

Mass provisioning to different-sex eggs within the laying sequence: consequences for adjustment of reproductive effort in a sexually dimorphic bird

GUILLERMO BLANCO*, JESÚS MARTÍNEZ-PADILLA*,
DAVID SERRANO‡, JOSÉ A. DÁVILA* and JAVIER VIÑUELA*

*Instituto de Investigación en Recursos Cinegéticos (CSIC-UCLM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain; and ‡Department of Applied Biology, Estación Biológica de Doñana (CSIC), Avda Maria Luisa s/n, 41013 Sevilla, Spain

Summary

1. In birds, the potential maternal ability to adjust resource allocation to different eggs in the clutch might have a major effect on the survival expectancies of particular nestlings or entire broods. We assessed whether sexually size-dimorphic Eurasian kestrels *Falco tinnunculus* (Linnaeus) are able to adjust their reproductive effort by adopting different strategies of egg mass provisioning according to egg sex and laying order.

2. Initial eggs bearing male embryos were heavier than initial eggs bearing female embryos, but no differences in egg mass associated to sex were detected for eggs laid subsequently. Furthermore, in clutches started with a male egg, egg mass declined in subsequent eggs, while in clutches started by a female egg the opposite trend in within-clutch egg-mass variation was found. This suggests differential deposition of resources invested in initial eggs of different sex leading to saved or depleted resources for subsequent eggs.

3. Daughters from initial eggs hatched earlier than sons from initial eggs, which may enhance survival of smaller siblings hatched later. These contrasting strategies of egg provisioning and hatching patterns depending on the sex of the first-laid egg were associated, respectively, with marked mass hierarchies and a lack of mass hierarchies at fledgling in broods initiated with eggs bearing sons and daughters.

4. Parental kestrels may allocate reproductive effort by promoting favouritism towards early hatched chicks or by avoiding any favouritism by producing siblings of each sex with similar mass. This may be achieved depending on the female ability both to identify egg sex and to partially reabsorb or differentially allocate resources to eggs accordingly to adjust reproductive investment. This may be a key mechanism to control sibling competition in birds with sexual dimorphism in mass.

Key-words: brood hierarchies, differential resource allocation, sexual egg dimorphism.

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Introduction

Life-history theory predicts that if offspring fitness is affected by sex, reproductive effort should concentrate investment in a particular sex or brood which sex composition deals with the highest fitness expectations per unit of parental investment (Bortolotti 1986; Stearns 1992). This may be enhanced if parents have some con-

trol on the sex composition of the progeny or if parents may identify offspring sex and then invest accordingly (Emlen 1997), which should be made at an early stage of propagule production to minimize costs and maintain the investment control (Stearns 1992). In birds, numerous studies have assessed the reproductive value of eggs of different mass within the laying sequence according to different parental strategies of resource allocation (Slagsvold *et al.* 1984). These strategies have been argued to be adopted by different species depending on adaptations and constraints characterizing their life-histories (Slagsvold *et al.* 1984; Viñuela 1997). For instance, some species lay a small final egg to enhance

Correspondence: Guillermo Blanco, Instituto de Investigación en Recursos Cinegéticos (CSIC-UCLM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain. Fax: 926 29 54 51; E-mail: gblanco@irec.uclm.es

the hierarchy imposed by hatching asynchrony, whereas others lay a relatively large final egg to enhance the survival of last hatched chicks (Slagsvold *et al.* 1984; Viñuela 1997, 2000). Reproductive effort has also been suggested to be potentially adjusted through sex-biased laying orders (Bortolotti 1986) and differential provisioning to different-sex eggs (Anderson, Reeve & Bird 1997). However, the value of different egg-mass allocation strategies for adjusting reproductive effort according to sex composition and sequence of the brood have not so far been addressed directly by any investigation.

