Hatching asynchrony and brood reduction influence immune response in Common Kestrel *Falco tinnunculus* nestlings

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The onset of incubation before the end of laying imposes asynchrony at hatching and, therefore, a size hierarchy in the brood. It has been argued that hatching asynchrony might be a strategy to improve reproductive output in terms of quality or quantity of offspring. However, little is known about the mediating effect of hatching asynchrony on offspring quality when brood reduction occurs. Here, we investigate the relationship between phenotypic quality and hatching asynchrony in Common Kestrel *Falco tinnunculus* nestlings in Spain. Hatching asynchrony did not increase breeding success or nestling quality. Furthermore, hatching asynchrony and brood reduction had different effects on nestlings' phytohaematogglutinin (PHA)-mediated immune response and nestling growth. In asynchronous and reduced broods (in which at least one nestling died), nestlings showed a stronger PHA-mediated immune response and tended to have a smaller body size compared with nestlings raised in synchronous and reduced broods. When brood reduction occurred in broods hatched synchronously, there was no effect on nestling size, but nestlings had a relatively poor PHA-mediated immune response compared with nestlings raised in asynchronous and reduced broods. We suggest that resources for growth can be directed to immune function only in asynchronously hatched broods, resulting in improved nestling quality, as suggested by their immune response. We also found that males produced a greater PHA-mediated immune response than females only in brood-reduced nests without any effect on nestling size or condition, suggesting that females may trade off immune activities and body condition, size or weight. Overall, our results suggest that hatching pattern and brood reduction may mediate resource allocation to different fitness traits. They also highlight that the resolution of immune-related trade-offs when brood reduction occurs may differ between male and female nestlings.

Keywords: brood reduction, cellular immunity, hatching asynchrony, nestling sex.

Because resources are limited, individuals have to optimize resource allocation among conflicting demands, resulting in trade-offs between fitness-related traits such as growth and survival (Stearns 1992). In birds, incubation commonly starts before clutch completion. This creates an age and size hierarchy within the brood and has a significant effect on nestling growth (Cotton *et al.* 1999, Clotfelter *et al.* 2000) and survival (Forbes *et al.* 1997, Viñuela 2000). Therefore, it is not surprising that evolutionary ecologists have attempted to understand the reasons why female birds seem to compromise their offspring fitness as a consequence of starting incubation before the end of laying. Multiple adaptive hypotheses, together

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with potential constraints, have been proposed to explain this pattern (Stoleson & Beissinger 1995). These hypotheses suggest that evolutionary pressures could be acting on different breeding stages or fitness traits, such as: (1) egg viability during incubation, (2) chick survival and growth during nestling or fledgling periods, or (3) parental survival and condition (reviewed by Stoleson & Beissinger 1995). Thus, different evolutionary pressures could be acting depending on the species (Stoleson & Beissinger 1995) or even within a species (Wiebe *et al.* 1998, Viñuela 1999). Specifically, experimental studies suggest that opposing selective pressures could regulate hatching asynchrony depending on prevailing conditions (Wiebe *et al.* 1998, Viñuela 2000).

The basic premise behind adaptive hypotheses to explain hatching asynchrony is that the observed pattern of hatching asynchrony should maximize fitness (Dawson & Bortolotti 2002). Available studies provide support that hatching asynchrony may depend on maternal condition prior to laying (Wiebe & Bortolotti 1994, Hanssen *et al.* 2002). However, most studies of hatching asynchrony in birds have estimated reproductive performance using classical variables such as growth rate, fledging mass or nestling survival (Stoleson & Beissinger 1995). Studies assessing the effects of hatching asynchrony on other aspects of fitness, such as physiological condition of fledglings, are scarce (Christe *et al.* 1998). It has been suggested that physiological condition, measured as cellular immunity, may be a good predictor of offspring quality (Moreno *et al.* 1998, Hõrak *et al.* 1999, Soler *et al.* 1999, Tella *et al.* 2000); thus, this could be a relevant trait to evaluate.

