Sex-Biased Hatching Order and Adaptive Population Divergence in a Passerine Bird

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Most species of birds can lay only one egg per day until a clutch is complete, and the order in which eggs are laid often has strong and sex-specific effects on offspring growth and survival. In two recently established populations of the house finch (*Carpodacus mexicanus*) in Montana and Alabama, breeding females simultaneously adjusted the sex and growth of offspring in relation to their position in the laying order, thereby reducing the mortality of sons and daughters by 10 to 20% in both environments. We show experimentally that the reduction in mortality is produced by persistent and sex-specific maternal effects on the growth and morphology of offspring. These strong parental effects may have facilitated the rapid adaptive divergence among populations of house finches.

Most species of birds lay one egg per day until a clutch is complete, and eggs hatch in approximately the order in which they are laid. Parents may modify the often strong effects of hatching asynchrony on survival of male and female offspring either by providing different resources to offspring of different laying order (*1*, *2*) or by changing the sex-ratio of offspring in relation to their position in the clutch (*3*, *4*). Thus, given the ubiquitous effects of hatching order on growth and survival of offspring, and environmental variation in the costs and benefits of raising sons and daughters (*5*, *6*), selection may favor the evolution of a mechanism that enables breeding females to adjust the sex and growth of offspring simultaneously in relation to their position in the laying order (*7*–*9*).

We examined the fitness consequences of hatching order in two recently established populations of the house finch (*10*). In both Montana and Alabama populations, male and female juveniles experience strong selection on their morphology, but the direction and intensity of this selection differs between sexes and populations (*11*, *12*). In only 20 to 30 years since these populations were established, they have diverged substantially (0.5 to 2.0 SDs) in most morphological traits of adults (*13*), in close agreement with the patterns favored by selection (*12*, *14*). However, the population difference in growth of offspring, although concordant with patterns of selection, is small, and cannot by itself account for the observed population divergence (*15*). Thus, the proximate mechanisms that allowed house finch populations to become established in two distinct environments and to respond so rapidly to local selection are not known.

Breeding females in both Montana and Alabama populations lay male and female eggs (*16*) in different sequences within clutches (Fig. 1), thus placing sons and daughters in the most advantageous positions for survival in that particular environment (Fig. 2, A and B). First-laid eggs produced mostly females in Montana (Fig. 1A) but mostly males in Alabama (Fig. 1B). This pattern was reversed between the populations for last-laid eggs (Fig. 1). In both populations, survival of juvenile males and females (*17*) was closely associated with their hatching positions, such that juveniles of the most strongly sex-biased hatching positions experienced the most sex-biased mortality (Fig. 2C). For example, males hatched in male-biased positions had higher survival than males hatched in female-biased positions (Fig. 2, A to C). On the population level, such sex-bias in the hatching order resulted in a 10 to 20% reduction of juvenile mortality (*18*) in both sexes compared with what would be expected if the sex ratio were equal with respect to hatching order (Fig. 2D).

The effects of hatching order on juvenile mortality were so striking because hatching order had a large effect on juvenile morphology (Table 1A), which in turn was the main determinant of juvenile survival (Table 1C).

Table 1. Tarsus size in relation to hatching order (parts A and B) and juvenile survival (C) in Montana and Alabama populations of the house finch.

Effect	Population					
	Montana			Alabama		
	A. Tarsus—Control nests					
	n = 26 nests, 119 birds			*n* = 40 nests, 153 birds		
	df	*F*	*P*	df	*F*	*P*
Year	3	19.46	<0.001	2	23.91	<0.001
Sex	1	1.02	0.36	1	0.63	0.56
Hatch order	4	2.88	0.04	4	3.65	0.01
Sex × hatch order	4	6.50	<0.001	4	4.44	0.006

