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Interaction between maternal effects: onset of incubation and offspring sex in two populations of a passerine bird

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Abstract Maternal phenotype and maternal environment can profoundly affect the phenotype and fitness of offspring. Yet the causes of variation in such maternal effects are rarely known. Embryos in avian eggs cannot develop without being incubated and this creates an opportunity for maternal control of duration and onset of offspring development. However, females might adjust the start of incubation (e.g., coincident with the first egg or delayed until after egg-laying) in response to environmental conditions that they experience at the time of breeding. We studied two populations of the house finch (*Carpodacus mexicanus*) that breed at the climatic extremes of the species' geographical range (Montana and Alabama) and found that in both populations, the timing of incubation onset was closely associated with the bias in the sequence in which male and female eggs were laid within a clutch. When females started incubation with the first egg, they produced sons and daughters in highly biased sequence, when females delayed the onset of incubation until after the egg-laying, the sequence of sons and daughters was not biased. Because in both populations, onset of incubation was associated with the ambient temperature, these results emphasize that maternal effects on offspring can be influenced by ecological conditions experienced by parental generation.

Keywords Ambient temperature · Egg-laying order · Incubation · Maternal effects · Sex-ratio

Introduction

The parental phenotype and the environment created by parents (maternal effects) often account for significant components of phenotypic variation in offspring traits (Kirkpatrick and Lande 1989). Depending on the nature of these effects, on the environmental context of offspring development, and on the patterns of selection, maternal effects can either reduce or enhance the fitness potential of offspring (Bernardo 1996). In species where maternal and offspring environments are highly correlated and parental care is extensive, maternal effects often are the most important aspect of offspring fitness, determining, for example, offspring sex, behavior, lifetime reproductive success, and colonization of novel environments (Trivers and Willard 1973; Fairbanks 1996; Mousseau and Fox 1998).

In birds, maternal effects have been extensively studied under the headings of nest-site selection and mate choice, egg-laying and incubation effects, post-hatching care, biased offspring sex-ratio, and, more recently, maternal transfer of growth and immunological factors into eggs (Price 1998). However, despite the ubiquity of maternal effects in birds, our understanding of the mechanisms behind their evolution is limited. The major obstacles have been the lack of a clear understanding of the adaptive value of maternal effects for both maternal and offspring generations and a failure to elucidate the causes of variation in maternal effects in natural populations. The precise nature of some adaptive maternal effects in birds (Komdeur et al. 1997, 2002; Cordero et al. 2001; Badyaev et al. 2002) is puzzling, especially when the environments of offspring are novel and variable. Yet precision in the adaptive consequences of maternal effects may be expected if females and offspring experience similar environmental pressures (Fear and Price 1998; Mousseau and Fox 1998; Wolf and Wade 2001; Komdeur and Pen 2002; Reece et al. 2002).

The onset of incubation in birds is a trait that has fitness consequences for both the maternal and offspring generations. Breeding females lay at most one egg per day

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until the clutch is complete, yet the onset of incubation, which is under maternal control, varies within and among species and is commonly viewed as an adaptive response of the breeding females to the external environment, e.g., food availability, predation risk, and temperature (Slagsvold and Lifjeld 1989; Stoleson and Beissinger 1995; Hébert 2002; Martin 2002; Sockman and Schwabl 2002). Females may begin incubation with the first egg or they can postpone incubation until after the clutch is complete. At the same time, the incubation decisions of females determine crucial aspects of offspring fitness – developmental time and environment – and thus are uniquely positioned to influence the fitness of both maternal and offspring generations.

We studied two populations of the house finch (*Carpodacus mexicanus*) that occur at the limits of this species' climatic distribution, in northwestern Montana and eastern Alabama. Both populations were established 20–30 years ago in the course of the rapid colonization of North America by the house finch (Badyaev and Hill 2000). Here, we document the association between the onset of incubation and the order in which male and female eggs are laid within a clutch in both populations.

Materials and methods

Study populations

Two recently-established resident populations in Montana and Alabama, (USA) have been monitored since 1995 and 1993 respectively (protocols in Badyaev et al. 2001). Resident adults were trapped and marked with a unique color band combination. We controlled for the effects of female's learning and previous breeding experience by including only females that bred at each site for the first time (young females hereafter), and only their first breeding attempts within a season. Female's age and breeding history was determined from the records of previous captures as juveniles (Montana) and breeding history at the study site (both populations). At both sites, all nests included in this study were found during building. Egg-laying and hatching were monitored closely and eggs were numbered sequentially on the day of laying and nestlings were marked within a few hours of hatching. In a part of 2000 and 2001 in both populations, eggs were not numbered during egg-laying and the laying order was inferred from the hatching order which were identical in these populations (Badyaev et al. 2002). In all nests eggs were laid every day – we did not observe female skipping a day during egg-laying period – although females varied in the time of egg-laying from one egg to the next within the 0700–1130 hours time period, and females differed in delay of egg-laying after the nest completion.