The death of the last hatched chick in a brood (brood reduction) is particularly common in raptors, and may be promoted by hatching asynchrony (Newton 1979). Several studies in Common Kestrels *Falco tinnunculus* have shown that an increased food supply reduces nestling mortality (Korpimäki 1989, Dijkstra *et al.* 1990, Wiehn & Korpimäki 1997), suggesting that brood reduction might reflect poor environmental conditions. Thus, nestling quality, particularly their immune capacity, might be compromised when food is scarce (Saino *et al.* 1998, Hõrak *et al.* 1999, Merino *et al.* 2000, Fargallo *et al.* 2002). Male and female nestlings differ in behaviour and physiology, and this becomes crucial in sexually dimorphic species such as Common Kestrels, as the sexes exhibit different patterns of growth and mortality (Fargallo *et al.* 2002, Laaksonen *et al.* 2004). In fact, recent studies demonstrate that inter-sexual differences in immune response are expected during the nestling period under stressful environmental conditions such as food shortage (Common Kestrels, Fargallo *et al.* 2002) or parasitism (Great Tits *Parus major*, Tschirren *et al.* 2003).

Lack (1947) suggested that hatching asynchrony might promote the death of the last hatched chick under food scarcity in order to maximize reproductive output. Under this scenario, the quality of surviving nestlings should be improved when mortality occurs in asynchronously hatched broods. However, the effects of hatching asynchrony and brood reduction on fitness components other than offspring production, such as immune capacity, remain unknown. In this paper, we explore the mediating effects of hatching asynchrony, nestling sex and brood reduction on Common Kestrel nestling quality, using phytohaematogglutinin (PHA)-mediated immune response as a proxy.

METHODS

Laying order and hatching pattern

The study was conducted in 2000–2002 in a Common Kestrel population breeding in nestboxes in the Campo Azálvaro region (40°40′N, 4°20′W, 1300 m asl), a homogeneous mountain grassland area in central Spain. In those 3 years, food abundance (voles, birds and lizards, the main prey in this area) was similar (our unpubl. data). Female Common Kestrels lay eggs every 2 days, so we monitored nestboxes every 1–2 days during the prelaying and laying periods to estimate laying date. Eggs were marked with pens as they were laid in order to identify the laying sequence. Adult females were captured by netting at the nestbox between a week after the end of laying and a week before the onset of hatching. We recorded their body mass to the nearest gram and wing chord as a proxy of wing length to the nearest millimetre. Four to 5 days before the expected date of hatching (25 days after the last egg was laid), we started to monitor nestboxes daily until we detected the first clues of hatching (chick sounds inside the unbroken eggshell). We then returned two or three times (as required) each day until hatching was complete. Hatching span was estimated as the number of hours elapsed between the first and the

last hatched egg in a brood (Viñuela 2000). Broods were considered asynchronous when hatching span was equal to or longer than 24 h, and synchronous broods were those in which all eggs in the brood hatched in <24 h (Stoleson & Beissinger 1995). In order to assign each nestling to its corresponding egg, hatchlings were identified by marking them with indelible ink on the hatching tooth when eggs were found during the hatching process, and subsequently on the head until ringing (Blanco *et al.* 2003a, 2003b). Twenty-four days after the first egg hatched, all nestlings were measured and a blood sample was taken. We chose to measure chicks at that time because (1) that is when we took measurements of nestlings in our population, allowing between-year comparisons and (2) it is the closest age to fledging without any risk that the nestlings fly off when we reached the nest. However, a small proportion of nestlings that had a delayed hatching in relation to the first egg hatched in the brood could have been younger than 24 days old (see below). All nestlings were weighed to the nearest gram and their wing chord was measured to the nearest millimetre. The difference in wing chord between nestlings to their largest sib in the brood was taken as the size difference. The blood sample was used to determine nestling sex by molecular procedures (Fridolfsson & Ellegren 1999) using DNA extracted from a drop of blood obtained by brachial venipuncture. We considered that brood reduction had occurred when at least one chick in the brood died.