In sexually dimorphic species, the offspring of the larger size sex generally have faster growth and increased metabolic rates, and thus a higher demand of resources, so they are usually more expensive to rear (Bortolotti 1986; Anderson *et al.* 1993). The establishment of a competitive brood hierarchy due to egg provisioning biased by laying order may promote that the larger sex further increases its competitive head start if hatched in first order (Bortolotti 1986; Bednarz & Hayden 1991; Blanco *et al.* 2002; Krebs *et al.* 2002). Therefore, the costs for parental fitness and offspring survival may be not independent of the gender composition and sequence of the brood (Bortolotti 1986). Sex-biased laying order may be a valuable mechanism of parental control of sibling competition, because the access to resources and then the survival probability of particular chicks over their siblings may thus be enhanced or reduced (Bortolotti 1986; Blanco *et al.* 2002). By logical extension, this potential control of sibling competition may be translated to the control of offspring number, quality and sex ratio in the brood. Females may adjust reproductive investment further by allocating resources to eggs according to the sex of the embryo because egg mass and composition may influence hatching success, hatchling mass and nestling growth, and thus survival and recruitment (Williams 1994; Schawbl 1996; Styrsky, Eckerle & Thompson 1999; Eising *et al.* 2001).

The potential maternal ability to adjust the resources allocated to different-sex eggs laid in different orders might have a major relevance in the life expectancies of particular nestlings or entire broods, although this has never been documented. Alternatively, a biased provisioning according to laying order and sex might not be the result of female control, but rather an epiphenomenon due to female behavioural and physiological changes from before to during laying. In the latter case, differential investment on different-sex eggs according to laying order might not cause any advantage to chicks depending on the eggs from which they hatched. To determine whether sexual egg dimorphism and sex-biased laying order are adaptive traits or non-adaptive consequences of an underlying process, it may be necessary to investigate the consequences of such bias for particular nestlings and entire broods. A study of sexual egg dimorphism in American kestrels (*Falco sparverius* L.) suggested that eggs pro-

ducing sons were larger than eggs producing daughters in each laying order to help parents to manipulate mass relationships between their offspring (Anderson *et al.* 1997). Other recent studies of sexual egg dimorphism have, however, not explored further the consequences of such dimorphism for nestlings because embryos were sexed after killing them before hatching (Cordero *et al.* 2000, 2001).

In this paper we assessed whether Eurasian kestrels *Falco tinnunculus* are able to adjust their reproductive effort by adopting different strategies regarding the control of mass provisioning to different-sex eggs laid in different orders. In birds that lay multiple-egg clutches, first-hatched chicks may influence growth and survival of their smaller siblings but chicks hatched later in the sequence generally do not affect to their larger siblings (Viñuela 2000; Blanco *et al.* 2002). In addition, first-laid eggs are often only significantly sex-biased, or sex bias is more marked than in eggs laid later in the laying sequence (Bortolotti 1986; Bednarz & Hayden 1991; Leroux & Bretagnolle 1996; Arnold, Griffith & Goldizen 2001; Blanco *et al.* 2002). Therefore, we predict an important role of the combination of sex and mass of initial eggs in the adjustment of reproductive effort in sexually dimorphic birds. To explore this prediction we assessed whether starting the clutch with a male or female egg, and to differentially allocate resources accordingly may be different parental strategies to enhance the optimal investment in offspring.

Methods

FIELD PROCEDURES

We monitored breeding of Eurasian kestrels (a sexually size-dimorphic raptor) nesting in nest boxes during the breeding season of 2000 in Campo Azálaro grasslands, central Spain (Fargallo *et al.* 2001, Blanco *et al.*, in press). Nest boxes were monitored during egg laying and eggs were marked as they were laid (one egg each 2 days, see also Wiebe, Wiehn & Korpimäki 1998), so laying date and laying sequence were known accurately. Eggs were weighed to the nearest 0.25 g on the day of laying. Before and 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 ink in the hatching tooth in the case of eggs found during hatching process, and later with ink on the head until banding. We visited the nests each 5 days after hatching of the last chick in an attempt to collect dead nestlings in order to sex them. Nestlings were weighed to the nearest 0.25 g the day of hatching and 24 days after hatching (just prior to fledging). Nestling sex was determined by molecular procedures using DNA extracted from a drop of blood obtained by brachial venipuncture. Nestlings that died and did not disappear from the nests ($n = 5$) were sexed by the same method using body tissues as a source of DNA.

STATISTICAL ANALYSES

The overall population sex ratio was analysed using the binomial test. Factors influencing brood sex-ratios were examined by fitting generalized linear models (GLM) with binomial error distribution and logistic link function (GENMOD procedure of SAS), where the response variable was number of males in a brood over brood size. Explanatory variables included laying date, clutch size, number of lost eggs and number of lost chicks.