Effect	Montana					Alabama				
	B. Tarsus—Experiment									
	n = 22 nests					*n* = 24 nests				
		Males *n* = 26		Females *n* = 21			Males *n* = 30		Females *n* = 32	
	df	*F*	*P*	*F*	*P*	df	*F*	*P*	*F*	*P*
Year	2	5.35	0.008	23.73	<0.001	1	1.10	0.31	0.45	0.51
Original hatch order	4	5.67	0.001	2.47	0.06	4	4.61	0.01	3.00	0.05
Change in order	1	0.30	0.58	1.98	0.19	1	3.34	0.11	5.97	0.03
	C. Juvenile survival									
		Males		Females			Males		Females	
	df	*F*	*P*	*F*	*P*	df	*F*	*P*	*F*	*P*
Year	3	1.64	0.18	5.31	0.003	2	3.82	0.02	1.91	0.18
Tarsus	1	7.77	0.006	3.74	0.05	1	6.84	0.01	5.29	0.03
Body mass	1	3.95	0.05	4.39	0.04	1	3.24	0.07	3.01	0.09
Hatch order	4	3.07	0.02	0.56	0.69	4	1.62	0.16	2.37	0.09

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In Montana, tarsus size decreased with hatching order in males, but increased in females (Fig. 3A). In contrast, in Alabama, tarsus size decreased with hatching order in females, whereas males hatched in the middle of the hatching sequence were the largest (Fig. 3B). To test the effect of hatching position on the morphology of juvenile males and females, we cross-fostered hatchlings by placing them in a different hatching order within a foster nest (*19*). This experiment allowed us to distinguish between the effects of the original hatching order and the effects of position within a brood during growth. In both sexes, the original hatching order strongly affected morphology at the end of growth, regardless of the experimental change in order during growth (Table 1B). The persistent effects of hatching order on final size of juvenile males and females were due to large differences in growth rates (*20*) among nestlings of different hatching positions. In Montana, growth rate decreased with hatching order in males, but increased in females (Fig. 3C). In contrast, in Alabama, growth rate decreased with hatching order in females, but was the highest for males in the middle of the hatching order (Fig. 3D). Thus, by joint modification of the sex and growth of offspring in relation to position within clutches, breeding females were able to reduce the mortality of sons and daughters significantly in both environments.

The proximate mechanisms enabling the joint optimization of growth patterns and offspring sex in relation to hatching order are not yet known, but several studies, including one of a closely related finch species (*21*), documented an environmentally dependent gradient of maternally transmitted growth enhancers or suppressors (most notably maternal testosterone and estrogen) relative to the position in which an egg was laid (*22*, *23*). As well as their long-lasting, and often sex-specific, effects on offspring growth (*24–26*), maternal steroids may affect the segregation of sex chromosomes during the first meiotic division of the oocyte and thereby influence the sex of an egg (*9, 27*), thus providing a potential mechanism behind the joint optimization of the sex and growth of offspring in relation to hatching order that we documented in this study.

The observation that the simultaneous adjustment of sex and growth of offspring in relation to hatching order facilitates adaptation to local environments provides the empirical support for the hypothesis that parental effects play a crucial role at the initial stages of population divergence by enabling establishment of populations in novel environments (*28*, *29*).