Immune response

To evaluate the efficacy of immune response, an antigen was injected to generate a local immune reaction. Twenty-four days after hatching of the first chick in a nest, all chicks were injected intradermally in the left wing web with 0.1 mL of a solution of PHA (3 mg PHA dissolved in 1 mL PBS–PHA; L8754-50MG, Sigma, St Louis, MO, USA) after measuring thickness at the point of injection. Twenty-four hours after injection, we measured wing web thickness again; we used the difference between initial and final measurement as an indicator of PHA-mediated immune response (Smits *et al.* 1999). Three measures were taken with a digital spessimeter (Mitutoyo Absolute ID-112B) to the nearest 0.01 mm before and after injection. The repeatability of initial ($F_{338,678} = 130.275$, $P < 0.001$) and final ($F_{338,678} = 205.384$, $P < 0.001$) measurements was high, so we used the mean values of the three measurements in the analyses. PHA-induced swelling generates a complex immune response involving acquired and innate components of the immune system in birds (Martin *et al.* 2006). Therefore, we refer in this study to PHA-induced swelling more generally as the 'PHA-mediated immune response', rather than the more traditional 'T-cell-mediated immune response'. The same person (J.M.-P.) took all measurements using the same spessimeter.

Statistical procedures

We ran two sets of models. In the first, we explored the factors that may explain hatching asynchrony and brood reduction in our population. The detected variables that explained hatching asynchrony and brood reduction were included in the second set of models to avoid potential confounding factors. The second set of models explored the effect of hatching patterns (synchronous or asynchronous hatched clutches) and brood reduction on nestling quality.

Logistic regression models were used to assess how different variables could explain hatching asynchrony and brood reduction. For the first model, hatching asynchrony (synchronous vs. asynchronous broods) was considered the dependent variable, and year, parent female mass and wing chord, laying date and clutch size were included as explanatory variables. Brood reduction was the dependent variable for the second model, with the following explanatory variables: brood size at hatching, hatching span and the same explanatory variables used in the first model. Thus, nest was the sample unit in both models.

We used general linear mixed models (GLMMs; SAS 1989–1996 Institute Inc., Cary, NC, USA) to assess the association between hatching asynchrony and brood reduction on nestling quality. Nestling sex was included as a factor in all models described from here on. As hatchlings and fledglings from the same nest had more similar characteristics than those from different nests due to the influence of sharing common parents and rearing environments, they were not considered independent samples. Therefore, we avoided pseudoreplication by including 'nest' nested in 'year' as a random factor (in all analyses $Z > 3.92$, $P < 0.001$). Females that bred several times during the study period were only considered in the analyses the first time they

did so. With this procedure, we could consider nestlings as the sample unit. We ran four GLMMs fitted to normal distribution of errors and an identity link function where five variables of nestling quality were considered: PHA-mediated immune response, body mass, wing chord, size differences and body condition. We considered body condition when body mass (dependent variable) was corrected by wing chord as a covariate in the models. In addition to including the explanatory variables described above in these models, laying order and hatching order were included as factors, and egg mass was fitted as a covariate. In Common Kestrels, eggs do not hatch in the same order as they are laid (Blanco *et al.* 2003b), so we consider these two variables as independent. Laying and hatching order were encoded as first-, last- and middle- (in between) laid/hatched eggs, allowing us to compare laying and hatching sequences across different clutch sizes. To explore hatching order, we only considered those chicks that could be assigned to the egg from which they hatched (84.1% of all hatchlings).

PHA-mediated immune response, nestling mass and wing chord were included as covariates when they were not the dependent variable. As PHA-mediated immune response shows daytime variation in Common Kestrels (Martínez-Padilla 2006), the hour of sampling was coded into three equal periods (Martínez-Padilla 2006: 1 or morning, from 08:30 to 12:30 h; 2 or midday, from 12:30 to 17:00 h; 3 or evening, from 17:00 to 20:55 h) and included as a factor when cellular immunity was the dependent variable. Hatching asynchrony may be affected by clutch size: clutches of three eggs ($n = 4$) always hatched synchronously and clutches of six ($n = 14$) and seven eggs ($n = 2$) always hatched asynchronously. Also, sample sizes for largest and smallest clutch sizes were relatively low. Thus, we only considered modal clutches of four and five eggs ($n = 86$, 78.7% of all broods studied) in this study; these were the only ones that could hatch synchronously or asynchronously (see Wiebe & Bortolotti 1994 for a similar procedure). Variance inflation factors (VIFs) suggest that our explanatory variables were not collinear (all VIFs < 2.89). All tests are two-tailed and means ± sd are given. Some of the explanatory variables could co-vary, so we fitted their effects to the observed data following a forward stepwise procedure in both set of models, testing the significance of each variable and adding only the variable that resulted in a better fit of the model.

