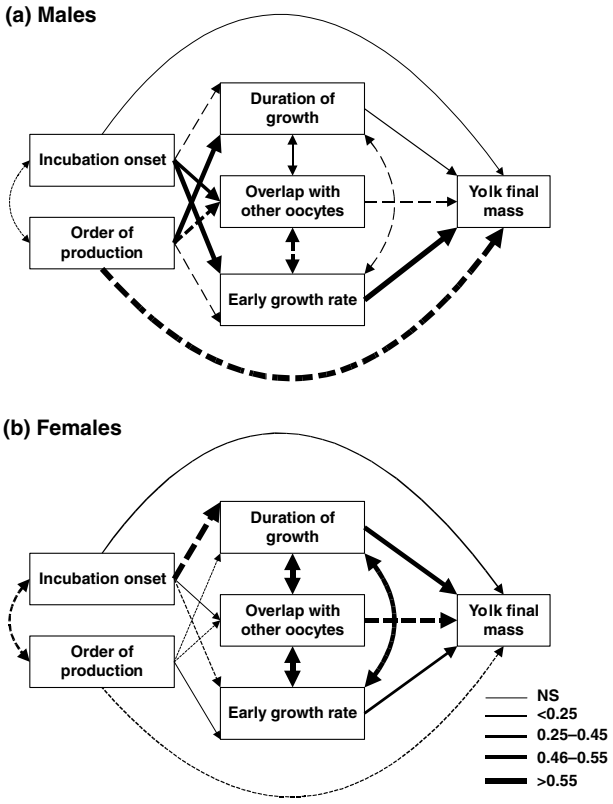

Fig. 5 Path diagram illustrating direct and indirect effects of maternal strategies (incubation onset, order of production) and oocyte growth patterns (duration, overlap with others, early growth rate) on the yolk mass during ovulation in (a) male and (b) female eggs. Double-headed arrows indicate covariation between two variables. Single-headed arrows show that change in the variable at the base of the arrow will cause a change in the one at the arrow's head. Positive effects are indicated by solid lines and negative effects by dashed lines. Thickness of arrows indicates the magnitude (in standard deviations) and the significance of the effect.

growth of concurrently developing offspring. One parental strategy to lower resource demand of multiple offspring is to reduce overlap in offspring developmental stages, e.g. by inducing growth asynchrony among siblings (Parsons, 1975; Schwabl, 1996; Royle & Hamer, 1998; Hillström, 1999; Eising *et al.*, 2001), or by spacing out the production of offspring with similar nutritional requirements, e.g. by modifying birth order of males and females within a clutch (Bortolotti, 1986; Meathrel, 1991; Krackow, 1995; Grau, 1996; Cordero *et al.*, 2001; Blanco *et al.*, 2002; Velando, 2002). Even when offspring are produced simultaneously and overlap in their developmental stages, parents can reduce the overlap in resource requirements by transferring growth-enhancing or growth-suppressing products to individual offspring (Schwabl *et al.*, 1997; Sasvári *et al.*, 1999; Lipar & Ketterson, 2000; Eising *et al.*, 2001; Parks, 2001).

Variable costs and benefits of raising sons and daughters among breeding environments lead to the evolution of sex-biased parental strategies that optimize parental reproductive investment in different ecological and social circumstances (e.g. Cordero *et al.*, 2001; Badyaev *et al.*, 2002; Blanco *et al.*, 2002; Krebs *et al.*, 2002; Anderson *et al.*, 2003, see also West & Sheldon, 2002). Consequently, sex-biased parental allocation of resources to offspring within a clutch is frequent and often most pronounced when the parental ability to acquire or store resources is constrained (Dijkstra *et al.*, 1990; Dzus *et al.*, 1996; Nager *et al.*, 1999; Krebs *et al.*, 2002; Velando *et al.*, 2002; Magrath *et al.*, 2003).