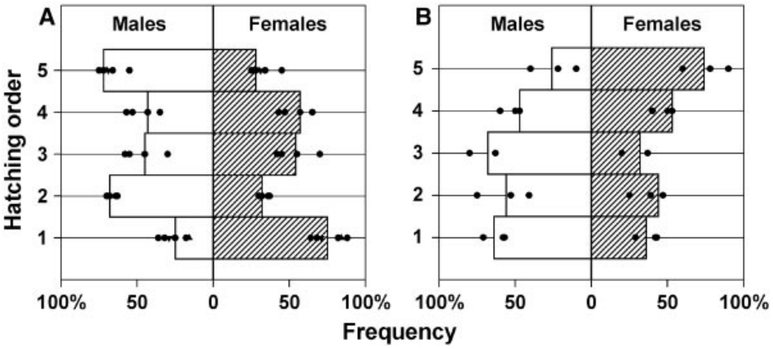

Fig. 1. The sex ratio in relation to hatching order in (**A**) Montana and (**B**) Alabama populations. Bars represent the means from four (Montana) and three (Alabama) years of observations (indicated by filled circles). In Montana, the sex-ratio of only first- and fifth- (last) hatched nestlings was determined for two additional years (1996, filled triangles, point up; 1997, filled triangles, point down). Populations differed in the sex of the first ($\chi^2 = 38.6$, $P < 0.001$), the third ($\chi^2 = 10.8$, $P = 0.001$), and the last-hatched nestling ($\chi^2 = 42.3$, $P < 0.001$). In Montana, the second- and the last-hatched positions were male-biased (both $\chi^2 > 13.0$, $P = 0.001$), whereas the first position was female-biased ($\chi^2 = 25.0$, $P < 0.001$). In Alabama, the first and third positions were male-biased (both $\chi^2 > 8.0$, $P < 0.01$), whereas the last-hatched position was female-biased ($\chi^2 = 26.0$, $P < 0.001$). The sex ratios of the whole brood were equal within (Montana, 0.51:0.49; Alabama, 0.52:0.48; P values > 0.7) and between populations ($Z = -0.17$, $P = 0.43$).

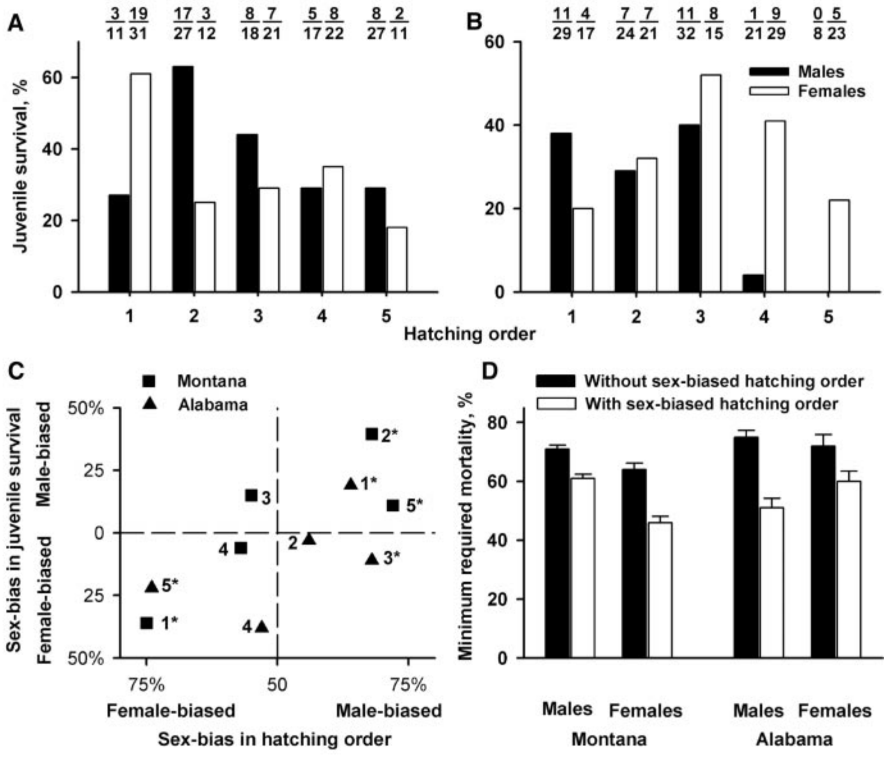

Fig. 2. Juvenile survival in relation to sex and hatching order in (**A**) Montana and (**B**) Alabama populations. Numbers above bars are sample sizes used to calculate juvenile survival (number after selection/number before selection). (**C**) Relation between sex-bias in hatching order and sex-bias in juvenile survival. Numbers by the symbols indicate the hatching order, asterisks indicate significant sex-bias in hatching positions. Birds hatched in the most sex-biased positions within a clutch had the most sex-biased survival (survival of males in male-biased positions versus in female-biased positions: 42.5 versus 14.0%, $t = 3.19$, $P = 0.03$; females: 39.8 versus 28.7%, $t = 1.12$, $P = 0.17$). (**D**) The amount of mortality needed to produce an observed selection intensity on juvenile morphology. Standard errors were generated by deleting with replacement individual tarsus measurements (within each sex and population) in pre- and post-selection samples and recalculating mortality for unbiased and observed hatching order. The required mortality assuming equal distribution of the sexes relative to the hatching order is higher (Montana: males and females t values > 4.9, P values < 0.001; Alabama: males and females t values > 3.06, P values < 0.02) than mortality calculated using the observed sex bias relative to the hatching order.