RESULTS

Hatching asynchrony and brood reduction

Of the total number of eggs laid in the 3 years of the study ($n = 439$), 83.6% hatched, 7.7% did not hatch and the rest (8.7%) were predated. From those eggs that hatched, 9.7% ($n = 43$) of chicks died during the nestling period. We were able to determine the hatching pattern for 86 broods. Time elapsed between the first and last egg hatching was $13 ± 9$ h for synchronous ($n = 26$) and $48 ± 20$ h for asynchronous ($n = 60$) broods. Within these broods, brood reduction occurred in 39.1% of asynchronous broods and in 10.7% of synchronous broods. Logistic models showed that hatching asynchrony was related only to laying date (Wald = 5.2876, $P = 0.022$). Asynchronous broods were more common than synchronous broods as the breeding season progressed. By considering only those nestlings for which we accurately knew day of death, we found that, on average, chicks died when they were $7.5 ± 4.6$ days old ($n = 35$) and 89.5% died before they were 15 days old. Logistic regression indicated that hatching span was the only variable significantly related to brood reduction (Wald = 16.182, $P < 0.001$), explaining 77.3% of the overall variance of the model. Most nests in which brood reduction occurred (75.9%) hatched asynchronously.

Offspring sex and phenotypic traits

Nestling wing chord was lower when brood reduction occurred (GLMM, $F_{1,155} = 4.24$, $P = 0.040$) and this relationship was different depending on hatching pattern (GLMM interaction: hatching asynchrony × brood reduction, $F_{1,155} = 4.28$, $P = 0.034$, Fig. 1a). Analysed separately, brood reduction led to a decrease in wing chord of 6.4% in nestlings hatched in asynchronous broods (GLMM, $F_{1,171} = 7.19$, $P = 0.006$, Fig. 1a) but not in synchronous broods (GLMM, $F_{1,45} = 0.25$, $P = 0.620$). When brood reduction occurred, nestlings from asynchronously and synchronously hatched broods had similar wing chord (GLMM, $F_{1,46} = 2.66$, $P = 0.075$). Female nestlings were bigger than males (GLMM, $F_{1,153} = 17.15$, $P < 0.001$), but nestling sex did not change any of the relationships described above (all $P > 0.342$).
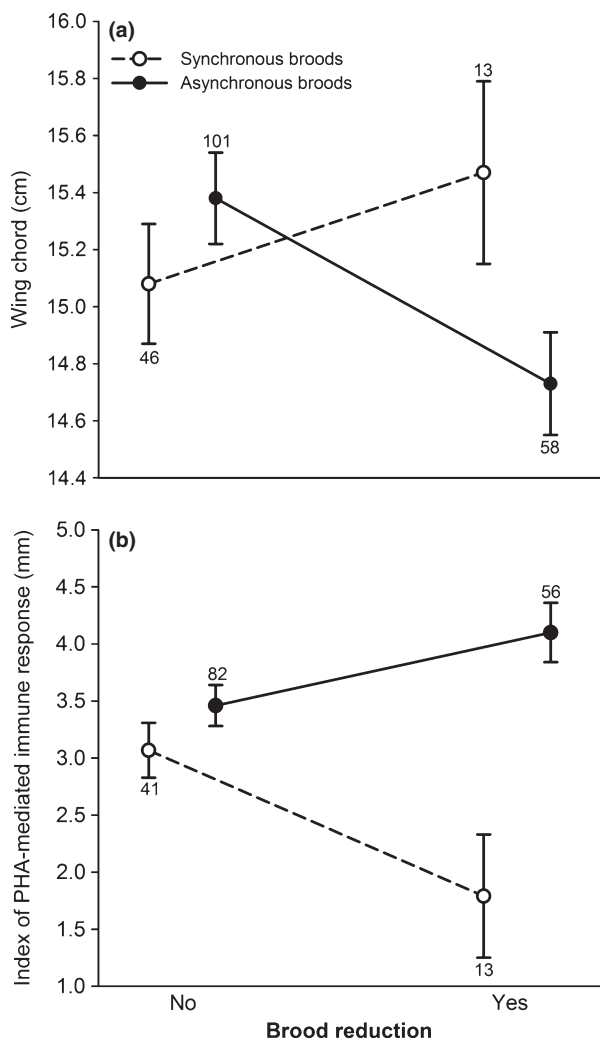
Figure 1. Mean ± se relative wing chord (a) and PHA-mediated immune response (b) of nestlings in relation to hatching pattern and mortality in Common Kestrels. Numbers above and below bars denote sample size.