Onset of full incubation was determined by daily monitoring of female presence on the nest during egg-laying and by inferring incubation patterns from thermocouples which were installed in each nest at the time of nest-building (Montana) and at the onset of egg-laying (Alabama) and were set to record egg temperature every 5 min (HOBO Pro Series, Onset Computer Corporation and iButton-TMEX, Dallas Semiconductor). The trials in 2002 showed that the observational and thermocouple data were identical, which together with the observations of egg development and hatching asynchrony (Badyaev et al. 2003) shows that the continuous presence of the female on the nest during egg-laying is indicative of the full incubation.

Molecular sexing

The sex of nestlings was determined molecularly by amplifying an intron of the CHD1 genes on the avian sex chromosomes (Griffiths et al. 1996). 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. 1998). Details of molecular work and tests of verification of the molecular sexing methods are in Badyaev et al. (2001). Sufficient data on both the onset of incubation and the offspring sex in relation to egg-laying order were available for 1997 and 1999–2001 in Montana and for 2000 and 2001 in Alabama.

Statistical analysis

To include both four- and five-eggs clutches, egg-laying sequence was recorded as the “first”, “second”, “third”, and the “last” (the fourth or fifth) egg. Ten clutches with three or two eggs, one with six, and one with seven eggs were excluded from this study. Onset of incubation was recorded as the proportion of the total number of eggs with which full incubation started (i.e., small values indicate early onset of incubation). To analyze the among-clutch trends in the egg-laying order while accounting for interdependency of egg-laying positions within each clutch and for the binomial error variance of the sex data, we used the generalized linear mixed models (SAS 8.12 macro GLIMIX modified from www.sas.com/service/techsup/faq/stat_macro/glimacr.html and PROC NLMIXED) with nest identity as a random effect (after Krackow and Tkadlec 2001). The components of the binomial variance of the sex of an egg were estimated with restricted maximum likelihood. The significance of the fixed effects of onset of incubation, nest-initiation date, and sex of preceding eggs on the sex of an egg in a laying sequence was evaluated with the Satterthwaite *F*-tests (Krackow and Tkadlec 2001).

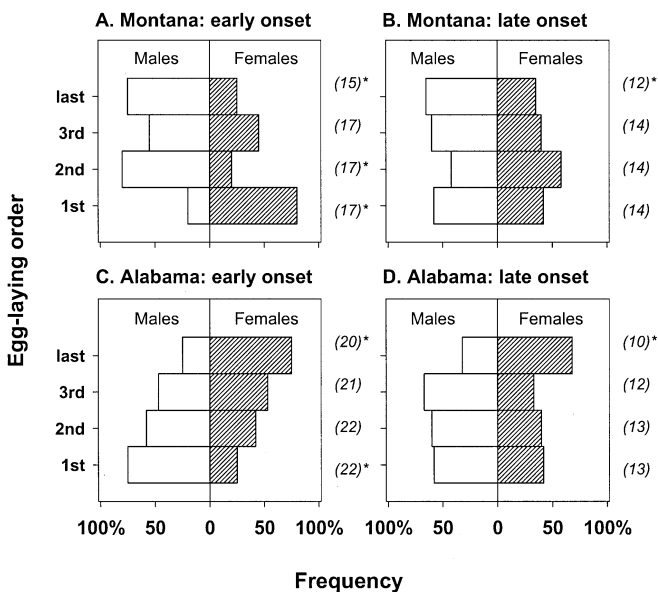
Results

The sex of eggs was highly biased in relation to the timing of incubation onset. In both populations, clutches that were incubated from the first eggs had highly sex-biased egg laying orders (Fig. 1AC; Table 1; Fig. 2). The bias was primarily due to sex of the first two eggs (Table 1; Fig. 1): in Montana the first egg produced mostly females (80%, binomial test $P=0.018$) and the second egg produced mostly males (80%, $P=0.018$), whereas in Alabama the first egg produced mostly males (82%, $P=0.014$; Montana versus Alabama: $Z=-3.48$, $P<0.01$), but the sex of the second egg was not biased (0.58:0.42, $P>0.25$; Montana versus Alabama: $Z=1.38$, $P=0.08$).