We performed generalized linear mixed models (GLMM) where log-transformed egg and chick mass were employed as response variables using a normal distribution of errors and an identity link function (GLIMMIX macro of SAS). To test for variables influencing egg mass, we considered egg sex and laying order as fixed factors and clutch size and laying date as covariates while controlling by the influence of the nest, which was treated as a random factor in the manner of a randomized complete block design to avoid pseudo-replication. Laying order was encoded in three categories (i.e. first, middle and last egg laid), which may potentially have different reproductive values according to different within-clutch egg-mass hierarchies and because there were different clutch sizes. We first confirmed that there was no difference in mass between eggs laid in different orders within the middle category (mixed model using the CONTRAST procedure available in SAS; all P -values > 0.05).

Some of the explanatory variables could covary, so we fitted their effects to the observed data following a forward stepwise procedure, testing the significance of each variable and adding only the variable that resulted in a better fit of the model. The significance of the remaining variables was tested again until no additional variable or interaction reached significance. Quadratic and cubic terms were also tested in the models to account for potential nonlinear relationships. 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

SEX RATIO AND LAYING ORDER

We sexed 136 hatchlings (65 males, 71 females) from 31 broods, and we did not find any bias in overall sex ratio of chicks (binomial test $P = 0.67$). This sample included five chicks that died by starvation (four males and one female), while other 10 chicks, probably also starved, disappeared rapidly from the nests, so they could not be sampled in order to sex them. Sex-ratio at fledgling (61 males, 70 females from 31 broods) did not significantly differ from a binomial distribution, both considering all broods (47% males, binomial test, $P = 0.49$) and broods where all eggs laid produced a fledgling (50% males, binomial test, $P = 1.00$, $n = 64$ chicks from 13 clutches). The sex ratio of eggs laid in each order did

not differ from parity (binomial tests, all P -values > 0.31). Similar results were obtained when egg order was encoded as initial ($P = 0.68$), middle ($P = 0.28$) or last laid ($P = 0.45$). Brood sex ratios were not related to clutch size, laying date and number of eggs or chicks lost when all broods were considered (GLM, all P -values > 0.32). Similarly, sex-ratio of broods where all eggs produced a fledgling was not related to laying date or clutch size (all P -values > 0.66).

SEXUAL DIMORPHISM IN EGG MASS

We were able to assign 108 marked eggs to its corresponding nestling (53 males, 55 females) from 28 broods, to compare the mass of eggs bearing embryos of different sex laid in different orders. Results of the mixed model showed a significant effect of laying order ($F_{2,74} = 5.01$, $P = 0.0091$), a marginally significant effect of egg sex ($F_{1,74} = 3.64$, $P = 0.060$) and a significant effect of the interaction between both factors on egg mass ($F_{2,74} = 3.63$, $P = 0.031$). These results indicated that egg mass tended to decline along laying sequence, and that sex affected mass only in first-laid eggs (tests of effect slices, first-laid eggs: $F_{1,74} = 9.39$, $P = 0.0030$; middle-laid eggs: $F_{1,74} = 0.01$, $P = 0.9395$; last-laid eggs: $F_{1,74} = 0.01$, $P = 0.9395$). Thus, first-laid eggs bearing a female embryo were lighter than first-laid eggs with a male embryo, and of similar mass to that of last laid eggs (Fig. 1), but there was no sexual dimorphism in the mass of eggs laid in middle or last orders (Fig. 1). In addition, egg mass decreased significantly as laying date increased ($F_{1,74} = 10.59$, $P = 0.0017$), but there was no significant effect of clutch size on egg mass ($F_{1,74} = 0.07$, $P = 0.79$). This model explained 22.55% of the original deviance without considering the deviance explained by parental and nest characteristics, which were controlled for by including the nest as a random term ($Z = 3.33$, $P = 0.0004$).

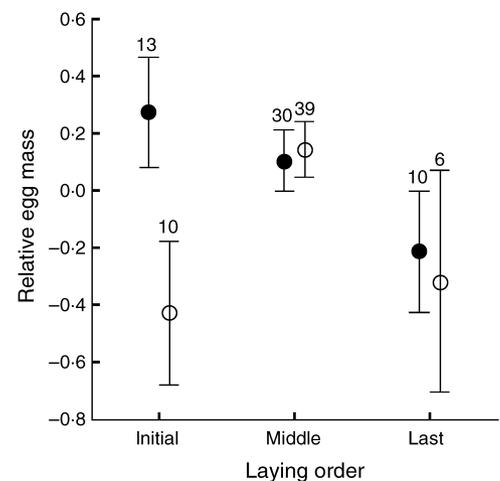


Fig. 1. Sexual dimorphism in mass of Eurasian kestrel eggs in relation to laying order. Egg mass was expressed as mean \pm SE relative egg mass (egg mass minus the clutch mean) to represent intraclutch variation. Full symbols represent males and open symbols represent females. Numbers above bars are sample sizes.

