

Egg size, offspring sex and hatching asynchrony in zebra finches *Taeniopygia guttata*

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Experimental synchronization of onset of incubation was employed in laboratory held zebra finches *Taeniopygia guttata* to study whether differential resource allocation and possible bias of offspring sex in subsequent eggs in the laying order could mitigate the effects of hatching asynchrony. We found that egg mass increased with laying order, but offspring sex was not related to laying order. Among synchronized clutches, eggs hatched more synchronously than eggs from control nests. Survival probability was related to egg mass, and as expected, this effect differed between experimental groups: it was positive among synchronized broods and not significantly related among asynchronous broods. This suggests that increase in egg mass with the laying order might reduce disparities between early and late hatching chicks. Female nestlings survived better than male nestlings. However their growth was impaired in synchronized broods, whilst growth of males was not affected by hatching synchronization.

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Hatching asynchrony is commonly observed in many altricial bird species and numerous hypotheses have been proposed to explain this phenomenon (reviewed by Magrath 1990, Stoleson and Beissinger 1995, Stenning 1996). It has been suggested to result from adaptive parental manipulation of hatching spread to increase fitness, e.g. by making incubation (Nilsson 1993), and provisioning of young (Hussell 1972), more efficient. Alternatively, hatching asynchrony has been proposed as a maladaptive artifact arising from parental necessity to start incubation before the clutch is completed which results in large age and size differences among nestlings within a brood (Veiga 1992). The latter argument has been supported by findings that in broods of experimentally achieved synchrony fledgling success was enhanced in 20 out of 24 studied species (see Nilsson 1995 for review). Thus, if hatching asynchrony is maladaptive, birds may use several possible methods to reduce disadvantages of this phenomenon. For example, the gradual increase of egg mass with laying order

observed in many species has been interpreted as an adaptive strategy to reduce competitive disparities between nestlings in the asynchronously hatching broods (Howe 1976, Clark and Wilson 1981). However, to our knowledge, such an adaptive role of increase in egg mass with laying order has never been evaluated experimentally. Egg size usually determines hatchling mass and may also affect subsequent nestling growth (Styrsky et al. 2000), and survival (Potti and Merino 1994). Females may differentially distribute nutritional resources and specific agents, such as hormones (Schwabl 1996, Eising et al. 2001), antioxidants (Royle et al. 2001, 2003) and immunoglobulins (Saino et al. 2001), which may be important for growth and development of chicks, resulting in faster or slower development in relation to their position in the laying order. Because differential resource allocation to subsequent eggs in the laying order may have different consequences for male and female offspring performance, the optimal strategy may involve the simultaneous adjustment of egg quality and

embryo sex. Indeed, there is growing evidence that offspring sex interacts with egg provisioning and laying order (in Eurasian kestrel *Falco tinnunculus*; Blanco et al. 2003) and that this interaction influences growth and survival of the offspring (in house finches *Carpodacus mexicanus*; Badyaev et al. 2002). Thus, one can expect that differential allocation to sons and daughters could serve to counteract negative effects of hatching asynchrony.

Here we aimed at studying whether the observed increase in egg mass with laying order in the zebra finches could potentially compensate for the negative effect of hatching asynchrony. If the increase in egg mass with laying order enhance growth and survival of late hatched chicks, this positive effect of egg mass may not be obvious under asynchrony, but should become detectable in synchronized broods. Thus, we performed a controlled experiment involving synchronization of incubation within a brood, and expected that among such broods egg mass should have more pronounced positive effect on nestlings. We chose zebra finches as a study object because in laboratory populations of this species hatching asynchrony is common, as parents usually start incubation well before clutch completion. In laboratory populations of this species egg mass increases with laying order (Williams 2001, Rutkowska and Cichoń 2002, Royle et al. 2003). Thus, female zebra finches may potentially provide late laid eggs with more resources, to reduce disadvantages of late hatching, by enhancing growth and survival of such nestlings. In the analysis we additionally introduced an effect of offspring sex to test whether it interacts with egg mass or laying order to determine offspring performance. In zebra finches female nestlings seem to be more sensitive sex as they exhibit higher mortality and slower growth rate under food shortage (de Kogel 1997, Martins 2004). Therefore, placing female eggs early in the laying order could also serve as a potential strategy to enhance brood survival. We aimed to test whether offspring sex is differentially allocated in relation to laying order, as reported by Kilner (1998) in zebra finches, or in relation to egg mass, and more importantly, whether male and female nestlings perform differently in asynchronous and synchronized broods.