In contrast, in nests where incubation started from the last-laid egg, the sex of the first and second eggs was not biased in either population (Fig. 1 BC; binomial *P*-values >0.4), but was distinct from the nests with early incubation onset in Montana (first egg: $Z=2.24$, $P=0.012$; second egg: $Z=2.20$, $P=0.014$) and for the first-laid egg in Alabama (first egg: $Z=1.46$, $P=0.07$; second egg: $Z<0.1$, $P>0.40$). The sex of the last-laid egg did not vary with the onset of incubation and was strongly biased in both populations; the last eggs produced mostly males in Montana (early and late onsets combined: 70%; $P=0.05$), and females in Alabama (early and late onsets combined: 72%, $P=0.04$; Fig. 1), and was strongly affected by the sex of the first two eggs, largely in compensatory manner (Figs. 1, 2; Table 1). In addition,

Table 1 Effects of incubation onset, nest-initiation date, and the sex of preceding eggs on the sex of an egg of a particular laying order in Montana and Alabama populations of the house finch (*Carpodacus mexicanus*)

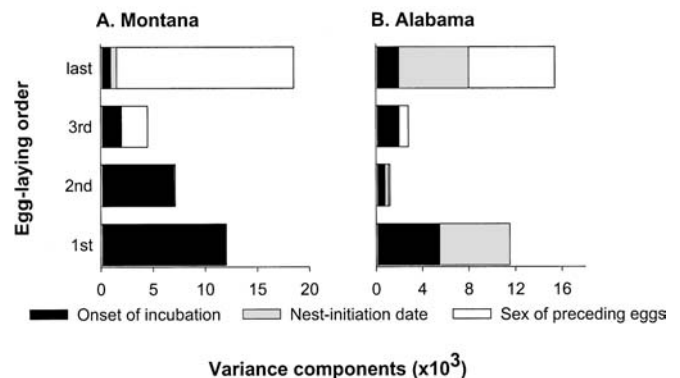
Egg-laying order	Effects									
	Incubation onset		Initiation date		Sex of preceding eggs					
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	1st-laid		2nd-laid		3rd-laid	
					<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Montana										
1st	13.39	0.003	0.36	0.55	–	–	–	–	–	–
2nd	11.21	0.001	0.33	0.56	0.34	0.52	–	–	–	–
3rd	4.56	0.04	0.25	0.62	1.49	0.22	4.55	0.04	–	–
Last	2.32	0.13	2.01	0.18	17.13	0.001	13.85	0.001	0.19	0.66
Alabama										
1st	4.78	0.04	6.25	0.01	–	–	–	–	–	–
2nd	2.63	0.11	0.61	0.43	1.98	0.19	–	–	–	–
3rd	5.98	0.01	1.35	0.24	2.90	0.07	0.80	0.37	–	–
Last	5.34	0.03	12.11	0.001	13.95	0.001	4.26	0.01	0.07	0.79

**Fig. 1** Sex-ratio in the house finch (*Carpodacus mexicanus*) in relation to egg-laying order in **A** nests in Montana with early onset of incubation (with first-laid egg), **B** nests in Montana with late onset of incubation (with the last-laid egg), **C** nests in Alabama with early onset of incubation, and **D** nests in Alabama with late onset of incubation. The number in parentheses shows the number of nests, asterisks indicate significantly biased sex-ratio. Early onset of incubation was associated with the strong sex bias of the first laid eggs in both populations. Only newly-breeding females are included

nest-initiation date accounted for significant variation in sex-bias of the first and the last-laid eggs in Alabama (Table 1; Fig. 2).

Discussion

In both Montana and Alabama populations, females that started incubation with the first egg, biased the sequence

**Fig. 2** Variance components for the sex of each egg-laying order due to the onset of incubation, nest-initiation date, and the sex of preceding eggs in **A** Montana, and **B** Alabama populations. Onset of incubation strongly contributed to variation in the sex of the first-laid eggs, while the sex of preceding eggs strongly affected the sex of the last-laid eggs in each clutch. Only newly-breeding females are included

in which they produced sons and daughters, especially early in the egg-laying sequence. In both populations, onset of incubation is closely associated with ambient temperature at the time of egg-laying (Badyaev et al. 2003), however the association between onset of incubation and offspring sex-ratio persisted despite the highly distinct climatic conditions between the populations. Previous study found that sex-biased egg-laying order can have strong consequences for the adaptive population divergence in this species (Badyaev et al. 2002). The results reported here suggest that maternal strategies that produce this sex-bias might be influenced by environmental conditions during breeding.

