

Food supply during prelaying period modifies the sex-dependent investment in eggs of Eurasian kestrels

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Abstract The theory of sex allocation suggests that if the reproductive value and the cost of producing/rearing offspring differ between male and female offspring, parents should invest differently in sexes depending on environmental conditions. Female parents could allocate more resources to eggs of one sex to compensate potential sex-dependent constraints later during the nestling period. In this study, we tested the influence of environmental conditions on sexual dimorphism in eggs of Eurasian kestrels (*Falco tinnunculus*) by experimentally manipulating food availability before laying. We found that an increase in food abundance before laying did not increase egg mass but changed sex-dependent resource distribution in eggs. In food-supplemented pairs, but not in control pairs, egg mass and hatchling mass were similar between males and females. In addition, we found, in the food-supplemented group, that the latest hatched females showed shorter hatching times than in the control group. In control pairs, female eggs, hatchlings and nestlings were heavier than males. In addition, male fledglings in the food-supplemented group gained less mass than those in the control group. As that food abundance was only increased until the onset of laying, female kestrels were expected to

invest in eggs taking food abundance before egg formation as a predictor of future conditions during brood rearing. Our study shows that environmental conditions before laying promote a subtle adjustment of the resources invested in both sexes of offspring rather than in other breeding parameters. This adjustment resulted in a shortening of hatching time of the last hatched females that possibly gives them advantages in their competitive capacity with respect to male nest-mates.

Keywords *Falco tinnunculus* · Food supplementation · Egg mass · Sex investment · Sexual size dimorphism

Introduction

The theory of sex allocation states that parents invest differentially in male and female offspring when fitness returns differ between offspring sexes (Charnov 1982; Clutton-Brock 1991). In birds, sex allocation operates by varying sex ratio and/or the amount of resources addressed to each offspring sex (Hasselquist and Kempenaers 2002). The different energetic demands and competitive capacity of sexes in sexually size-dimorphic species suggest that offspring sex can be differentially vulnerable to conditions of limited food resources, and hence, parents should vary the investment in each sex according to a given situation of food availability (Trivers and Willard 1973). In oviparous animals, females must invest the resources required for embryonic development in discrete units (eggs); hence, the maternal resource allocation at the time of egg formation is an important trait in avian life histories. If sexes are differentially vulnerable to the prevailing environmental circumstances (Clutton-Brock et al. 1985; Bortolotti 1986; Fargallo et al. 2002, 2006), parents able to identify

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offspring sex and to invest accordingly during the production of propagules would minimise costs and maximise benefits (Stearns 1992). Thus, females might optimise maternal allocation of resources by giving advantages (or compensating disadvantages) to one sex over the other during egg formation to improve their fitness (Blanco et al. 2003a).

Analysing the variation in egg size and composition can be a method to quantify units of parental investment. Food availability has been suggested as a major factor influencing egg mass or size (Nager and Zandt 1994; Korpimäki and Wiehn 1998; Aparicio 1999; Evans et al. 2005). Relative egg size can influence hatching success (Croxal et al. 1992; Magrath 1992; Valkama et al. 2002), hatchling mass (O'Connor 1984; Ricklefs 1993), nestling body condition (Cunningham and Russell 2000; Fargallo et al. 2006), time for hatching (Fargallo et al. 2006) and nestling survival (Ankey 1980; Bolton 1991; Ricklefs and Starck 1998). Thus, manipulating egg size according to laying order has been suggested as a potential mechanism to control sibling competition (Blanco et al. 2003a). Hence, egg size can define, to some extent, offspring fitness (Williams 1994; Smith and Bruun 1998). However, numerous studies manipulating food abundance before laying failed to find any effect on egg size in a wide range of species (Christians 2002); thus, the dependence of egg size on food conditions before laying is not yet clear.