Female nestlings were heavier (GLMM, $F_{1,164} = 13.54$, $P < 0.001$), and overall nestling mass was similar between brood-reduced and non brood-reduced nests (GLMM, $F_{1,164} = 1.88$, $P = 0.172$). The effect of brood reduction on nestling mass did not depend on hatching pattern (GLMM, $F_{1,164} = 0.25$, $P = 0.618$) or sex (GLMM, $F_{1,164} = 0.01$, $P = 0.952$). Nestling body condition was similar between synchronous and asynchronous broods (GLMM, $F_{1,194} = 1.84$, $P = 0.360$), and the pattern was similar when brood reduction occurred (GLMM, $F_{1,198} = 0.65$, $P = 0.421$). Nestling sex or its interaction with other explanatory variables did not affect any of these relationships (all $P > 0.124$).

We assessed PHA-mediated immune response of 369 chicks from 69 different broods. The average immune response of nestlings was 3.31 ± 1.11 mm (2000: 3.81 ± 1.20 mm, $n = 101$; 2001: 2.87 ± 1.08 mm, $n = 135$; 2002: 3.41 ± 0.75 mm, $n = 133$). We assessed PHA-mediated immune response only in those chicks from clutches of four or five eggs ($n = 192$ chicks). After controlling for the significant effect of nestling mass ($F_{1,156} = 35.58$, $P < 0.001$; Table 1), GLMMs showed that the relationship between immune response and hatching asynchrony varied depending on the occurrence of brood reduction (Table 1, Fig. 1b). Specifically, we found that PHA-mediated immune response was higher when brood reduction occurred in asynchronous broods ($F_{1,80} = 2.69$, $P = 0.025$). In contrast, PHA-mediated immune response was lower in synchronous broods where at least one nestling died than in synchronous broods where all chicks fledged ($F_{1,33} = 8.18$, $P = 0.007$). In relation to sex, the significant effect of the interaction between brood reduction and sex suggests that differences in PHA-mediated immune response were different in relation to brood reduction (Table 1, Fig. 2). Specifically, in females when brood reduction occurred, PHA-mediated immune response was lower than when all nestlings survived ($F_{1,104} = 20.8$, $P = 0.039$, Fig. 2), but not different in males ($F_{1,104} = 0.01$, $P = 0.991$, Fig. 2). The interactions of sex with any other explanatory variables were not significant (all $P > 0.101$).

Analysing the covariance between wing chord and PHA-mediated immune response, we found that hatching pattern mediated this relationship (wing chord × hatching asynchrony × brood reduction, $F_{2,106} = 4.09$, $P = 0.046$). To analyse the significance of this interaction, we explore it separately in relation to hatching patterns. In asynchronously hatched broods, the relationship between PHA-mediated immune response and wing chord changed depending on whether the brood was reduced or not (brood reduction × wing chord, $F_{1,65} = 8.46$, $P = 0.005$). Specifically, PHA-mediated immune response and wing chord were negatively related in asynchronous and reduced broods ($F_{1,29} = 6.64$, $P = 0.015$), but not in those in which brood reduction did not occur ($F_{1,53} = 0.23$, $P = 0.629$). We did not find any pattern in synchronously hatched broods (brood reduction × wing chord, $F_{1,32} = 0.01$, $P = 0.943$).