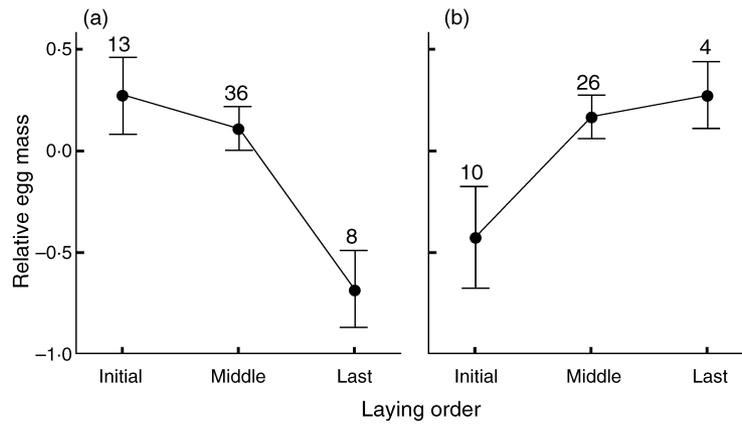


Fig. 2. Mean \pm SE relative egg mass (egg mass minus the clutch mean) according to laying order in (a) clutches initiated with an egg bearing a son or (b) a daughter. Numbers above bars are sample sizes. Unsexed eggs within the categories ‘middle’ and ‘last’ eggs have been included.

Table 1. GLMM with normal error and identity link function on mass of Eurasian kestrel eggs, where sex of the first-laid egg in each nest and laying order (first egg laid, middle and last egg laid) were included as fixed factors. This model explained a 24.84% of the original deviance, without considering the deviance explained by parental and nest characteristics which were controlled for by including the nest as a random term ($Z = 2.98$, $P = 0.0015$). Other potentially influencing variables such as clutch size and egg sex, and the remaining interactions between the variables, were not significant ($P > 0.88$). Parameter estimates and SE for the levels of fixed factor were calculated considering a reference value of zero for last order level in the variable ‘laying order’ and for the female level in the variable ‘sex of initial egg’

Variables	Parameter estimate (SE)	F	d.f.	P
Sex of initial egg (male)	-0.00207 (0.03727)	1.84	1, 69	0.1793
Laying order (initial)	-0.03936 (0.02141)	1.42	2, 69	0.2475
Laying order (middle)	-0.00885 (0.01982)			
Sex of initial egg (male) \times laying order (initial)	0.08549 (0.02663)	5.50	2, 69	0.0061
Sex of initial egg (male) \times laying order (middle)	0.04781 (0.02420)			
Laying date	-0.00440 (0.00216)	4.16	1, 69	0.0452

CONSEQUENCES OF CLUTCH INITIATION WITH EGGS BEARING SONS OR DAUGHTERS

Given that only initial eggs were sexually dimorphic, we explored which possible consequences for broods could have that females started the clutch with an egg bearing a son or a daughter. We have also explored whether starting the clutch with an egg bearing a son or a daughter influenced the mass of eggs laid subsequently. For these analyses, we excluded five clutches where the sex of the initial egg was unknown because we failed to assign it to their corresponding hatchling. Given that no sexual dimorphism was found for middle or late orders, we have included in the analyses all eggs measured within those categories, even those unsexed (larger sample sizes in Fig. 2 with respect to Fig. 1). There was no significant difference in laying date ($t_{21} = 0.16$, $P = 0.87$) and clutch size (Mann–Whitney U -test, $Z = 0.14$, $P = 0.93$) between nests initiated with eggs bearing sons ($n = 13$) and daughters ($n = 10$). We conducted a GLMM where log-transformed egg mass was the response variable, egg sex, laying order, and the variable ‘sex of the initial egg in each nest’ were fixed factors, and considering the effect of other potentially influencing variables (Table 1). Results showed a significant interaction between ‘sex of initial egg’ and

laying order (Table 1), suggesting that the relationship between egg mass and laying order was different for clutches initiated with eggs bearing sons or daughters. Thus, females starting the clutch with an egg bearing a son decreased the mass of the eggs laid subsequently in the laying sequence (Fig. 2a), while the opposite strategy was adopted by females initiating the clutch with an egg bearing a daughter (Fig. 2b). In addition, we found that egg mass decreased with laying date (Table 1).