In non-siblicidal sexually size-dimorphic birds, dimorphism in size contributes to the competitive capacity of the sexes (Anderson et al. 1993; Oddie 2000; Hipkiss et al. 2002; Fargallo et al. 2003, 2006). This has led to the suggestion that female American kestrels *Falco sparverius* may reduce the competitive disparity between offspring by laying larger eggs of the smaller sex (Anderson et al. 1993). Further, it has been suggested that in the Eurasian kestrel *F. tinnunculus*, a sexually size-dimorphic species (females 20% heavier than males), females are able to adjust their reproductive effort by promoting sex differences in the mass of first-laid eggs (Blanco et al. 2003a), where sexual differences in the size of first-laid eggs defined the pattern of within-brood mass hierarchies of fledglings. This supports the possibility of a female capacity to manipulate sibling competition by a sex-dependent egg investment. However, the same species in different environmental conditions may alter its sex-dependent investment in eggs (Badyaev et al. 2002a, b, 2003) and different environmental conditions may affect male and female nestlings differentially (Fargallo et al. 2002). Thus, it is expected that different food conditions experienced before laying could alter maternal investment in male or female eggs. Only one study on zebra finches *Taeniopygia guttata* has specifically investigated the effect of food conditions on the sex-dependent mass of the eggs in zebra finches (Rutkowska

and Cichon 2002). However, although this study suggests an effect of food on the proportion of male to female eggs according to laying order, the authors did not find sexual differences in egg mass or size.

In this paper, we assessed whether Eurasian kestrels are able to adjust their reproductive effort by adopting different strategies based on food conditions before egg formation. We supplemented female Eurasian kestrels until the onset of laying and compared them with a control group to avoid an undesired enlargement of the clutch size (Aparicio 1994). We analysed the effect of the experiment on egg mass and chick condition in relation to sex. If sex-dependent investment in first-laid eggs is a strategy to control sibling competition (Blanco et al. 2003a) and females are able to manipulate it, our experiment should modify the relationship between sex and egg mass of the first-laid eggs. Previous studies in kestrels have shown that nestling males show a lower competitive capacity and are more vulnerable under adverse conditions (Fargallo et al. 2002). Thus, if sex-dependent investment in eggs of sexually size-dimorphic species modifies within-brood size hierarchies (Blanco et al. 2003a) to optimise offspring sibling competition under food shortage (Forbes et al. 2002), we should expect a weaker sexual size-dimorphism of eggs in the food-supplemented group.

Materials and methods

Field procedures

The study was carried out in a population of Eurasian kestrels in Campo Azávaro (40°40'N, 4°20'W, 1,300 m.a.s.l.), central Spain, during the breeding season of 2002. The study area is a montane grassland where kestrels breed mainly in nest boxes provided since 1994 (Fargallo et al. 2001). We monitored nest boxes every 2 days during egg laying and marked eggs as they were laid to accurately determine laying date and laying sequence (Blanco et al. 2003a, b). Eggs were weighed to the nearest 0.25 g on the day of laying or the day after. To record hatching order, we started visiting nests every day from 25 days after the end of laying and up to three times everyday from when we detected the first evidence of hatching until all eggs of the clutch hatched. We marked the chicks with indelible ink on the hatching tooth, on the head at hatching and remarked every 5 days until they were ringed at 17–20 days. This allowed us to determine the egg origin for each chick (Blanco et al. 2003a, b). We encoded laying and hatching order as initial (first), last (last) and middle (rest) laid/hatched eggs (Blanco et al. 2003a, b). We estimated differences in hatching time (hatching span) of each egg as the number

of hours elapsed between the hatching of the first hatched egg and the hatching of a given egg (Viñuela 2000). Therefore, hatching time of the first hatched eggs was 0. Hatching asynchrony of the nest was defined as the time elapsed between last and first hatched eggs. Dead nestlings ($n=12$) were collected during regular visits for a posteriori sexing. Nestlings were weighed to the nearest 0.25 g and wing length measured to the nearest 1 mm on the day of hatching and at 24 days old. Weight of the brood was defined as the total weight of the nestlings at 24 days old in a given nest. Nestling sex was determined by molecular procedures (Fridolfsson and Ellegren 1999) using a pinprick blood sample from the brachial vein at 24 days old or a sample of muscular tissue in dead chicks. On some occasions, when we observed chicks with poor body conditions at early ages, we took a blood sample for later sexing.

