

First evidence of sex differences in the duration of avian embryonic period: consequences for sibling competition in sexually dimorphic birds

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Parental favoritism in birds would be enhanced if parents can control any egg feature influencing the ontogeny of the embryo during incubation. Egg size and composition may influence the duration of incubation and hatching periods, and eggs bearing embryos of different sex may differ in size and composition. Therefore, the sex of the embryo could also influence its ontogeny before hatching. We tested this prediction by investigating the duration of the embryonic period of different-sex embryos in the Eurasian kestrel (*Falco tinnunculus*), a sexually dimorphic raptor in which adult females are approximately 20% heavier than are adult males. We found the first evidence of sex differences in the duration of the embryonic period in avian eggs. Female embryos had a shorter embryonic period than did male embryos, which allowed females to hatch earlier in the hatching sequence and assume a higher rank than that of males in the intrabrood size hierarchy. Embryos with a fast growth and development resulted in hatchlings with greater residual reserves and thus larger mass, which suggests that a shorter embryonic period requires less maintenance metabolism relative to growth. Our results also indicated that early hatching may be advantageous to gain a high rank in the size hierarchy within the brood independently of the effect of sex on fledgling mass. Sex differences in avian egg ontogeny may therefore be a factor shaping life-history traits associated with parental control of sibling competition, which should be addressed in any future work on optimal reproductive investment. *Key words*: avian egg, embryo ontogeny, parental favoritism, sex differences. [*Behav Ecol* 14:702–706 (2003)]

Life-history theory predicts that if offspring fitness differ, parental reproductive investment should be concentrated on a particular offspring or brood composition that provides the highest fitness expectations per unit of parental investment (Frank, 1990; Stearns, 1992). Parent birds may adjust reproductive investment efficiently by controlling the hatching patterns and the size of chicks at hatching, which in turn affect the relative survival of offspring within a brood owing to the establishment of within-brood size hierarchies and sibling competition (Mock and Parker, 1997; Viñuela, 2000; Wiebe et al., 1998). Embryonic growth may determine mass at hatching and duration of incubation, thus affecting the delay in hatching time with respect to earlier hatched siblings, as well as overall hatching asynchrony (Viñuela, 1997). Hatching asynchrony and hatchling mass are clear determinants of individual competitive ability within a brood and of the probability of survival in a critical stage, because most mortality in nonprecocial avian broods affects to the youngest chicks at a short age (Amundsen and Slagsvold, 1991; Gibbons, 1987; Mock and Parker, 1997; O'Connor, 1984; Viñuela, 2000; Williams, 1994). Thus, strong selective pressures on growth rate during incubation period may be expected (Skutch, 1974), especially in species with sexual dimorphism in size.

Differences in posthatching growth dynamics between sons and daughters in sexually dimorphic birds are well known (Bortolotti, 1986; O'Connor, 1984). In these species, a particular sex may be more costly to produce for parents, so the costs for parental fitness and offspring survival may not be

independent of the gender composition of the brood (Bortolotti, 1986; Clutton-Brock, 1991; Fisher, 1930; Oddie, 2000). More specifically, the larger size sex may suffer higher mortality during the nestling period, because its higher demand for resources, and this may cause sex-biased mortality and sex ratio at fledging when resources are limited (Clutton-Brock et al., 1985; Oddie, 2000). However, available evidence suggests that in most cases, sex ratio at fledging in nonprecocial birds does not differ from unity, even in highly dimorphic species (Clutton-Brock, 1986; Hasselquist and Kempenaers, 2002; Krackow, 1999). Thus, parent birds may compensate for the disadvantage of higher resource demand by the larger sex in some way, but the mechanisms for achieving this are poorly known (Oddie, 2000). One possible mechanism could be sex-biased hatching order, because earlier hatched chicks have better access to resources. Thus, depending on the sex, the probability of survival of later-hatched chicks may be enhanced or reduced (Blanco et al., 2002; Bortolotti, 1986).

The adjustment of reproductive investment would be also enhanced if parents can control any egg feature influencing the ontogeny of the embryo during incubation. For instance, egg size and composition may influence the duration of incubation and hatching periods (Eising et al., 2001; Sockman and Schwabl, 2000). Given that eggs bearing embryos of different sex may differ in size (Anderson et al., 1997; Cordero et al., 2001) and composition (Petrie et al., 2001), the sex of the embryo could also influence its ontogeny before hatching. The recent finding of sex differences in avian yolk hormone levels (Petrie et al., 2001) is remarkable, because it suggests possible sex differences in prehatching ontogeny, as yolk hormones may influence embryonic growth rate and the onset of hatching (Eising et al., 2001; Ricklefs, 1993; Sockman and Schwabl, 2000). However, sex differences in avian embryo