Methods

Breeding pairs in our experiment were progeny of birds originating from several local breeders. Birds were kept in a climatized room at $21 \pm 2^\circ\text{C}$, under a 13:11 h incandescent light:dark photoperiod, lights on at 7 a.m. They were fed *ad libitum* with a standard mixture of seeds (Minifit, Perbo), along with a mixture of hard-boiled eggs chopped with biscuits and finely grated carrot. Birds also received a cuttlebone and grit. Rearing

conditions were kept constant during the experiment. Initially, all birds were maintained in a common aviary, where they reared one brood. As we used young birds that never bred before, this served only to increase their breeding experience. Sexes were then separated for a month and paired again with the same partner in visually separated, individual cages (75×30 cm and 40 cm high) equipped with external nest-boxes and nesting material. Each pair contributed only one brood to the analyses.

Following pairing, nest-boxes were inspected every morning between 9 a.m. and 10 a.m. to record nest building and egg laying. All eggs were numbered and weighed with an electronic balance (± 0.01 g) shortly after laying (up to three hours after egg laying). Every second pair that started a clutch was allowed to incubate their eggs throughout the laying period. Other pairs were assigned to the group with synchronized incubation in which newly laid eggs were removed from the nest within three hours after laying and replaced by clay models. Removed eggs were stored at 11°C and returned to the nest the day after the clutch was completed. This storage temperature seems to be the optimal one to preserve the viability of eggs (Olsen and Haynes 1948). A similar method was successfully applied in other studies, e.g. egg storage in 8°C for one week affected neither hatching success, nor mass of nestlings of pied flycatcher *Ficedula hypoleuca* at hatching, or 4 days afterwards (Siikamäki 1998).

At the day of expected hatching, nests were inspected hourly whereas during nights (between 8 p.m. and 8 a.m.) eggs were transferred to separate compartments in an incubator chamber (humidity $\sim 70\%$, temperature 36.4°C). This enabled determination which hatchling came from which egg. Newly hatched chicks were weighed to the nearest 0.01 g, marked by nail clipping and returned to the nest. Nestlings were weighed every second day after hatching with an electronic balance: on day two to the nearest 0.01 g, and subsequently to the nearest 0.1 g until they were 12 days old. At the age of two weeks, nestlings were ringed with individually numbered aluminum rings. Nestling survival was followed up to 50 days of age, when they were separated from their parents.

The sex of offspring was determined by plumage characteristics (86 individuals), or by using molecular techniques (30 individuals), if embryos failed to hatch or nestlings died before their sex could be assessed by plumage. DNA was extracted from a piece of tissue using Chelex (Bio-Rad; Walsh et al. 1991). The CHD-W and CHD-Z genes from sex chromosomes were amplified using polymerase chain reaction with primers P2 and P8 (Griffiths et al. 1998).

Seventeen broods were assigned to the asynchronous group and the same number of broods was experimentally synchronized. In two asynchronous clutches eggs

were unfertilized and one clutch with laying gaps was not incubated. In the synchronized group one male died just after clutch completion, two clutches were unfertilized and four clutches were not incubated and did not hatch at all. All these clutches were excluded from statistical analyses. Thus, results presented here include 14 asynchronous and 10 synchronized clutches, totaling 141 eggs. 116 eggs were unambiguously sexed, the others had either no visual signs of embryo development (9), were broken or missing (9), results of molecular sexing ambiguous (2) or nestlings mixed up (5 nestlings in 2 broods).