Experimental design

We randomly assigned 16 pairs for food supplementation and 17 pairs as controls. We visited nests of both groups with the same frequency. We supplemented food every 2 days with 120 g of farmed Japanese quail *Coturnix c. japonica* as previously reported for kestrels (Aparicio 1999). Feeding started at least 18 days before egg laying, thus, covering the entire period of egg formation in this species (Meijer et al. 1989). Thus, we assumed that food supplementation started before egg formation. Food supplementation, however, before and during egg laying in Eurasian kestrels can increase clutch size (Aparicio 1994), rendering groups incomparable. Thus, because we were interested in the effect of food availability on egg size and its subsequent effect on chicks, we ceased food supplementation when the first egg was laid to avoid this possible effect. By doing so, we altered the perception of females about the real food abundance and gave them extra food to study between-group differences in the distribution of resources. We confirmed that quails were eaten because their remains were found in or around food-supplemented nests. All female kestrels were captured after food-supplementation during incubation by netting at the nest box between 1 week after the end of laying and 1 week before hatching to explore the effect of the experiment on their body mass and condition. Therefore, the time elapsed from when we stopped food supplementation until female capture varied from 17 to 30 days (kestrels lay eggs every other day). They were weighed to the nearest 2 g, and their tarsus and wing length were measured to the nearest millimetre.

Statistical analyses

As eggs and chicks from the same nest share parents and environment, we used the egg/nestling as the unit and the

nest as a random factor in general linear mixed models (GLMM) performed in SAS statistical software (SAS 1989–96 Institute, Cary, NC, USA). By doing so, we avoided pseudoreplication and ensured that the effects of the fixed variables were not influenced by common parental or nest characteristics. We did three GLMM models where egg mass, hatchling mass and nestling mass were the dependent variables. In each model, treatment, sex and sex of first-laid egg were considered as fixed terms. We also considered clutch size as a response variable in all models. Satterthwaite approximation for degrees of freedom was used. Sex ratio variation, hatchling success (HS) and fledgling success (FS) were assessed using generalised linear mixed models (GLIMMIX, macro of SAS) with binomial error and logit function. The probabilities of belonging to a given sex, to hatch or to fledge were analysed by including these variables as binomial variables considering the egg/chick as the unit and including the nest as a random factor. Sex of initial laid eggs and brood sex ratio were not related (GLIMMIX, $F_{1,70}=1.72$, $p=0.194$), allowing us to use both variables in the same model. Some of the explanatory variables could covary, thus, we fitted their effects to the observed data after forward and backward stepwise procedures, testing the significance of each variable one by one and adding only the variable that best fits the model. The result is the minimum adequate model for explaining the variance of the response variable where only significant explanatory variables and significant interactions were retained. To explore the effect of treatment on reproductive parameters and female body measurements, we used general linear models (GLM). Means \pm SE are given.

Results

Food supplementation

The average length of supplementary feeding before the onset of laying was 34.45 ± 5.07 days. Female parent mass, wing, tarsus length and condition (body mass corrected by wing length) did not differ between treatments (GLM, all $p>0.192$). We did not find any effect of food supplementation on clutch size, brood size, egg mass, laying date, nestling mass or weight of the brood (GLM, all $p>0.173$). Twenty-three out of 186 laid eggs did not hatch (control, $n=13$ eggs; food-supplemented group, $n=10$ eggs). We were able to assign the egg of origin in 132 (76%) out of those 163 hatchlings (66 in food-supplemented group and 66 in control group). Of these 132 hatchlings, only 12 did not survive until fledging (five in food-supplemented group and seven in control group), although we were able to sex them. We found that egg mass varied significantly with laying order (first laid, 21.22 ± 0.28 , $n=41$; middle laid eggs, 21.21 ± 0.17 ,

$n=116$; last laid eggs, 20.60 ± 0.28 , $n=40$, GLMM, $F_{1,141}=5.01$, $p=0.027$) and did not differ between control and food-supplemented clutches (GLMM, treatment \times laying order, $p=0.785$). HS and FS were not significantly different between groups (GLMMIX, HS \times treatment and FS \times treatment, both $p>0.240$). We did not find any effect of the treatment on hatching time of each egg (in relation to the first hatched egg), on egg size or hatching asynchrony (GLM, all $p>0.635$). Considering all nests, we did not find any effect of our treatment on secondary sex ratio (GLMMIX, $p=0.690$).