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Table 1
Factors affecting the duration of the embryonic period and hatching and fledging mass

Response term	Explanatory term	Rejected term	Estimate	SE	F	df	p	
Embryonic period	Sex (male)		0.08439	0.03798	4.94	1,78	.0292	
	Laying order		-0.07214	0.01233	34.24	1,78	<.0001	
	Clutch size		-0.29650	0.14140	4.40	1,78	.0392	
		Laying date		-0.02380	0.01195	3.80	1,77	.0548
		Egg mass		0.01869	0.02327	0.65	1,77	.4242
Hatching mass	Embryonic period		-0.01814	0.00589	9.49	1,76	.0029	
	Egg mass		0.05442	0.00449	147.23	1,76	<.0001	
	Clutch size		-0.04572	0.01528	8.95	1,76	.0037	
	Laying date		-0.00477	0.00132	13.09	1,76	.0005	
		Sex (male)		-0.01671	0.01146	2.13	1,74	.1491
		Hatching order		0.00273	0.00394	0.48	1,73	.4901
		Laying order		0.00114	0.00539	0.04	1,72	.8336
Fledging mass	Sex (male)		-0.09321	0.01611	33.49	1,72	<.0001	
	Egg mass		0.02178	0.00737	8.72	1,72	.0042	
	Hatching order		-0.01288	0.00533	5.84	1,72	.0182	
		Clutch size		0.02204	0.20380	0.69	1,72	.4104
		Brood size		-0.02283	0.01495	2.33	1,72	.1310
		Laying order		0.00781	0.00697	1.25	1,71	.2666
		Laying date		0.00069	0.00234	0.09	1,67	.7696
		Hatching mass		0.00296	0.00827	0.13	1,67	.7217
		Embryonic period		0.00170	0.00944	0.03	1,66	.8606

Explained deviance: embryonic period (32.56%), hatching mass (18.24%), and fledging mass (60.70%). All interactions between the explanatory terms were not significant ($p > .05$).

ontogeny have not, to our knowledge, directly addressed so far by any investigation.

In this article, we tested the prediction that the sex of the embryo may influence its ontogeny before hatching in the Eurasian kestrel (*Falco tinnunculus*). Thus, we investigated differences in the duration of the embryonic period of different-sex embryos and assessed their consequences for hatchlings and fledglings.

METHODS

We monitored breeding in the Eurasian kestrel, a sexually dimorphic raptor in which adult females are approximately 20% heavier than adult males (Fargallo et al. 2002). The study was performed during the breeding season of 2000 in Campo Azalvaro region, a montane grassland area in central Spain (Fargallo et al., 2001). Nest-boxes were monitored daily during egg laying, and eggs were marked as they were laid, so laying sequence was accurately known. Eggs were weighed on the day of laying with a Pesola balance to the nearest 0.25 g. During hatching, nests were visited at least twice each day to assign each marked egg to its corresponding nestling. Hatchlings were identified by marking them with indelible and nontoxic color felt pens in the hatching tooth in the case of eggs found during hatching process, and later on the head until banding. The embryonic period of each particular egg was defined as the number of days between the start of incubation and the day of hatching. There is some variation in the onset of incubation, but females typically incubated 95–100% of the time (full incubation) on, or on the day after, the penultimate egg was laid (Wiebe et al., 1998). Given that some embryo development could have occurred before full incubation started (Wiebe et al., 1998), we considered that incubation began on the day before the penultimate egg was laid, when partial incubation has occurred in all nests as determined by subjective recording of apparent temperatures by hand (see also Wiebe et al., 1998). The extent to which possible embryo development could have occurred before full

incubation started may differ between nests and between eggs within nests, which may contribute to increase the variance. Therefore, our results on sex differences in the duration of the embryonic period were conservative in this respect.

Overall, we were able to assign 108 marked eggs to its corresponding nestling (53 males, 55 females) from 28 broods. Nestlings were weighed to the nearest 0.25 g on the day of hatching and 24 days after hatching (just before fledging). Nestling sex was determined by molecular procedures (Fridolfsson and Ellegren, 1999) by using DNA extracted from blood obtained by brachial venipuncture. Nestlings that died were sexed by the same method by using body tissues as a source of DNA.