Table 1. GLMM testing the effect of hatching asynchrony on immune response of Common Kestrel nestlings. The model explained 26.3% of the original variance.

Response term	Explanatory term	Rejected term	*F*	df	*P*†	Estimate ± se
PHA-mediated immune response	Brood reduction*		1.46	1,104	0.229	−0.111 ± 0.608
	Nestling mass		16.81	1,104	**<0.001**	0.016 ± 0.004
	Hatching asynchrony*		15.54	1,104	**<0.001**	−2.368 ± 0.729
	Hatching asynchrony × brood reduction		7.59	1,104	**0.007**	1.837 ± 0.659
	Sampling time*		5.10	2,104	**0.008**	0.966 ± 0.161
	Nestling sex*		0.77	1,104	0.383	0.462 ± 0.328
	Hatching asynchrony × nestling sex		0.70	1,104	0.405	0.261 ± 0.310
	Brood reduction × nestling sex		5.12	1,104	**0.026**	−0.811 ± 0.357
		Hatchling mass	0.71	1,103	0.103	−0.065 ± 0.031
		Brood size	2.49	1,102	0.117	−0.173 ± 0.200
		Wing chord	0.64	1,101	0.427	−0.009 ± 0.010
		Laying order*	0.59	2,99	0.551	−0.058 ± 0.579
		Clutch size	0.20	1,98	0.656	−0.086 ± 0.375
		Size differences	0.08	1,84	0.776	0.001 ± 0.222
		Hatching order*	0.07	2,80	0.935	0.039 ± 0.583
		Laying date	0.00	1,79	0.983	−0.001 ± 0.015

*Parameter estimates for levels of a fixed factor were calculated considering a reference value of zero. In the final model 'hatching asynchrony', 'brood reduction' and 'nestling sex' were maintained to explore interactions.
†Values in bold are significant.
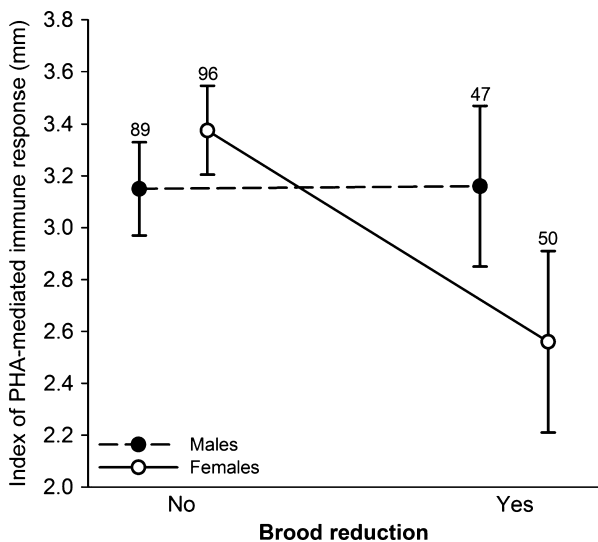
Figure 2. Mean ± se relative immune response in relation to nestling sex and mortality in Common Kestrels. Numbers above bars denote sample size.

DISCUSSION

Our results suggest that brood reduction was more likely to occur in asynchronous than in synchronous broods, and that hatching asynchrony was more frequent later in the breeding season. In addition, PHA-mediated immune response and wing chord showed opposite patterns when brood reduction occurred in asynchronously or synchronously hatched clutches. Specifically, nestlings in asynchronous broods had higher immune response but were smaller when brood reduction occurred.