Sexual differences in egg and hatchling masses

The sex of eggs was not correlated with hatching order or laying order, and there was no interaction among these three variables (GLMMIX, all $p>0.960$). Results of mixed models showed that female eggs were heavier, although not significantly, than male eggs (Table 1) but that the interaction between sex and treatment on egg mass was significant (Table 1; Fig. 1). In control nests, female eggs were significantly heavier (3.4%) than male eggs (GLMM, $F_{1,41}=5.73$, $p=0.021$; Table 1; Fig. 1), but not so in the food-supplemented group (GLMM, $p=0.780$; Table 1; Fig. 1). Food-supplemented females produced female eggs with no significant difference in mass to control females, and likewise for male eggs (GLMM's, both $p>0.410$). Egg masses with respect to laying order and sex were similar between groups (GLMM, treatment \times sex \times laying order, $p=0.302$). Sex of initial eggs did not affect the egg mass of middle or last laid eggs in control clutches (GLMM, sex initial egg \times laying order, $p=0.292$), nor were they affected by our treatment (GLMM, sex initial egg \times laying order \times

treatment, $p=0.786$). We did not find any effect of sex of the initial egg on hatchling mass within the brood (GLMM, sex initial egg \times hatching order, $p=0.635$), nor was this influenced by the experiment (GLMM, sex initial egg \times hatching order \times treatment, $p=0.913$).

Sexual differences found in eggs were maintained in hatchlings in the control group (Table 1). Female hatchlings were significantly heavier than males (males, 14.28 ± 0.33 g, $n=26$; females, 15.64 ± 0.30 g, $n=29$), whereas no differences were observed in the food-supplemented group (males, 14.85 ± 0.29 g, $n=32$; females, 15.06 ± 0.29 g, $n=29$; Table 1). Controlling for egg mass, female hatchlings were still heavier than males, although the difference was not significant (GLMM, $F_{1,78}=3.72$, $p=0.057$; egg mass as covariate, $F_{1,78}=121.31$, $p<0.001$).

We found that hatching span between males and females differed between groups and hatching order (GLMM, treatment \times nestling sex \times hatching order, $F_{2,79}=4.23$, $p=0.018$). Specifically, we found that hatching span between males and females differed between groups in last hatched eggs (GLM, treatment \times nestling sex, $F_{1,26}=6.93$, $p=0.014$), but not in middle hatched eggs (GLMM, $p=0.271$). We found that hatching time of the last hatched female in relation to the first hatched chick in the clutch was shorter in the food-supplemented group than in the control group (GLM, $F_{1,12}=3.19$, $p=0.008$; Fig. 2).

Effect on male and female offspring

Nestling mass was positively related to egg mass (GLMM, $F_{1,87}=20.58$, $p<0.001$). This relationship was not different between groups (GLMM, egg mass \times treatment, $p=0.801$). We found that nestling mass at 24 days old differed between sexes and groups (Table 1; Fig. 3). Females were larger and heavier than males in both treatments, although nestling mass was different between sexes and groups (Table 1; Fig. 3). Male nestlings of food-supplemented broods weighed 11.9 g less (5.9%) than males in the control group (GLMM, $F_{1,38}=2.77$, $p=0.033$; Fig. 3). We did not find between-group differences in mass of nestling females (GLMM, $p=0.378$).

Discussion

Our experiment showed that prelaying environmental conditions influenced maternal distribution of resources by modifying investment between male and female eggs. We also found that the latest hatched females showed shorter hatching times in the food-supplemented group than in the control group. A third result showed that males from the food-supplemented group gained less mass than in the control group. However, food supplementation during the prelaying