We found a strong link between the onset of incubation and the sex of eggs that were laid early in a clutch. The physiological mechanisms behind this link may be related to the changes in the hormonal profile of a female during egg-laying. In birds, the onset of incubation is associated with an increase in prolactin (Crisostomo et al. 1998;

Vleck 2002). In several species, females that incubate from the first egg versus from the last egg differ in their prolactin profiles. Initiation of incubation from the first-laid egg coincides with a surge in female's plasma prolactin, whereas in females that incubate from the last egg, prolactin increases gradually and reaches a maximum 2–3 days after the onset of incubation (Sockman et al. 2000; R.A. Duckworth and A.V. Badyaev, unpublished data). During egg-laying, a rise in circulating prolactin is associated with variation in circulating testosterone (Schwabl 1996a; Vleck 2002) and both these hormones have been suggested as affecting the segregation of sex chromosomes during meiosis of avian oocytes and the release of Z- and W-bearing ova during ovulation (Krackow 1995).

However, more likely the observed pattern is an active maternal manipulation of offspring sex ratio in relation to optimization of both onset of incubation and growth patterns of sons and daughters in the two populations (Badyaev et al. 2002). Differential maternal allocation of growth enhancers or suppressors into eggs in relation to the sex of the eggs and laying order is often documented (Schwabl 1996b; Eising et al. 2001; Royle et al. 2001) and can either mitigate or exaggerate the effects of variable developmental times of offspring from different egg laying positions in a clutch (Anderson et al. 1997; Cordero et al. 2001; Reed and Vleck 2001; Hanssen et al. 2002). Changes in the plasma prolactin and testosterone levels of females during egg-laying and the onset of incubation may account for temporal variation in the ability of breeding females to transfer particular hormones into eggs in relation to their laying order (Schwabl 1999). Because of the strong and often sex-specific effects of hormones on growth (Henry and Burke 1999) and the fact that the opposite growth patterns are favored in the two environments (large females and small males in Montana, small females and large males in Alabama [Badyaev et al. 2002]), the adjustment of sex-ratio in relation to incubation onset may allow females to achieve a compromise between the need to maintain both egg-viability during egg-laying and adaptive patterns of offspring growth. In the closely related canary (*Serinus canaria*), exposure to increasing photoperiod caused a systematic change in hormonal profile of females and, in turn, in the amount of steroids transferred into eggs (Schwabl 1996a), thus providing a link between environmental conditions experienced by both generations. In addition, the opposite sex bias of the adjacent egg positions provides an argument against sex-ratio adjustment as a passive consequence of a female's hormonal profile during the onset of incubation because the rapid reversal of hormone profiles during several hours after laying of the preceding egg seems unlikely.

Our finding that the sex bias in the last-laid eggs is influenced by the sex of preceding eggs, and that sex bias of the eggs in the adjacent laying positions is highly distinct (Fig. 1; see also Bortolotti 1986; Legge et al. 2001; Blanco et al. 2002; Krebs et al. 2002) corroborates the argument that, even in species with chromosomal sex

determination, the ability of females to modify the sex of offspring can be highly precise when the environmental conditions that favor such modifications are predictable (as they are for the first and the last eggs in relation to the onset of incubation) and the costs of sex adjustment are low (as they are here because of the limited sexual dimorphism in the house finch) (Komdeur and Pen 2002; West and Sheldon 2002).

Joint modification of onset of incubation and sex-ratio in relation to laying order enables females to control growth periods of male and female offspring. Because male and female offspring differ in sensitivity to environmental condition (Sheldon et al. 1998; Badyaev 2002), breeding females can mitigate the effects of early incubation onset by producing sexes of different environmental sensitivity in different positions within a laying sequence (Bortolotti 1986; Albrecht 2000; Blanco et al. 2002; Badyaev et al. 2003). More generally, whereas seasonal variation in sex-ratio, including in relation to egg-laying order, is commonly attributed to seasonal variation in fitness of male and female offspring (Daan et al. 1996; Cordero et al. 2001; Krebs et al. 2002) here we showed that the effects may be a compensatory response to environment of the maternal generation. Overall, the present study shows that environmental pressures on females for when to begin incubation can have profound effects on offspring generation.

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