Hatching asynchrony, i.e. the number of days that elapsed between the hatching of the first and the last chick, did not differ between clutches initiated with eggs bearing sons ($n = 13$) and daughters ($n = 10$) (Mann–Whitney U -test, $Z = 0.032$, $P = 0.97$). There was no significant difference in the number of eggs lost (Mann–Whitney U -test, $Z = 0.41$, $P = 0.74$), number of chicks hatched ($Z = 0.37$, $P = 0.74$), nestlings lost ($Z = 0.41$, $P = 0.74$) or number of chicks fledged ($Z = 0.60$, $P = 0.56$) between nests initiated with eggs bearing sons ($n = 13$) and daughters ($n = 10$). The number of sons (2.85 ± 0.90) and daughters (1.77 ± 1.01) fledged from clutches initiated with an egg bearing a male were, respectively, higher ($t_{21} = 3.65$, $P = 0.002$) and similar ($t_{21} = 1.54$, $P = 0.14$) than those fledged from clutches initiated with an egg bearing a female (sons: 1.50 ± 0.85 , daughters: 2.50 ± 1.27).

Table 2. GLMM with normal error and identity link function on mass of Eurasian kestrel fledglings, where fledgling sex, sex of the first-laid egg in each nest and hatching order (first hatched, middle and last hatched) were included as fixed factors. This model explained a 46.78% of the original deviance, without considering the deviance explained by parental and nest characteristics which were controlled for by including the nest as a random term ($Z = 2.47$, $P = 0.0068$). Other potentially influencing variables such as laying date, clutch size, laying order, hatching mass, number of brothers and sisters and the remaining interactions between the variables, were not significant ($P > 0.23$). Parameter estimates and *SE* for the levels of fixed factor were calculated considering a reference value of zero for last order level in the variable 'laying order', female level in the variable 'sex of initial egg', and for the female level in the variable 'fledgling sex'

Variables	Parameter estimate (SE)	<i>F</i>	d.f.	<i>P</i>
Sex of initial egg (male)	-0.14330 (0.05014)	4.10	1, 63	0.0471
Fledgling sex (male)	-0.08794 (0.01704)	26.63	1, 63	< 0.0001
Hatching order (initial)	-0.01104 (0.04037)	2.18	2, 63	0.1217
Hatching order (middle)	-0.00966 (0.03744)			
Sex of initial egg (male) × hatching order (initial)	0.13550 (0.05248)	3.33	2, 63	0.0420
Sex of initial egg (male) × hatching order (middle)	0.09311 (0.05033)			
Egg mass	0.01637 (0.00796)	4.23	1, 63	0.0440

However, sex ratio at fledging in nests initiated with eggs bearing sons (61% males, $n = 59$) and daughters (38% males, $n = 40$) did not differ from parity, although opposite trends were apparent (binomial test, $P = 0.12$ and $P = 0.16$, respectively).

Given that eggs of different sex laid in different orders might differ in laying and hatching order in nests initiated with eggs bearing sons or daughters, we performed a mixed model (logistic link function, binomial error) where the sex of the eggs was the response variable. In this form, we assessed simultaneously whether laying and hatching order were sex-biased in both kind of nests. We controlled by the influence of the nest, which was treated as a random factor. This analysis revealed that the sex of the eggs was not biased by laying order ($F_{1,72} = 0.72$, $P = 0.35$), but that females hatched earlier than males ($F_{1,72} = 7.25$, $P = 0.009$). In addition, there was a significant effect of the variable 'sex of initial egg in the clutch' showing that the number of hatched chicks of each sex differed between broods from clutches initiated with eggs of each sex ($F_{1,72} = 7.84$, $P = 0.007$). That is, there were more sons among nestlings hatched in nests initiated with a male egg, and more daughters in nests initiated with eggs bearing females. In addition, sex ratio of chicks hatched in each order did not differ from parity in broods from clutches initiated with an egg bearing a son or a daughter (binomial test, all P -values > 0.13), except for first-hatched eggs from clutches initiated with eggs bearing daughters, which were female-biased (76% females, $n = 17$, binomial test $P = 0.049$).