We examined the growth pattern and overlap in development among male and female oocytes in the house finch, a species where strong and precise sex-biased maternal effects on offspring growth are adaptive for both parental and offspring generations in at least two

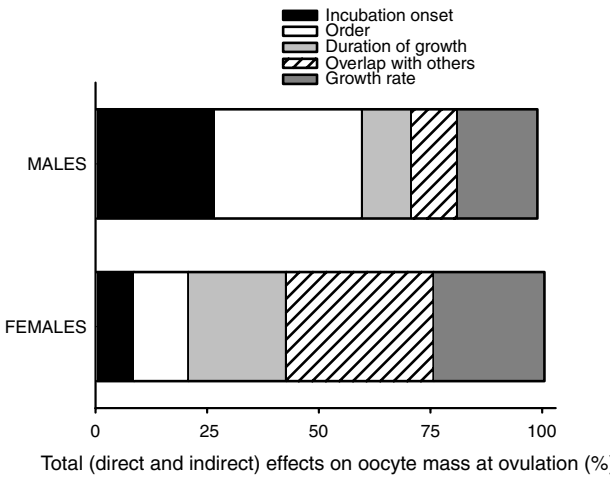

Fig. 6 Contribution of the total effects (direct and indirect) to the mass of male and female oocytes in the house finches. Shown are percentages of the total variation accounted by the full path models in Fig. 5.

populations (Badyaev *et al.*, 2002, 2003a). We found that male and female oocytes were generally similar in size at ovulation, but differed strongly in duration and rate of growth. Male oocytes grew faster and reached ovulation size earlier than female oocytes. Because of their shorter and faster growth, male oocytes overlapped less with other oocytes compared with female oocytes. Consequently, the size of male oocytes at ovulation was mostly influenced by the order of ovulation and growth rate (both are likely direct maternal effects, see below) and was largely unaffected by overlap with other ova during growth, whereas the size of longer-growing female oocytes was negatively affected by greater overlap with concurrently growing ova (Figs 4–6, Tables 1 and 2). To our knowledge, this is the first documentation of sex-specific growth patterns of prefertilization oocytes in birds.

The discovery that male and female ova grow differently from the onset of development, that they are affected by different factors (Figs 5 and 6), and that oocyte growth is variable throughout the preovulation period (Figs 2 and 3) raises several important questions. First, why should mothers modify growth of male and female oocytes? Secondly, how can mothers distinguish between male and female oocytes and affect their growth at such early stages? Finally, what are the implications of these findings for the often documented sex-biased maternal transfer of growth and immune factors to offspring?

Modification of growth of individual ova might be adaptive when it minimizes the overlap in maternal resource allocation among growing follicles. For example, mass of ovulating oocytes was negatively correlated with shared supply of yolk precursors in the European starling (*Sturnus vulgaris*) (Challenger *et al.*, 2001, see also Royle *et al.*, 1999). Similarly, allocation of maternal plasma carotenoids, which depends strongly on maternal diet, into individual follicles was affected by overlap in egg development in several species (Surai *et al.*, 1998; Bortolotti *et al.*, 2003; Koutsos *et al.*, 2003). On the contrary, when overlap among hierarchically maturing oocytes was small, as is the case in some shorebirds with large ovulation intervals, or when food supply was abundant, the growth rate of ova tended to be uniform both within and among clutches (Astheimer & Grau, 1990; Meathrel, 1991; Grau, 1996). In poultry species, which are selected for short ovulation and laying intervals, yolk growth rates are often variable among follicles (Shahabi *et al.*, 1975; Redshaw & Follet, 1976; Robinson & Etches, 1986) presumably because of the fine-tuned control of yolk precursor and carotenoid uptake at the level of each oocyte (Barber *et al.*, 1991; see also Challenger *et al.*, 2001; Bortolotti *et al.*, 2003).

To our knowledge, no study has examined directly the sex-specific growth in prefertilization ova, however many studies have found pronounced differences between male and female growth as early as a few hours post-fertilization [e.g. at the 32 cells stage in some mammals (Mittwoch, 1993; Ray *et al.*, 1995)]. For example, in birds, males and females can differ in the rate and duration of early embryonic growth (Burke, 1992; Blanco *et al.*, 2003; Magrath *et al.*, 2003) and developmental stages at hatching (Bray, 1965; Burke, 1989; Badyaev *et al.*, 2001a; Rutkowska & Cichon, 2002). Moreover, in several species, male and female eggs differ in size and this has strong adaptive consequences for offspring growth and survival (Mead *et al.*, 1987; Anderson *et al.*, 1997; Cordero *et al.*, 2000, 2001; Magrath *et al.*, 2003). If larger eggs have larger yolks, the findings of these studies imply sex differences in oocyte growth. In our study, male and female oocytes did not differ in size at ovulation with the exception of the first laid eggs (Fig. 2). Thus, differential growth of eventually similarly sized male and female oocytes in the house finch might be more closely related to the processes taking place during early follicular development. One such process might be temporal variation in hormonal profiles of both the mother and individual oocytes.