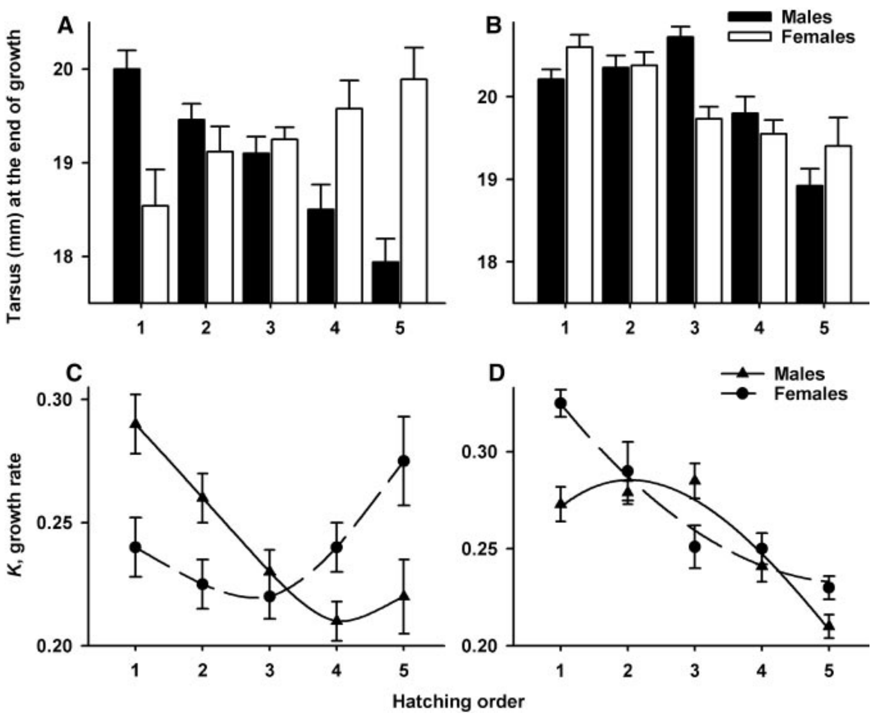

Fig. 3. Final tarsus size and tarsus growth in relation to hatching order in (**A** and **C**) Montana and (**B** and **D**) Alabama juveniles. In Montana males, growth rate and final size decreased with hatching order [positions significantly different after sequential Bonferonni adjustment ($n = 5$ orders): growth and size: 1st $>$ 3rd, 1st $>$ 4th, 1st $>$ 5th, and 2nd $>$ 4th; F values > 8.0, $P < 0.001$; Table 1A]. In Montana females, growth and final size increased with hatching order (difference in size: 5th $>$ 1st, 5th $>$ 2nd; Table 1A; difference in growth: 5th $>$ 3rd; F values > 6.7, $P < 0.001$). In Alabama males, growth and final size peaked in the 3rd hatching position (size and growth: 3rd $>$ 5th and 3rd $>$ 4th, F values > 5.3, $P < 0.01$; Table 1A). In Alabama females, growth rates and final size decreased with hatching order (size: 1st $>$ 3rd, 1st $>$ 4th; Table 1A; growth: 1st $>$ 5th, 1st $>$ 4th, 1st $>$ 3rd, and 2nd $>$ 5th, F values > 8.0, $P < 0.001$).

