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Egg size and offspring performance in the collared flycatcher (*Ficedula albicollis*): a within-clutch approach

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Abstract Adaptive within-clutch allocation of resources by laying females is an important focus of evolutionary studies. However, the critical assumption of these studies, namely that within-clutch egg-size deviations affect offspring performance, has been properly tested only rarely. In this study, we investigated effects of within-clutch deviations in egg size on nestling survival, weight, fledgling condition, structural size and offspring recruitment to the breeding population in the collared flycatcher (*Ficedula albicollis*). Besides egg-size effects, we also followed effects of hatching asynchrony, laying sequence, offspring sex and paternity. There was no influence of egg size on nestling survival, tarsus length, condition or recruitment. Initially significant effect on nestling mass disappeared as nestlings approached fledging. Thus, there seems to be limited potential for a laying female to exploit within-clutch egg-size variation adaptively in the collared flycatcher, which agrees with the majority of earlier studies on other bird species. Instead, we suggest that within-clutch egg-size variation originates from the effects

of proximate constraints on laying females. If true, adaptive explanations for within-clutch patterns in egg size should be invoked with caution.

Keywords Cross-fostering · Intraclutch · Maternal effects · Nestling growth · Offspring fitness

Introduction

Within-clutch allocation of resources by a laying female is an important topic in evolutionary ecology. In addition to studies examining the allocation of resources in relation to laying order (e.g. O'Connor 1979; Slagsvold et al. 1984; Wiggins 1990; Williams et al. 1993a; Cichoń 1997; Viñuela 1997; Hillström 1999), increasing attention is being paid to possible adaptive allocation in relation to egg sex (Weatherhead 1985; Leblanc 1987a; Mead et al. 1987; Teather 1989; Andersson et al. 1997; Cordero et al. 2000, 2001; Rutkowska and Cichoń 2002; Blanco et al. 2003; Cichoń et al. 2003; Magrath et al. 2003). By allocating resources differentially in relation to laying order, females may enhance/impair survival of the later hatching chicks (Slagsvold et al. 1984) or favour chicks with the highest reproductive value (Williams et al. 1993a). By targeting resources to eggs of a particular sex, the female may also obtain two types of benefits. First, in sexually dimorphic species, she may boost performance of the smaller sex to prevent it from starvation due to competition for food with the larger sib of the other sex (Anderson et al. 1997). Second, when in good condition she may increase her fitness by investing selectively resources to the sex with larger variance in reproductive success (Trivers and Willard 1973).

The critical assumption of the adaptive allocation of resources within a given clutch is that the amount of the invested resources has consequences for offspring fitness. For example, it is usually assumed that the larger the egg, the higher the fitness of the offspring that hatches from this egg. This assumption has been most often tested by the cross-fostering approach when eggs/nestlings are swapped

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between nests and performance of offspring in relation to mean egg size of the clutch is analysed (we know of 16 such studies; e.g. Bize et al. 2002; Pelayo and Clark 2003). However, to test the assumption of the within-clutch adaptive allocation specifically, it is better to examine effects of within-clutch deviations in egg size on the performance of individual offspring. First, variation in egg size is typically much greater between females than within clutches of individual females (Christians 2002). Thus, cross-fostering studies work with the egg-size variability that is most probably not available to laying females when allocating resources within a clutch. Second, direct competition between sibs for resources supplied by parents, monopolisation of these resources by dominant sibs and selective parental feeding in relation to offspring size are common within broods (Budden and Wright 2001). Cross-fostering studies working on the between-female level do not take into account these within-family relations and thus may not reliably estimate egg-size effects present on the within-brood level (see also Nilsson and Svensson 1993). Third, positive covariation between direct and maternal pathways of the determination of offspring phenotype may exist, which would lead to overestimation of egg-size effects in cross-fostering design (Krist and Remeš 2004).

Studies testing effects of within-clutch deviations on offspring performance have been done less frequently than cross-fostering studies (we know of nine studies; e.g. Howe 1976; Amat et al. 2001). They often compared average egg size of surviving versus non-surviving siblings instead of looking at individual offspring. Moreover, they did not, for the most part, control for the factors that are known to affect offspring performance. First, all nest-mates may be affected in the same way by brood-level factors (between-year variation in the quality of breeding conditions, advancement of breeding season, territory quality). These factors may be included in the analyses of egg-size effects on offspring performance to reduce unexplained variation and thus increase statistical power of the main test. Second, nest-mates may differ in performance due to hatching asynchrony (Magrath 1990), laying order (Ylimaunu and Järvinen 1987), sex (Becker and Wink 2003), paternity (Sheldon et al. 1997) or concentration of androgens in eggs (Schwabl 1993). These individual-level factors may be, in contrast to brood-level factors, correlated with within-clutch differences in egg size and as such directly confound any relationship between the latter and offspring performance.