Generalized linear mixed models with normal error and identity link function (GLIMMIX macro of SAS, Littell et al., 1996) were used to investigate the effect of sex on the duration of the embryonic period, and the effects of sex and duration of the embryonic period on hatching and fledging mass. The nest was included as a random term in the manner of a randomized complete block design to avoid pseudo-replication (Hurlbert, 1984). In addition, by introducing this random term, we ensured that the effects of the fixed variables were not influenced by characteristics of the parents or the nest. Statistical tests associated with random terms denoted significant nest variation in the three response variables examined (embryonic period: $Z = 3.47$, $p = .0003$; hatching mass: $Z = 1.76$, $p = .0394$, fledging mass: $Z = 2.86$, $p = .0021$). We included other possible influencing variables as explanatory terms in the models (Table 1). Some of the explanatory variables could covary, so we fitted their effects to the observed data following a modification of the traditional forward stepwise procedure, testing the significance of each variable and adding only the variable that resulted in a better fit of the model (Donazar et al., 1993). The significance of the remaining variables were tested again until no additional variable or interaction reached significance. The result is the most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. All tests are two-tailed.

RESULTS

We compared the duration of the embryonic period (from incubation start to hatching) of different-sex embryos and found that female embryos hatched sooner than did male embryos (Figure 1), after controlling for other possible confounding variables (Table 1). As in other studies (Vince, 1968; Viñuela, 1997) we also found that the duration of the embryonic period decreased with laying order and clutch size (Table 1). To examine whether these differences influenced the sex sequence at hatching, we conducted a log-linear analysis with laying order, hatching order and sex as factors (three-way interaction, $p = \text{ns}$; fit of the model $G = 11.45$, $df = 25$, $p = .90$). Results showed that eggs generally did not hatch in the order they were laid (laying order \times hatching order, $G = 110.74$, $df = 25$, $p < .0001$) and that, although laying order was not sex-biased (laying order \times sex, $G = 7.83$, $df = 5$, $p = .17$), females hatched earlier than did males (hatching order \times sex, $G = 12.17$, $df = 5$, $p = .033$).

Hatching mass (mean \pm SD: 15.05 ± 1.61 , $n = 108$) increased with egg mass (20.96 ± 1.63 , $n = 108$) (Table 1) and decreased as the duration of embryonic period increased (Table 1) after controlling for hatchlings sharing nests (Table 1), but there was no effect of hatchling sex (Table 1). In addition, hatchling mass decreased as clutch size (5.06 ± 1.03 , $n = 28$) and laying date increased (Table 1). After controlling for parental and nest effects, we found that fledgling mass was higher in females (216.82 ± 3.31 , $n = 53$) than in males (192.64 ± 2.25 , $n = 55$) (Table 1), increased with egg mass (Table 1), and decreased with hatching order (Table 1).

DISCUSSION

We compared the duration of the embryonic period of different-sex embryos and found that female embryos hatched sooner than did male embryos. This result is, to our knowledge, the first evidence of sex differences in the duration of the embryonic period in avian eggs, and suggests that female embryos grew faster, hatched at an earlier stage of development than did male embryos, or both. The shorter embryonic period of female embryos allowed them to hatch earlier in the hatching sequence and thus probably assume a higher rank than that of males in the intrabrood size hierarchy. Early hatching may be favorable for growth during the nestling period, as reflected by the effect of hatching order on fledgling size, once removed the effects of sex or other confounding variables.

It has been demonstrated that early-hatched chicks have survival advantages over their nest mates (O'Connor, 1984; Ricklefs, 1993). If earlier hatching is the result of a faster ontogeny at the egg stage, we would expect earlier-hatched offspring to have more yolk or fat reserves. Presumably, hatchlings with larger energy reserves (e.g., a larger yolk sac) can withstand more prolonged food restriction (O'Connor, 1984; Ricklefs, 1993). On the other hand, an early hatching may be associated with hatching at an earlier stage of development, which may represent developmental or energetic costs for hatchlings, i.e., on their motor abilities, which needs further be investigated. Our results showed that mass at hatching increased with egg mass and decreased as the duration of embryonic period increased. This is the first study to our knowledge that shows that hatchling mass may vary according to the duration of the embryonic period after controlling for parental and nest effects, egg mass, and other influencing variables. Presumably, a shorter development period requires less maintenance metabolism relative to growth (Wittow, 2000), and then embryos with a fast

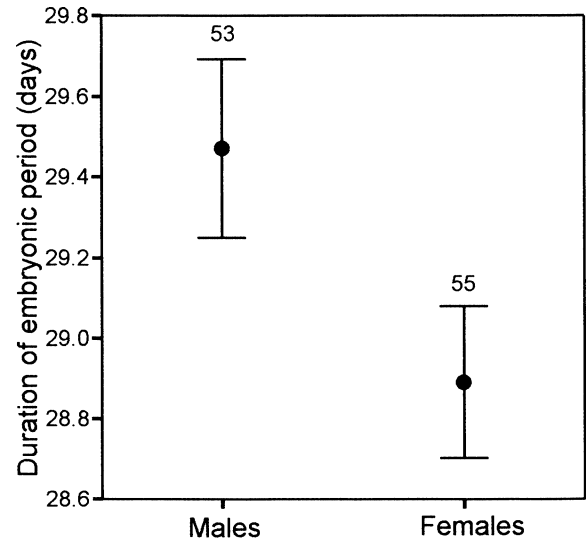


Figure 1

Mean \pm SE duration (in days) of the embryonic period of male and female Eurasian kestrel eggs. Numbers above bars are sample sizes. Note this is an univariate comparison (see Table 1 for statistics).