FLEDGING MASS IN BROODS INITIATED WITH EGGS BEARING SONS AND DAUGHTERS

We explored whether the observed differential investment in egg mass according to sex of initial eggs and laying order influenced the mass of particular fledglings. Therefore, the mass of fledglings of each sex from nests initiated with an egg bearing a male or a female embryo was compared by conducting a GLMM where fledgling sex and 'sex of the initial egg in each nest' were

fixed factors, and also considering other potentially influencing variables (Table 2). Hatching order was included as a factor with three levels (first, middle and last order) because of the predictable differences in fledgling mass with hatching order according to brood-survival or brood-reduction strategies potentially adopted by different females. Results showed that fledgling mass was higher in females than males (Table 2), as expected, due to reversed sexual dimorphism, and that fledgling mass increased as did egg mass (Table 2). In addition, there was a significant effect of the interaction between hatching order and the variable 'sex of initial egg' (Table 2). This interaction indicated that fledgling mass decreased from first- to last-hatched chicks in nests initiated with eggs bearing sons (Fig. 3a), while fledgling mass of chicks hatched in different orders did not differ in nests initiated with eggs bearing daughters (Fig. 3b).

Discussion

SEXUAL DIMORPHISM IN EGG-MASS BIASED BY LAYING ORDER

We found that females may potentially adjust reproductive investment by differentially allocating resources to eggs according to the sex of the embryo. To our knowledge, only five other studies have reported sexual size-dimorphism in size of avian eggs (Mead, Morton & Fish 1987; Anderson *et al.* 1997; Cordero *et al.* 2000, 2001; Cunningham & Russell 2001). Furthermore, we found evidence that female kestrels may allocate resources to eggs differentially depending simultaneously on sex and laying order. That is, initial eggs bearing a male embryo were heavier than initial eggs bearing a female embryo, but no differences in mass associated to sex were found for eggs laid later in the laying sequence. To our knowledge, this striking result provides the first evidence of sexual egg dimorphism biased by laying order.

Parental favouritism expressed in differential resource allocation to different-sex eggs may be a mechanism to adjust allocation for both the primary and secondary

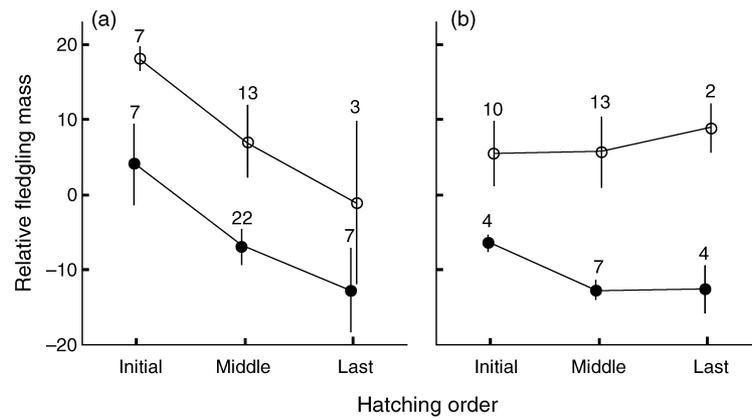


Fig. 3. Mean \pm SE relative fledgling mass (fledgling mass minus the brood mean) according to hatching order and sex (open symbols are females, full symbols are males) in (a) clutches initiated with an egg bearing a son or (b) a daughter. Numbers above bars are sample sizes. Note that there were male and female chicks hatched in first order in both nests initiated with eggs bearing sons and daughters because laying and hatching order of particular eggs may differ (see Results).

sex-ratio of clutches in order to increase parental and offspring fitness (Cordero *et al.* 2000, 2001). We found that differential investment in eggs bearing sons or daughters was not related to the proportion of the sexes in the first vs. subsequent eggs, and that sex-ratio was not biased by laying order. Other studies have shown that first-laid eggs are only significantly sex-biased or more biased than later-laid eggs (Emlen 1997). Laying order may be biased towards the larger (Leroux & Bretagnolle 1996; Arnold *et al.* 2001) or the smaller sex (Bednarz & Hayden 1991; Blanco *et al.* 2002) in sexually size-dimorphic birds. Furthermore, opposite trends have been found in different populations of the same species (Clotfelter 1996; Kilner 1998) and between years with contrasting food conditions (Dzus, Bortolotti & Gerrard 1996). Our results suggest that differential investment in offspring may also be reached by differential provisioning of eggs according to the sex of the first-laid egg, rather than biasing sex ratios of initial eggs, although we cannot discard that female kestrels could be able to choose the sex of first-laid eggs.