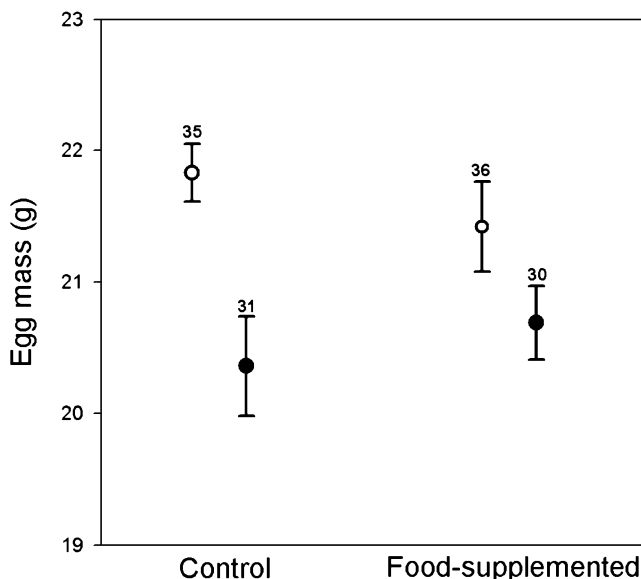


Fig. 1 Mean (\pm SE) egg mass according to egg sex and treatment corrected by nest. Full symbols represent males, and empty ones represent females, and numbers show the sample size

Table 1 GLMM with normal error and identity link function on egg mass, hatchling mass and nestling mass

| Response term | Explanatory term | <i>F</i> | df | <i>P</i> |
|----------------|------------------|----------|------|----------|
| Egg mass | Treatment | 0.22 | 1,89 | 0.644 |
| | Treatment × sex | 4.99 | 1,89 | 0.028 |
| | Sex | 3.37 | 1,89 | 0.070 |
| Hatchling mass | Treatment | 0.00 | 1,80 | 0.983 |
| | Treatment × sex | 6.22 | 1,80 | 0.015 |
| | Sex | 11.43 | 1,80 | 0.001 |
| Nestling mass | Treatment | 20.14 | 1,87 | <0.001 |
| | Treatment × sex | 5.41 | 1,87 | 0.022 |
| | Sex | 0.12 | 1,87 | 0.732 |

The model retained the variance of nests introduced into the model as a random term (in all models, the random term was significant, $Z > 3.33$, $p < 0.001$; see “Materials and methods” for more details). Other interactions between the variables related to treatment were not significant.

period did not result in a modification of breeding parameters such as laying date, clutch size, brood size, nestling mass, weight of the brood, hatching or nestling success. In previous experimental studies in Eurasian (Beukeboom et al. 1988; Aparicio 1994, 1999; Korpimäki and Wiehn 1998) and American kestrels (Wiebe and Bortolotti 1994), food supply at early stages of reproduction had strong effects on different reproductive parameters. However, food supplementation in these studies was prolonged until the onset of incubation (Beukeboom et al. 1988) or at least until the end of laying (Wiebe and Bortolotti 1994; Korpimäki and Wiehn 1998; Aparicio 1999). Because we finished feeding when the first egg was laid, we suggest that food supplementation before laying in our study was not enough for females to develop more follicles.

This experimental design did not result in a mass gain in parent females probably due to the time elapsed between the end of food-supplementation and the capture of females (see “Materials and methods”). We find unlikely that adult males may explain the absence of effect of our food supply experiment on body mass of their mates by taking part of the extra food we provided. During 2005 and 2006, we have observed kestrel behaviour during the non-fertile, pre-fertile and fertile stages before egg laying in our study area (P. Vergara and J.A. Fargallo, unpublished data), and adult females were always in nest surroundings (they do not or rarely hunt), and males spent most of time hunting far from the nest (same pattern for British kestrels; Village 1990). Thus, although it is expected that males may eat part of the food supplemented, the fraction consumed by them is