To test the assumption of adaptive within-clutch allocation of resources, we examined effects of within-clutch deviations in egg size on individual offspring performance in the collared flycatcher (*Ficedula albicollis*), a small migratory passerine. Besides egg-size effects, we also followed effects of other individual-level factors including hatching asynchrony, laying order, offspring sex and paternity, which makes our study well suited for separating an independent effect of egg size on offspring performance. We examined effects of these factors on nestling survival, weight, fledgling condition, structural

size and offspring recruitment to the breeding population. In addition, we also controlled statistically for some brood-level covariates to render the analyses of egg-size effects more powerful.

Materials and methods

Field methods

The study was conducted in Velký Kosíř forest (49°32'N, 17°04'E, 370–450 m a.s.l.), central Moravia, the Czech Republic, in 2001–2003. In the study area, there were five plots with the total number of about 350 nest-boxes. Three plots were located in coniferous (*Picea abies*) and the other two in deciduous (*Quercus petraea*) forest. Approximately 60 pairs of collared flycatchers bred in the nest-boxes each year.

The study area was visited daily during the breeding season. Each egg was numbered with a waterproof felt-pen and measured to the nearest 0.01 mm with a digital calliper on the day it was laid. Egg volume was calculated using the formula: $\text{volume} = 0.51 \times \text{length} \times \text{width}^2$ (Hoyt 1979). Two measures of width were taken in two perpendicular directions and their average was used as a measure of width. After 10–13 days of initiation of incubation, eggs were taken from nests, put into a thermo-box and then within 10 min of transfer placed into individual compartments in an incubator. Plastic dummy eggs were put into the nests for females to incubate. The method was successful since only one out of 38 artificial clutches was abandoned. Temperature in the incubator ranged between 37 and 39°C, humidity between 40 and 70%. The incubator was checked for newly hatched young at least every 3 h throughout the day and night. Hatching time was recorded for each chick. When hatching was not directly observed, hatching time was approximated as the midpoint between the check when the egg was hatched and the preceding check when the egg was still unhatched. As soon as possible, hatchlings were returned to their nest of origin. The mean time (\pm SD) which elapsed between hatching and the return of the hatchling to the nest was 2.95 ± 2.33 h (range 10 min–10 h). The longer time periods occurred when the young hatched in the evening and starved until sunrise, which is also the case under natural conditions. To ensure that the delay did not affect our results, we included the time elapsed between hatching and returning the hatchling to the nest ("time to return" hereafter) as a covariate into our models (see below). Before their return, the claws of hatchlings were marked by nail-varnish to enable individual recognition. Nestlings were checked daily until they were 13 days old, i.e. close to fledging. Every day, nestlings were weighed to the nearest 0.25 g with a Pesola spring balance and re-marked if needed. Nestlings were ringed when about 7 days old, blood sampled (about 25 μ l) by brachial venipuncture at 10–13 days and their tarsi were measured (to the nearest 0.01 mm) at 13 days. Blood samples were transferred to 1 ml of Queen's lysis buffer (Seutin et al. 1991). Dead nestlings were taken from nests and conserved in 70% ethanol. Putative parents were caught with nest-box traps while feeding nestlings and their blood was sampled in the same way as for nestlings.

Each year nearly all adults breeding in the study area were captured and checked for rings. Thus for the young fledglings in 2001, 2 years of potential recapture as breeding adults were available, but only 1 year for the young fledgling in 2002. It is certain that some individuals that had ultimately recruited to the breeding population were not discovered by us and thus we erroneously treated them as non-recruits. However, under the assumption that the dispersal and the probability of starting breeding in the second year of life are not biased with respect to egg size, our subsample of the recruits is representative. The first part of the assumption seems to be realistic as breeding dispersal is unbiased with respect to other offspring traits such as fledgling weight or tarsus length in this species (Pärt 1990). Concerning the second part of the assumption, it is possible that superior individuals already

start breeding in the second year of life while individuals in bad condition are “floaters” at this time but are recruited a year later, in their third year of life. This would lead to over-representation of individuals in good condition in our sample of recruits and thus overestimation of egg-size effects on recruitment (to the extent that egg size positively affects condition and probability of early breeding). However, this possibility makes our conclusions even more conservative (see below).

Sex and paternity

Nestling sex and parentage were determined using standard methods for the collared flycatcher (Sheldon and Ellegren 1996). In short, DNA was extracted from blood or tissue samples using the phenol-chloroform method. Sex was determined by polymerase chain reaction amplification of the CHD gene using primers P2 and P8 (Griffiths et al. 1998), followed by polyacrylamid electrophoresis. The method was completely accurate: sex of about 60 adults of known sex was determined rightly in all cases. Parentage was determined by comparing genotypes of putative parents and nestlings at three microsatellite loci: FhU2, FhU3 and FhU4. Their combined exclusion power is about 96% in the collared flycatcher (Sheldon and Ellegren 1996). This means that in about 4% of cases nestlings sired by an extra-pair male are erroneously concluded to be sired by the pair male. It was not possible to determine sex and parentage in three offspring due to their disappearance from the nest or decay of tissues.