SEX OF INITIAL EGGS AND EGG-MASS HIERARCHIES WITHIN CLUTCHES

Intraclutch egg-mass variation may result from physiological or nutritional constraints on the laying female, but it may also be an adaptive response to enhance the parental control of sibling competition (Slagsvold *et al.* 1984; Viñuela 1997). These alternatives are not mutually exclusive for explaining within-clutch egg mass variation between species, as it may depend on how the resources for laying are gathered (Viñuela 1997). Different strategies of intraclutch egg-mass variation might also occur within species if individuals differ in how and how many resources for laying are gathered and allocated within the clutch, as suggested by experimental food supplementation before and during laying altering egg-mass hierarchies within clutches (Simmons 1994; Wiebe & Bortolotti 1996; Aparicio 1999). Our

results indicate that females starting the clutch with an egg bearing a daughter increased mass investment on eggs laid subsequently in the clutch, while females laying a first egg bearing a son invested a decreasing amount of nutrients in subsequent eggs. This is the first evidence suggesting that different individuals of a given population may adopt different strategies of intraclutch egg-mass variation depending on the sex of initial eggs.

Constraints such as 'gearing up' physiologically for starting egg production may explain relatively small initial eggs (Parsons 1976; Leblanc 1987), while prolactin influence on developing follicles during incubation or depletion of reserves at the end of laying may explain relatively small last-laid eggs (Leblanc 1987; Arnold 1991). However, these proximate mechanisms cannot explain why a relatively large or small initial egg should be associated with a particular sex and furthermore, why this association should influence the pattern of investment on subsequent laid eggs in the clutch. Our results suggest that resources invested on initial eggs bearing daughters would be partially reabsorbed or differentially provisioned when passing through the oviduct to result in relatively small eggs, and saved resources should be then invested in relatively larger eggs laid subsequently in the laying sequence independently of their sex. On the other hand, resources invested in relatively large initial eggs bearing sons would be not restricted and then reserves should be increasingly reduced due to their depletion during the laying period.

THE DILEMMA OF THE INITIAL-EGG SEX AND ITS CONSEQUENCES FOR OFFSPRING

Different strategies of resource allocation according to egg sex and laying order may be different forms of investment in the progeny by influencing the degree of sibling competition. According to the 'brood-survival' hypothesis, females may allocate a relative large amount of resources on eggs laid in last order to decrease the probability that the last-hatched nestling will starve.

Alternatively, females may invest a relatively larger amount of nutrients on initial eggs to enhance some mortality due to the hierarchy imposed by hatching asynchrony under unpredictable food shortages ('brood-reduction' hypothesis). In our study, different patterns of intraclutch egg-mass variation were not found being translated to differences in egg losses, hatching asynchrony or offspring mortality. Brood reduction in this population of kestrels is relatively rare (< 10% of hatched chicks died), due probably to high food availability and optimal hunting habitat, and thus the possible effects of intraclutch egg mass variation on brood reduction may be difficult to detect. Alternatively, our results suggest that parents may be able to invest in their progeny through different egg-mass hierarchies, depending on the sex of the first-laid eggs, and this may allow to reduce possible nonadaptive mortality induced by increasing sexual dimorphism in the mass of chicks along growth (Bortolotti 1986).

Regarding brood composition, we found that clutches initiated with an egg bearing a male fledged more sons than those initiated with an egg bearing a female. The opposite trend, albeit not reaching significance, was found for the number of daughters. Even if these trends influenced the distribution of sexes among fledglings from clutches initiated with different-sex eggs, sex-ratios at fledgling did not differ from parity in both kinds of nests, and perhaps these non-significant results could be caused by the relatively small sample size in these analyses (see West & Sheldon 2002). The small difference between the number of sons and daughters raised in both kinds of nests may be a simple effect of considering the sex of the initial egg as the grouping variable. Alternatively, these patterns may be due to differences in environmental or parental features, such as condition, age, breeding experience or the onset of incubation start, influencing the ability to raise broods with different sex ratios. We lack data on parental characteristics to test these predictions, but a negative relationship between female condition and proportion of male offspring (the cheaper sex) have been found in this species (Korpimäki *et al.* 2000) and in the closely related American kestrel (Wiebe & Bortolotti 1992). Therefore, it remains possible but untested that different sex allocation strategies associated with different egg-mass hierarchies and sex of initial eggs depend on parental characteristics.