Different temporal patterns of growth of male and female follicles might expose them to distinct maternal hormonal profiles and might also enable mothers to differentially provision male and female oocytes with carotenoids, hormones, and antibodies. The main source of steroid hormones to which the preovulatory oocytes are exposed is the theca and granulosa cell layers of the follicular walls, and steroid production in these tissues varies during follicle maturation (Redshaw & Follet, 1976; Bahr *et al.*, 1983; Etches & Duke, 1984; Porter *et al.*, 1989). When male and female oocytes differ in growth rate and duration, as we found in this study, male and female ova might accumulate different concentration of hormones. For example, in male oocytes,

the most rapid growth rates occurred during early stages (Fig. 3). If, in the house finch, fully grown follicles synthesize and absorb steroids at a lower rate than earlier stage follicles [as was shown in chickens (*Gallus domesticus*) (Robinson & Etches, 1986)], then sex differences in temporal patterns of growth might result in differential synthesis and absorption of steroids (Shahabi *et al.*, 1975).

If accumulation and metabolism of steroids in follicular tissues depend on the hormonal state of the breeding female (Schwabl, 1996, but see Doi *et al.*, 1980), then temporal variation in male and female follicle sequestration and growth can expose the follicles to different concentration of maternal hormones. For example, environmentally induced onset of incubation is associated with the increase in plasma prolactin (Crisostomo *et al.*, 1998; Maney *et al.*, 1999; Sockman *et al.*, 2000), which in turn can affect steroidogenesis in the ovaries and the allocation of steroids into developing follicles (Zadworny *et al.*, 1986; Sockman & Schwabl, 1999; Sockman *et al.*, 2001). The modification of male and female follicle growth in relation to the onset of incubation documented in this study (Figs 5 and 6) could be a consequence of a maternal strategy to bias the sequence of production of males and females in relation to hormonal fluctuations caused by environmentally induced incubation onset and might provide a mechanism behind the close and consistent linkage of the onset of incubation and sex-biased ovulation order in this species (Badyaev *et al.*, 2003b).

The results reported here suggest that breeding females either can discriminate between developing male and female oocytes and thereby influence their provisioning and ovulation schedule, or they can directly and simultaneously affect both sex and growth of a oocyte. Crucial to distinguishing between these alternatives is knowledge of the timing and the patterns of chromosome segregation during the meiotic divisions in this species. Histological studies of several nonpasserine and passerine birds suggested that the first meiotic division occurs 0.5–3 h prior to ovulation, after most of the oocyte growth is completed (Warren & Scott, 1935; Olsen & Fraps, 1944; Birrenkott *et al.*, 1988; Johnson, 1996). However, these studies also describe strict hierarchy of the follicular development (Bissonnette & Zujko, 1936; Sturkie, 1986), whereas our results show that follicles within the same clutch had different rates and duration of growth, revealing sex-specific resource allocation several days prior to ovulation (Figs 2 and 3).

Twenty years ago, Ankney (1982) and Ryder (1983) suggested that sex-biased laying order in birds could be a consequence not of meiotic drive, but of different developmental times of male and female preovulation oocytes (see also Krackow, 1995). Ankney (1982) further suggested that sex differences in sequestration, growth of follicles, and ovulation might be due to their different sensitivity to maternal hormones. Here we provide the first empirical support for these suggestions. It is possible that premeiotic oocyte exposure to different hormonal states of females induces meiotic drive and thus biases oocyte sex determination at the same time as it facilitates its sex-specific growth (see also Bowden *et al.*, 2000; Eising *et al.*, 2001; Petrie *et al.*, 2001; Lovern & Wade, 2003). As a result of such exposure, variation in concentration of yolk steroids, especially in the outer layers of oocyte, might influence the elasticity and movement of the meiotic spindles thereby biasing chromosome segregation during the cell division (Olsen, 1942; Yoshimura *et al.*, 1993a,b).

Our findings show that, at least in this species, the assumption of a strict hierarchy of follicle development, a constant within-clutch rate of growth, and, consequently, a consistent temporal overlap (and thus resource requirement for production) might be incorrect. In this study, some oocytes accumulated most yolk at early stages, whereas others grew at the same rate for longer periods. Distributing the cost of egg production and lowering the peak demand of resources by modifying the sequestration and growth of oocytes lowers the cost of clutch production and might account for the variable relationship between current environmental condition and egg mass documented in several studies (e.g. Magrath, 1992; Williams & Cooch, 1996). Moreover, our results show that maternal modification of overlap among oocytes may enable breeding females to produce, within the same clutch, several offspring with distinct hormonal requirements (Uller, 2003).

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