growth and development should result in hatchlings with greater mass. Some internal egg features, such as shape and composition (e.g., yolk hormones), or external factors such as incubation pattern and temperature, which may be susceptible of female control if they may distinguish individual eggs, may be behind this association. In addition, hatchling mass decreased as clutch size and laying date increased. The effects of larger clutch and later laying date on this relationship may be owing to higher temperatures in small clutches later in the season owing to incubation efficiency, as higher temperatures may result in higher respiration compared with tissue accumulation (Wittow, 2000).

Early hatching may place female nestlings at the top of within-brood competitive feeding hierarchies induced by hatching asynchrony and mass at hatching. A higher competitive rank in the brood sequence may result in faster growth rate or greater fledgling mass (Ricklefs, 1993), which is likely to have important implications for offspring fitness, as it has been found to be related to postfledgling survival and recruitment (for review, see Magrath, 1991). In this study, we showed that fledgling mass was higher in females than in males, probably because of the genetically determined adult sexual dimorphism. Fledgling mass also increased with egg mass, as predicted by the egg-size hypothesis (Clutton-Brock, 1991; Styrsky et al., 1999), and decreased with hatching order. These results indicate that early hatching may be advantageous in order to gain a high rank in the size hierarchy within the brood independently of the effect of sex on fledgling mass, and that intrabrood competition may be a strong selective factor affecting duration of incubation of birds, as suggested by Skutch (1976).

A trade-off between growth rate and energy requirements has been suggested to govern the evolution of posthatching ontogeny (Ricklefs, 1993). In sexually dimorphic species, the larger sex may be more susceptible to starvation owing to its higher food demands (Bortolotti, 1986; Ricklefs, 1993). Therefore, selective forces that act on embryo ontogeny in sexually dimorphic birds should favor more rapid embryonic development and greater residual reserves to enhance survival of the larger sex when food is scarce just after hatching, or to enhance the competitive ability of the large sex within broods.

The relatively small difference in hatching time in favor of females may be sufficient to compensate for their higher requirements compared with that of males if food is scarce just after hatching. Alternatively, the larger mass at fledgling for females does not necessarily imply that females need more food than do males, but may reflect differences in body composition, growth rate and duration of growth period, growth priorities, or activity levels between the sexes (see Newton, 1986). However, previous studies of kestrels found that male kestrel chicks had poorer body condition than that of female chicks in nest with food shortage (Fargallo et al., 2002) and that female nestlings had competitive superiority to access food over their male nest mates (Fargallo et al., 2003). These results suggest that the earlier hatching of female kestrel chicks, induced by their shorter incubation period, give them a head start in the intrabrood size hierarchy that may be important for survival of the larger-size sex when resources are scarce. Thus, this would constitute a mechanism allowing raising broods with unbiased sex ratios, albeit large sexual size dimorphism, and this agrees with the fledging sex ratio remarkably similar to 1:1 found in this population.

Sex differences in the duration of embryonic period may be a form of parental favoritism if they are influenced by internal egg features or differential incubation. Alternatively, sex differences may be strictly a function of embryo adaptations to enhance competitive ability. Distinguishing between these possibilities may provide fruitful research avenues for understanding the evolution of parental control of sibling competition and parent-offspring conflict over brood reduction. There is growing evidence that steroid hormones are involved in the control of both embryonic and posthatching growth (Eising et al., 2001; Schawbl, 1993, 1996; Sockman and Schawbl, 2000), and recent evidence of sex differences in avian yolk hormone levels (Petrie et al., 2001). Therefore, egg hormones are good candidates to be involved in differential ontogeny of different-sex embryos. However, more research is needed, because there is contradictory information about the effect of steroids on growth of birds (Whittow, 2000).

Furthermore, although interspecific variation in incubation period of eggs of birds seems to be well explained by differences in egg size, eggshell structure, environmental temperatures, incubation behavior, or predation pressure (Skutch, 1976; Starck and Ricklefs, 1998; Whittow, 2000), little is known about these relationships within a species (Christensen et al., 2001). There are intriguing differences in the intraspecific variation of incubation period. Thus, in some species, but not in others, incubation period is affected by laying order, laying date, clutch size, or egg size. This may be partly owing to differences in the duration of embryonic period depending on the sex of embryos that, up to now, had not been detected in birds, and that should be examined in future studies of variation in incubation period.

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