Hatching asynchrony was not correlated with nestling survival at the end of the nestling stage or with body condition of either the parents or nestlings, as previously suggested for northern populations of American Kestrels *Falco sparverius* (Wiebe & Bortolotti 1995) and Common Kestrels at least in low food years (Wiehn *et al.* 2000). Different studies have described opposite patterns of nesting success according to hatching pattern: synchronous (Wiebe & Bortolotti 1994) or asynchronous broods (Wiehn *et al.* 2000) could maximize reproductive output under certain environmental conditions. In those studies, only nestling mass was evaluated as a measurement of nestling quality. However, other indices of individual quality, such as immune response, could help us to understand better the effects of hatching asynchrony in birds. Although nestling mass could indicate that nestling quality was similar, offspring quality may vary for other fitness traits. In particular, PHA-mediated immune response can be considered a good index of offspring fitness (Hõrak *et al.* 1999, Soler *et al.*

1999, Tella *et al.* 2001, Moreno *et al.* 2005), despite the effects of year (Jovani *et al.* 2004) and hour of sampling (Martínez-Padilla 2006) that were controlled for in this study. Consistent with previous studies, we also found that nestling mass and PHA-mediated immune response were positively associated (Tella *et al.* 2000, Fargallo *et al.* 2002, Jovani *et al.* 2004), supporting the idea that this index can be considered a good proxy of individual quality. We did not find any effect of hatching or laying order on PHA-mediated immune response as previously shown (Christe *et al.* 1998, Müller *et al.* 2003, Banda & Blanco 2008). Other factors acting on PHA-mediated immune response during nestling growth such as brood reduction and hatching pattern may hide more subtle effects than those related to hatching or laying order. Brood reduction mediated the PHA-mediated immune response depending on the hatching pattern of the brood. Specifically, when brood reduction occurred, PHA-mediated immune response was stronger in asynchronously hatched broods and weaker in synchronous ones. This might suggest that nestling mortality in asynchronously hatched nests could be advantageous only when considering physiological condition of the chicks. In contrast to some previous studies suggesting that hatching asynchrony could be maladaptive (Amundsen & Slagsvold 1991, Wiehn *et al.* 2000), we suggest that it may mediate resource allocation priorities. Our results also showed that PHA-mediated immune response and wing chord were negatively associated only in asynchronous broods that also experienced brood reduction, suggesting that hatching asynchrony may promote the trade-off between growth and PHA-mediated immune response.

It has been suggested that maintaining good cellular immunity is energetically costly (Hillgarth & Wingfield 1997) and that there could be a trade-off between cellular immunity and growth (Merino *et al.* 2000). In this sense, chicks from asynchronous broods with brood reduction were smaller but had a stronger PHA-mediated immune response. A similar pattern has been described in House Martins *Delichon urbica* (Christe *et al.* 1998). A growth delay in second clutches was associated with stronger cellular immunity, suggesting a potential trade-off between growth and immune response at the intraspecific level (Merino *et al.* 2000), although this trade-off might disappear at the interspecific level because the cost of

growth is larger relative to immune function (Van del Most *et al.* 2011). Asynchronous broods with brood reduction in Common Kestrels could increase their survival prospects by having a higher immune response (Tella *et al.* 2000) and delayed growth (Merino *et al.* 2000). This may be particularly important late in the breeding season when asynchronous broods were most common and when susceptibility to parasite infection may be higher (de Lope & Møller 1993, Merino & Potti 1995). In synchronous broods, there was no correlation between brood reduction and wing chord or mass, but chicks in these broods had relatively poor immune responses. Thus, we suggest that nestling fitness in these broods could be expressed in other traits not considered in this study. Alternatively, these combined results suggest that the potential trade-off faced by chicks in reduced broods between investing in growth or immune response may be resolved in different ways depending on hatching pattern.

We suggest that there are two more likely, but not mutually exclusive, explanations that account for our results, although we cannot rule out other potential explanations because of the descriptive nature of our study. First, it is possible that this flexibility may be a result of sibling competition. Chicks in asynchronous broods, where size hierarchies are well established, could delay growth but keep a stronger immune system because they do not need to grow quickly to compete with their siblings and, therefore, can allocate those resources to immune functions. The opposite pattern would be found in synchronous broods: chicks may invest more in growth to maintain a similar size to their siblings. Secondly, a physiological mechanism may also explain our results. Corticosterone helps individuals recover from environmental perturbations, but keeping high levels of corticosterone or being unable to metabolize it causes immunosuppression (Buchanan 2000) and, ultimately, affects fitness (Romero & Wikelski 2010). It has previously been seen in Common Kestrels that corticosterone levels compromise growth when food is not restricted (Müller *et al.* 2009). Under food restrictions (brood reduction), it is possible that hatching pattern may reduce competition between sibs, reducing the deleterious effect of corticosterone on growth and/or immunity. Thus, nestlings in asynchronous and reduced broods might pay the cost of corticosterone in terms of growth, but nestlings in synchronous and reduced broods might pay the