A fine-tuned egg mass vs. laying sequence strategy has been suggested to be unlikely to evolve in American kestrels because females seem not to have the ability to always hatch eggs in the same order they are laid (Bortolotti & Wiebe 1993). Eurasian kestrels have been suggested to be able to hatch eggs in the order they were laid (Wiebe *et al.* 1998). However, the possibility that the lack of matching between laying and hatching orders would enhance the adjustment of parental effort when eggs of different sex are provisioned differently has been not addressed before. We found that daughters from initial eggs, which were relatively lighter than

the remaining eggs in the clutch, hatched earlier than sons from initial eggs, which were relatively heavier than the remaining eggs in the clutch, due probably to differences in the duration of incubation associated to sex (Blanco *et al.* in press). Therefore, females may be adjusting egg-mass provisioning to initial eggs of different sex in an attempt to control sex-sequence at hatching and then possible mass hierarchies within the brood (Bortolotti 1986; Blanco *et al.* 2002; Krebs *et al.* 2002). Thus, first-laid eggs bearing daughters would be relatively lighter, to compensate for faster growth rate of female chicks if hatched in first order (see Blanco *et al.* 2002). In this case, daughters from initial eggs may not take advantage of the brood hierarchy due to the mass of the eggs from which they hatch. In contrast, initial eggs bearing sons would be relatively heavier in an attempt to compensate for their lower growth rate and delayed hatching.

Different strategies of egg provisioning and hatching patterns were associated, respectively, with mass hierarchies and the lack of mass hierarchies without differential mortality in broods initiated with eggs bearing sons and daughters. Thus, fledgling mass decreased with hatching order in nests initiated with eggs bearing sons, while fledgling mass of chicks hatched in different orders did not differ in nests initiated with eggs bearing daughters. Overall, mean mass of both male and female fledglings did not differ between both kinds of broods. However, fledgling mass differences due to hatching order in nests initiated with a male egg could result in differential mortality during breeding seasons with a food shortage or after fledgling. We do not know if females were able to control the sex of initial eggs, but we have showed that they were apparently able to identify their sex and provision them accordingly to presumably maintain the subsequent control of investment. This control may be achieved due mainly to the clear influence that egg mass has on hatchling mass (Williams 1994; Christians 2002), and then the potential influence that egg-mass allocation within the clutch may have on the brood mass hierarchy (Slagsvold *et al.* 1984; Viñuela 1997). In addition, this control may be achieved through the influence that first-hatched chicks may have on growth and survival of their siblings in sexually dimorphic species with variable degrees of hatching asynchrony (Bortolotti 1986; Blanco *et al.* 2002; Krebs *et al.* 2002).

In conclusion, the combination of small initial eggs and the larger mass of subsequent eggs laid in the clutch may smooth intrabrood mass hierarchy when the first egg laid bears an embryo of the larger-size sex. In contrast, relatively heavier initial eggs, and the lightest mass of final eggs in the clutch, enhances intrabrood mass hierarchy when the first egg laid bears an embryo of the smaller-size sex. These strategies may potentially be adopted to (1) compensate differences in mass induced by genetically determined sexual differences in growth rate, thus avoiding possible maladaptive intrabrood mass hierarchies induced by asynchronous hatching, while (2) maintaining a given degree of intrabrood

mass hierarchy favouring early hatched chicks. However, the outcome of these strategies may vary depending on environmental or parental conditions, or both, which need be investigated further. The main conclusion of this study reinforces the evidence that egg mass may potentially be controlled depending on the female ability both to identify egg sex and to differentially allocate resources accordingly to adjust reproductive investment. More research is needed on the role of the combination of sex and mass of eggs laid in particular orders, especially initial eggs, in the adjustment of reproductive effort in birds with marked sexual dimorphism in mass.

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