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10. Two recently established resident populations in northwestern Montana and in east-central Alabama have been monitored since 1995 and 1993, respectively. The data used in this study were collected in 1998–2001 in Montana and in 1998, 2000, and 2001 in Alabama. We marked nestlings individually within a few hours of hatching (*11*). In 2 years in Montana and Alabama, we examined the concordance between the sequence in which individual eggs were laid and hatched, by numbering each newly laid egg with a permanent nontoxic marker and recording subsequent hatching sequence. In all years, the egg-laying sequence was the same as the egg-hatching sequence, thus only hatching order was used in this study.
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16. The sex of nestlings was determined by a molecular sexing technique that amplifies an intron of the *CHD1* genes on the sex chromosomes of birds. We used polymerase chain reaction (PCR) primers P8 and P2, which anneal to conserved exonic regions and amplify across an intron in both *CHD1-W* and *CHD1-Z* genes (*30*). We collected a 5- to 10-μl blood sample when each nestling was 8 days old. DNA extraction, PCR, and electrophoresis were performed as described (*11*). We verified this sexing technique by both amplifying the DNA of 20 different known sex adults (10 males and 10 females) and by comparing the results of molecular methods with the sex of post-molt juveniles. In all cases the molecular method correctly identified the sex of these birds.
17. Juvenile survival was estimated by recapturing a cohort of marked juveniles 40 to 50 days after they left the nest (*11*). Survival in relation to hatching order was calculated for both sexes by dividing the number of juveniles of a particular hatching order alive at the time of recapture by the total number of fledglings of this hatching order. The effects of dispersal are likely to be minimal as no significant juvenile dispersal takes place before finches are 70 to 80 days of age [see (*11*) for details].
18. To assess the expected population-wide changes in mortality due to the sex-biased hatching order, we first calculated intensity of selection (I) acting on the morphology of juvenile males and females [$I = s/\sigma$, where s is the difference in the means of tarsus length before (16- to 18-day-old birds) and after (70- to 80-day-old birds) selection and σ is the standard deviation in the population before selection] (*11*). Second, we estimated the minimum required mortality (*31*) needed to produce the observed selection intensity. Mortality was estimated in two ways: by assuming no sex-biased hatching order and calculating the expected unweighted distribution of tarsus size at fledging (from Fig. 3, A and B) and by using the observed (i.e., sex-biased in relation to hatching order) distribution of tarsus size for males and females at fledging. Under the assumption of truncation selection, mortality was calculated from the mean selection intensities and a normal distribution. We then assessed the differences in mortality under the two scenarios to estimate the net benefit of the sex-biased hatching order. Body mass was included in the mixed-model analysis of variance of juvenile survival (Table 1C) to account for the effects of individual condition on survival.
19. To separate the effects of initial hatching order and position within a brood during growth, we conducted cross-fostering experiments. We moved newly hatched nestlings ($<$24 hours of age) between nests where hatching had started on the same day. The sex of nestlings was not known at the time of exchange, but by chance the sex-ratio was approximately equal at the end of experiment (Table 1B). In the "no-change-in-order" treatment, nestlings were moved into the foster brood such that their new position was the same as the original (e.g., a first-hatched nestling was moved to the position of the first-hatched nestling in the foster nest). In the "change-in-order" treatment, nestlings were moved into the foster nest where their position was different from their original hatching order (e.g., a first-hatched nestling was moved to occupy the fourth-hatched position in the foster nest). In most cases, the proportion of own and foster nestlings was equal among the broods under the experiment. All exchanges were among nests situated less than 50 m apart in Montana and 15 to 1200 m apart in Alabama. In 1999–2000 in Montana and in 2000 in Alabama, 55 and 60% of all exchanges were the "no-change-in-order" treatment, whereas in 2001 in both populations 70 and 85% of all exchanges were the "change-in-order" treatment. We performed mixed-model analyses of variance on the data from both unmanipulated and experimental nests. In the full model, the main effects were year, sex, and hatch order (all random effects; Table 1A). Reduced models for experimental nests were fitted for each sex separately because of the sample size limitations. In these models, the effects were year, hatch order (random effects), and experiment ("change" or "no change," fixed effect; Table 1B).
20. We measured left and right tarsus of nestlings with an accuracy of ±0.02 mm. Two people measured all nestlings [repeatabilities reported (*11*)]. Nestlings were measured every second day starting on the day of hatching and continuing until they left the nest (days 16 to 18). To control for seasonal variation and nestling mortality, we only used the subset of nestlings (sample sizes in Table 1) from the first breeding attempts (late February to late April), that were measured throughout the entire nestling period. For each nestling, we fitted the Gompertz curve to the longitudinal growth data to obtain the initial specific growth rate constant K. Tarsus length attains adult size by the day young leave the nest (*11*).
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