The effects of the experimental group, offspring sex (class variables), egg mass and egg laying order (covariates), and the effects of their interactions on probability of survival and nestling growth were the central interest of this study. To analyze the sex of eggs and offspring survival, we used a generalized linear mixed model (GLMM) procedure in SAS (SAS 2000), that assigned binomial error variance with logit link (Krackow and Tkadlec 2001). Changes of egg mass with the laying order were analyzed using ANCOVA, and changes in body mass (=growth rate) of nestlings were assessed using repeated measures ANOVA. Nestling body mass was log-transformed to satisfy requirement of linearity. Brood identity was included in all models. It was nested in the experimental group in ANOVAs or introduced as a random factor in GLMM. Interactions of the forth and third order were not significant and were removed from the models to increase power of the test. Interactions of the second order that were the primary interest of this study remained in the model even if non-significant.

Results

Clutch size in synchronized ($\bar{X} \pm \text{SE}$: 6.0 ± 0.3) and asynchronous broods (5.8 ± 0.2 eggs), did not differ ($F_{1,22} = 1.10$, $P = 0.74$). In both groups egg mass increased with laying order and last-laid eggs (5–7) were ca. 15% heavier than the first eggs (ANCOVA accounting for brood identity, $\beta = 0.72$, $F_{1,116} = 126.8$, $P < 0.01$). Sex of the egg was not related to laying order (GLMM; $F_{1,104} = 0.66$, $P = 0.4$, brood ID: estimate \pm SE = 0.10 ± 0.26 , $Z = 0.38$, $P = 0.35$), and sexes did not differ in egg mass ($F_{1,91} = 0.29$, $P = 0.59$; brood ID: $F_{23,91} = 12.0$, $P < 0.0001$).

Experimental synchronization of incubation was effective, and resulting in a significant reduction in hatching spread (from the first to the last hatching egg within a brood), with the average (\pm SE) of 1.7 ± 0.2 days for synchronized broods in comparison to 4.8 ± 0.3 days for asynchronous broods. Hatching success did not differ between synchronized and asynchronous broods (93% versus 84% respectively; GLMM; $F_{1,21.9} = 1.34$, $P = 0.26$)

which ensures that storing eggs from synchronized broods under cold conditions was effective, and had no negative side effects.

Nestling survival calculated from hatching to 50 days of age was lower among synchronized broods than it was in asynchronous ones (71% versus 85%; Table 1, Fig. 1). As predicted, egg mass significantly affected nestling survival, and it seemed to have larger effect in synchronized broods, although the expected interaction of experimental group \times egg mass was marginally non-significant ($P = 0.074$, Table 1). In the analyses performed separately within the experimental groups, egg mass had a significant effect on nestling survival within synchronized broods (GLMM; $F_{1,38.8} = 8.75$, $P = 0.005$), but the effect was not significant within asynchronous broods (GLMM; $F_{1,58} = 0.57$, $P = 0.45$). Laying order had an independent significant effect on probability of survival: it decreased with the egg laying order in both groups. Thus, other factors related to egg laying order, not only egg mass, seem to be important determinants of nestling survival. Female nestlings appeared to survive better than male nestlings (88% versus 76%, respectively, Table 1). Moreover, interactions of sex \times egg mass and sex \times laying order significantly affected offspring survival.

We found that male and female nestlings from the two experimental groups showed different growth patterns as indicated by the significant interaction between experimental group and sex (Table 1, Fig. 2). Therefore, we performed additional analyses of the effect of synchronization treatment separately for male and female nestlings. We found that brood synchronization negatively affected growth of female nestlings while it had no effect on growth of male nestlings (Repeated measures ANOVA; $F_{1,10} = 6.69$, $P = 0.027$ and $F_{1,10} = 1.76$, $P = 0.21$, respectively; Fig. 2). Egg mass or egg laying order had no significant effect on nestling growth rate (Table 1).