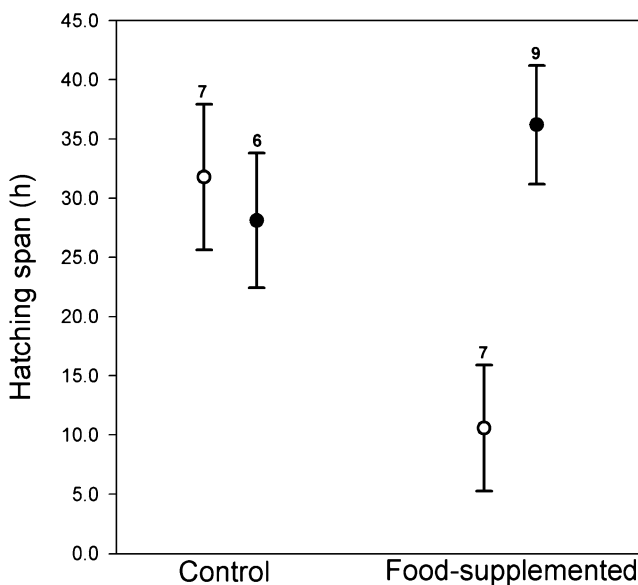


Fig. 2 Mean (\pm SE) hatching span with respect to the treatment and the sex of the last hatched chick. Full symbols represent males, and empty ones represent females. Numbers above bars show the sample size

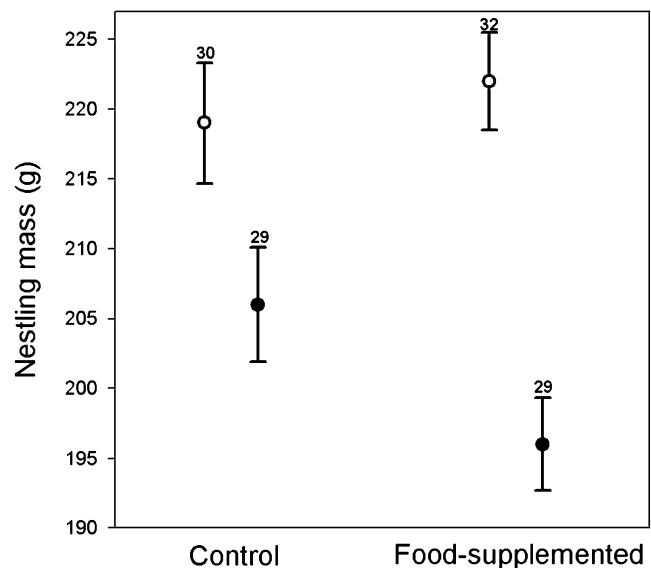


Fig. 3 Mean (\pm SE) nestling mass at 24 days of age according to egg sex and treatment corrected by nest. Full symbols represent males, and empty ones represent females. Numbers above bars show the sample size

also expected to be small. Also, despite the fact that male nestlings reached a lower body mass than those in the control group, no differences in the total offspring biomass were observed. This is due to the fact that the food-supplemented group fledged, overall, more chicks (24-day-old nestlings) than the control group, although this difference was not enough to reveal between-group significant differences in brood size. Thus, we do not have any evidence that overall parental investment differed between groups. These results show that food supply did not alter egg mass in the clutch independently of chick sex. Chick sex could therefore be an important component of maternal investment.

Higher investment in particular eggs within a clutch has been previously reported in several bird species (Mead et al. 1987; Cordero et al. 2000, 2001; Cunningham and Russell 2001; Magrath et al. 2003; Arnold and Griffiths 2003; Müller et al. 2005; Anderson et al. 1997). Our results indicate that when females perceive that breeding will be performed under good food conditions, they decrease sexual size-dimorphism in eggs, and consequently also in hatchlings, which is expected to lower the degree of sibling competition (Anderson et al. 1997; Blanco et al. 2003a). However, this decrease occurs by reducing the investment in eggs of the larger sex (females) and increasing it in eggs of the smaller sex (males). This is unexpected because American and Eurasian kestrels seem to invest more resources in eggs of the smaller sex (only in first-laid eggs; Blanco et al. 2003a). We do not have a clear explanation for this result, as what we would expect is that when adult females predict more food for breeding, they increase their investment in eggs of the smaller sex without reducing investment in the larger sex. One possibility is that egg size of each sex can only be enlarged until a given threshold such that the only option for mothers to reduce the intersexual differences in egg size is to modify the investment in both sexes rather than only by increasing it in the smaller sex. However, there is no support for this idea in previous studies, and this should be tested.