cost in terms of immunity. Overall, our results suggest a potential trade-off between growth and immunity which is mediated by hatching pattern, as previously suggested (Merino *et al.* 2000). However, although the effects of PHA-mediated immune response have previously been established (Tella *et al.* 2000, 2002), in our study we have not determined how fitness was affected by these patterns. Nonetheless, it is intriguing to speculate why individuals may sacrifice their growth. Post-fledging parental care in Common Kestrels operates for a month during which parents deliver the prey and siblings have to compete to get it. It has been suggested that in the post-fledging period smaller siblings may have an advantage in competing for prey provided by their parents (Vergara & Fargallo 2008). It is possible that reducing growth might be a disadvantage during the nestling period but might be beneficial during the post-fledging period.

Brood reduction had a sex-dependent effect on PHA-mediated immune response. It has been shown in Common Kestrels that female nestlings can compete better than their male sibs for food, which makes them able to produce a stronger PHA-mediated immune response under food shortage (Fargallo *et al.* 2002). Experimentally enlarged broods in that study were created to reproduce food-shortage conditions, which resulted in higher mortality and female nestlings having better body condition and PHA-mediated immune response. Our results may contradict this idea as females show weaker PHA-mediated immune response. However, brood reduction in our study did not have a sex-dependent effect on nestling body mass, condition or size, suggesting that brood reduction might not be comparable with the sex-dependent effect of food-shortage conditions described above. It is possible that brood reduction in our study might have been caused by food shortage, but the early death of the last hatched chick might have masked inter-sexual differences on nestling condition, size and weight between the surviving nestlings from brood-reduced nests and those from non-reduced nests. We show here that females produced a lower PHA-mediated immune response than males only when nestling mortality occurred. The reason for this inter-sexual difference is unclear but suggests that females may allocate fewer resources to immune purposes than males when nestling mortality occurs, perhaps trading off

immune activities and body condition, size or weight. This might be plausible because growth can be more energetically costly than immune defence (Van del Most *et al.* 2011), especially in female Common Kestrels as it is the bigger sex and may need more resources to grow. Thus, it is possible that males and females face stronger growth-immunity or different trade-offs when brood reduction occurs and may have different strategies to resolve them. However, if female nestlings are better competitors than males in reversed sexually size-dimorphic species (Anderson *et al.* 1993, Fargallo *et al.* 2002), it is still intriguing why females, and not males, had weaker PHA-mediated immune response. The reason is unclear and beyond the scope of this study, but a higher physiological cost for females (the bigger sex) to grow under stressful conditions (Martinez-Padilla *et al.* 2004), sex-dependent access to prey items of different sizes (Anderson *et al.* 1993, Fargallo *et al.* 2003) or production/maintenance of sexual characters (Gil *et al.* 1999, Verhulst *et al.* 1999) are potential explanations for this sex-dependent variation of PHA-mediated immune response between studies.

We suggest that hatching pattern could be linked to different strategies to maximize nestling quality when brood reduction occurs by maintaining nestling size in the case of synchronous broods or improving cellular immunity in the case of asynchronous ones. We have found evidence for these effects by considering a physiological indicator of nestling quality and traditional biometrical variables, suggesting that other variables of nestling quality could also be affected when the effect of hatching asynchrony on nestling quality is explored. Within the context of the potential benefits of a given hatching pattern, we suggest that more evaluations of nestling quality and health indices must be considered for a better understanding of the evolutionary meaning of hatching asynchrony.

in different ways. Jessica Haines greatly improved the English text.

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