Discussion

In this study, the experimental synchronization of incubation was applied to examine whether an increase in egg mass with laying order, observed in captive zebra finches, and possibly sex allocation, could compensate negative effects of hatching asynchrony. The effects of differential investment into eggs laid early and late in the laying order should be more easily detected in synchronized broods, in which the negative effects of a large hatching spread are minimized. Thus, we expected that egg mass would differently affect nestling survival and growth rate in synchronized, and asynchronous broods. Indeed, nestling survival increased with egg mass in synchronized broods but not in asynchronously hatching broods (Fig. 1). This seems to support the idea that

Table 1. Analyses of factors influencing nestling survival and growth in the broods of synchronized, and asynchronous hatching. Nestling survival was analyzed with generalized linear mixed model (see Methods). Nestling body mass was log-transformed and the growth rate was analyzed with repeated measures ANOVA. Because all nestlings gain weight with time, only between subject sources of variation are shown.

Source of variance	Nestling survival			Nestling growth		
	df	F	P	df	F	P
Experimental group	1, 59	4.12	0.047	1, 30	0.05	0.817
Sex	1, 87.1	5.70	0.019	1, 30	0.02	0.894
Egg mass	1, 85.8	9.99	0.002	1, 30	1.03	0.318
Laying order	1, 90	12.12	0.0008	1, 30	0.05	0.820
Experimental group \times sex	1, 80.8	2.34	0.130	1, 30	6.44	0.017
Experimental group \times egg mass	1, 61.5	3.29	0.074	1, 30	1.07	0.309
Experimental group \times laying order	1, 92	1.82	0.180	1, 30	0.72	0.401
Sex \times egg mass	1, 87.5	7.29	0.008	1, 30	0.04	0.847
Sex \times laying order	1, 86.1	6.72	0.011	1, 30	0.66	0.421
Egg mass \times laying order	1, 88.8	13.58	0.0004	1, 30		
	estimate \pm SE	Z	P			
Brood	11.8 \pm 5.2	2.26	0.012	19, 30	2.66	0.008

increase of egg mass with laying order may indeed counteract negative effects of hatching asynchrony as suggested by Howe (1976), and Clark and Wilson (1981). Probability of survival decreased with egg laying order in both groups. It may indicate that when the effect of egg mass is removed, other factors related to egg laying order, not only egg mass, are important. For example, androgens and antioxidants have been shown to decrease with egg laying order in zebra finches (Gil et al. 1999, Royle et al. 2003) but whether these substances affect nestling survival in this species is unknown.

In this study we also expected that male and female offspring might perform differently in synchronized and asynchronous broods. It could happen if females adjust offspring sex in relation to laying order and egg mass to maximize brood survival under asynchrony. Thus, the more vulnerable sex should be positioned in the first laid eggs. Although we did not find any evidence that embryo sex was related to laying order, nor to egg mass, the sex-

specific effect on offspring performance was indeed revealed in the analysis of growth rate (Fig. 2). Growth of the female but not the male nestling was impaired among synchronized broods. This corroborates the earlier findings, which show impaired growth of female nestlings under stressful conditions (Martins 2004). However, contrary to earlier studies (de Kogel 1997, Kilner 1998), female nestlings had higher survival than male nestlings. Regardless of the experimental treatment, survival of female nestlings was related to egg mass and egg laying order in a different way than the survival of male nestlings, as indicated by the significant interactions (Table 1).

Most of previous field studies in which brood asynchrony was experimentally altered showed that such manipulation improves fledgling success (Nilsson 1995). In our study synchronization of the onset of incubation had negative effect on nestling survival (Table 1, Fig. 1), suggesting that in our population of zebra finches brood asynchrony may not be entirely maladaptive. Lower survival of nestlings from synchronized broods could have resulted from higher hatching success: lower quality offspring do not die at the embryo stage, but later during the nestling stage. Shortened hatching span could also enhance sibling competition in such broods, as the overall food demand of brood consisted of nestlings of similar age, was perhaps larger than that of the asynchronous brood. However, synchronized and asynchronous broods did not differ significantly in the mean number of hatchlings (and number of hatchlings, introduced to the analyses as an independent variable appeared non-significant). There is also a possibility that a prolonged incubation period of experimentally synchronized clutches may have negatively affected parents, that in turn provisioned the nestling less effectively. However, 3.4 days longer incubation of synchronized clutches should not have such a large