Our results suggest that prelaying food conditions affected kestrel females' decisions when investing in eggs, depending on egg sex. In addition, in an 8-year study in Finland (Laaksonen et al. 2004, although see Dijkstra et al. 1990b), food condition did not either affect sex ratio variation. Both results suggest a weak capability of kestrels to manipulate offspring sex ratios at fecundation. Interestingly, our results suggest that food supplementation did not mean an overall increase in egg mass, but rather a subtle adjustment of the investment in male and female eggs. Previous studies assumed a strong heritability of egg size, as environmental conditions rarely affect egg mass or size (Williams 1994) and had suggested this heritability in kestrels (Martínez-Padilla 2006). However, none of the

studies tested the effect of sex. Thus, our study stresses the importance of sex to explore maternal investment in eggs because a subtle degree of maternal manipulation of egg size may appear when sex is considered.

It has been previously suggested in kestrels that male eggs are heavier than female eggs in the same study area, although this is based only on first-laid eggs in 2000 (Blanco et al. 2003a). Two years later, in the same population, we found that female eggs were larger than male eggs in our control group. Previous findings suggest that females of *Carpodacus mexicanus* under different environmental conditions may change their investment between male and female embryos, as the reproductive value is different under different environmental conditions (Badyaev et al. 2002a, b). Within this context, we do not know the reproductive value of male and female nestlings so we cannot suggest an explanation for this swapping pattern. However, environmental (weather) conditions may help to explain why investment in male and female eggs was different between 2000 and 2002.

It has been suggested that rainy conditions may constrain foraging effort in American kestrels (Dawson and Bortolotti 2002), which may be crucial when adult males are feeding their mates in April in our study area (unpublished data). In 2000, rain in April was the highest recorded in the area ($1,616.0 \text{ mm}^3$) in 13 years (1993–2005), while in 2002 (485.5 mm^3), it was lower than the average (518.3 mm^3). Our experiment does not allow us to elucidate the reason why females changed their investment in male and female eggs, but we suggest that weather may constrain male foraging effort, and hence, the food provided to females in the prelaying period, potentially influencing female allocation decisions. This reveals the necessity for more studies manipulating food abundance at different breeding stages to understand what proximate and ultimate factors affect maternal decisions on egg investment.

We also found that last-hatched females hatched earlier than males in the food-supplemented group but not in the control broods. However, this difference did not result in a between-group difference in the overall hatching asynchrony probably because the variance of hatching span between males and females was lower than the variance due to hatching asynchrony between nests. We speculate that sex-dependent hormone allocation may play a key role, as they may modify hatching patterns (Sockman and Schwabl 2000; Lipar and Ketterson 2000; Eising et al. 2001). We can also not rule out a carry-over effect of supplementation on female condition where a potential improvement of incubation efficiency may influence hatching patterns. Our results suggest a maternal capacity to manipulate sex-dependent hatching patterns associated with food resources, as suggested previously but never experimentally tested. This may explain why males in the food-supplemented

group showed lower body mass than the control group. The smaller nest-mates or sex in kestrels can lose body mass or deteriorate in health condition under food shortage (Dijkstra et al. 1990a; Fargallo et al. 2002). Shortening hatching time between eggs in the clutch involves increasing levels of sibling competition that can have adverse effects for smaller nest-mates (Stoleson and Beissinger 1995). The diminished growth of male nestlings in the food-supplemented group with respect to males in the control group is understood as an erroneous prediction by adult females about the real abundance of food resources, which was an unexpected effect of the experiment.

In conclusion, our experiment failed to find any effect on classic reproductive parameters such as clutch size, egg mass, laying date, hatching success or fledging success, but found a more subtle sex-dependent resource distribution in eggs that might influence the dynamics of sibling competition. Our study also provides evidence of the capability of female kestrels to reallocate resources invested in each offspring sex at the time of laying, which has not been previously demonstrated using experimental approaches. This reorganisation may have promoted different patterns of hatching that resulted in a differential growth of the offspring sexes. The low probability of nestling mortality (only 12 out of 163 nestlings) may indicate the high quality of our study area. It is likely that food supplementation over a certain threshold may imply subtler effects as described in our study rather than those previously shown in kestrels (Beukeboom et al. 1988; Aparicio 1994, 1999; Korpimäki and Wiehn 1998). Thus, further experiments are necessary to determine the proximate mechanism that modulates sexual differences in maternal investment under different environmental conditions.

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