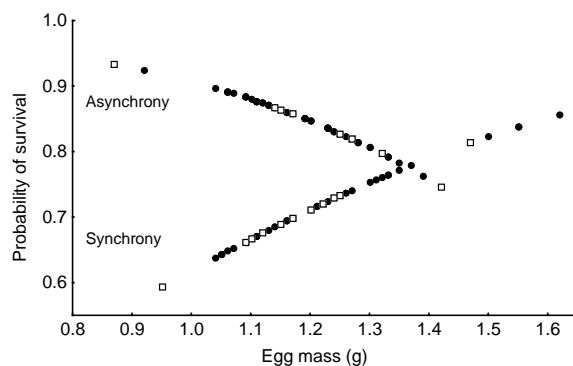


Fig. 1. Probability of nestling survival in experimentally synchronized and asynchronous clutches in relation to egg mass. Probabilities of survival of chicks that originated from an egg of a given mass were calculated from the logit functions fitted to the data separately for the two groups. Filled circles represent surviving nestlings, and open squares represent non-surviving nestlings.

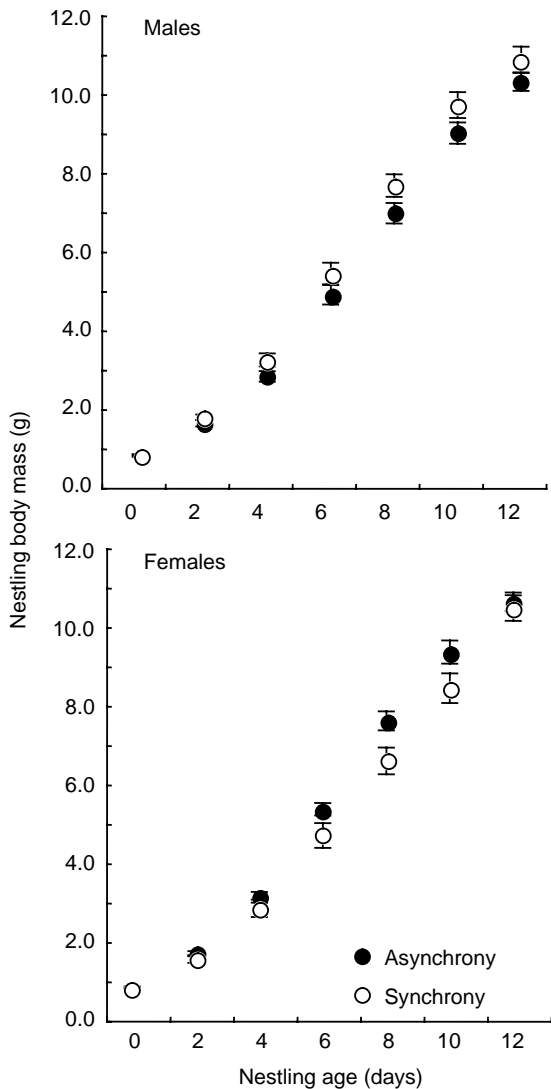


Fig. 2. Growth trajectory of female and male offspring in asynchronous and experimentally synchronized broods. Mean body mass \pm SE are presented.

effect on parents rearing broods under laboratory conditions with *ad libitum* access to food.

To our knowledge, this is the first experimental study, which shows that an increase in egg mass with laying order that has been observed in a number of bird species, may serve to mitigate the negative effects of hatching asynchrony. We also show that hatching synchronization may have negative effects on growth of female nestlings. However, future studies are needed to verify whether sex allocation is adjusted to meet competitive disparities between male and female offspring during asynchrony.

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