Samples and statistics

Out of 224 artificially incubated eggs originating from 38 nests, 180 hatched, which represents hatchability of 80.4%. Only nests in which either all or all but one young hatched were used in this study. This ensured a natural level of sibling competition in the studied nests. Mean egg volume of the clutch did not differ between the two groups of nests (nests with high hatchability, mean egg volume \pm SE=1620.9 \pm 23.5 mm³, $n=29$; nests with low hatchability, mean egg volume \pm SE=1635.3 \pm 42.1 mm³, $n=9$; $t=0.3$, $P=0.77$). Further, only nests where both parents were captured, allowing the determination of parentage, were used. Consequently, 121 chicks hatched in 22 nests remained for the analyses. Hatchability in these nests was 92.4%, which equals the natural level (Cramp and Perrins 1993; M. Krist, unpublished data). Clutch sizes were six, five and seven eggs in 19, two and one nest, respectively. All clutch sizes were pooled for the analyses. Nevertheless, results were virtually the same when only six-egg clutches were used (results not shown). Analyses of tarsus length, nestling mass and fledgling condition (residuals from the regression of 13-day body mass on tarsus length; weight=0.852+0.669 tarsus, $n=89$, $P=0.008$, $r^2=0.079$), were based only on nestlings that subsequently fledged, because nestlings that died did not exhibit normal growth for several days before death (i.e. their mass remained constant or even decreased when the mass of their sibs increased). The only exception were young from three nests that were abandoned at the end of the nestling phase, probably due to depredation of parents. These young grew normally before their abandonment and were included in the analysis of nestling mass up to the day before strong mass recession was recorded.

Because the aim of this study was to analyse the effects of intraclutch egg-size variation, egg volume was converted to relative egg volume (hereafter termed “egg size”). This was computed as egg volume minus the mean egg volume of the clutch (i.e. centring). In this way, between-clutch variation is removed and relative egg volume then represents egg-size variation within clutches. To enable comparison between nests, hatching time was computed for every nestling as follows. The value of zero was assigned to the first-hatched young. Time (in hours) elapsed between hatching of the first young and every subsequent nest-mate was assigned to the latter. The resulting variable is hereafter termed “hatching asynchrony”.

To assess the effect of egg size on offspring performance, five models were fitted. The response variables in these models were nestling survival (binomial variable; fledged versus not fledged), recruitment to the breeding population (binomial variable; recruited versus not recruited; only young that fledged successfully were used for this test), fledgling tarsus length, fledgling condition and nestling mass, respectively. The predictor variables were as follows. Firstly, egg size, hatching asynchrony, laying sequence, sex and paternity were retained in all the models as fixed effects of interest. The only exception was the model for nestling survival, which was fitted without paternity because all extra-pair young fledged. In this latter case, maximum likelihood estimates of effects may not exist and thus the validity of the model fit would be questionable. The reason for including the above variables in all final models was that their effects on offspring performance after controlling for the other factors are not known and that is why they may be of interest. Secondly, mean egg volume of the clutch, year, advancement of the breeding season (standardised between years by subtracting the median date of egg-laying in the particular year from the actual egg-laying date), and the time elapsed between hatching and returning of the hatchling to the nest were included in initial models as fixed-effects covariates. To test also for the possibility that the effect of egg size on the response variables depends on brood-level variables, interactions of egg size with year, breeding season and mean egg volume of a clutch were initially fitted in all models. Covariates and their interactions with egg size were selected according to Akaike’s information criterion (AIC). The final model was that with the lowest number of parameters from the series of models which had AIC between the smallest value and the smallest value+2 (see Burnham and Anderson 1998). Thirdly, nest was included as a random effect to control for dependence of data points within nests. Denominator *df* were computed using Satterthwaite’s method. Recruitment and survival were analysed using GLIMMIX macro of SAS (generalised linear mixed model with binomial error and logit link), the other models were fitted using PROC MIXED.

The model for nestling mass was more complex than the other models. First, nestlings were weighed each day until the brood was 13 days old (brood age zero is the day the first egg of a clutch hatched). Hence, an individual nestling was treated as a second random factor nested within nest (i.e. higher-level factor) and age of the brood as an additional fixed effect (for the rationale of the model, see Singer 1998). Second, all the interactions of brood age with fixed effects of interest were initially included in the model to investigate whether the effect of independent variables changes as young grow. Interactions were selected according to AIC as described above.

Hatching asynchrony, laying order and egg size were positively correlated (Fig. 1). Such correlations between independent variables in multiple regression (multicollinearity) could reduce the power of the analyses. To assess the influence of multicollinearity on our significance tests, we looked at variance inflation factors (VIF) for individual predictors. Predictors with VIF<10 are generally accepted as giving unbiased results (Chatterjee et al. 2000). Recently, it was suggested that even VIF as small as 2 might bias results (Graham 2003). In our analyses, VIF for paternity in the three models with the response normally distributed were between 2 and 2.5, VIF for all other predictors in all models were <2. Thus, multicollinearity should not have seriously biased our significance tests. Moreover, in contrast to significance tests, parameter estimates are always unbiased even when multicollinearity is high (Freckleton 2002). All tests are two-tailed and were computed in SAS (SAS Institute 2000).

Results

Egg size was more variable between than within clutches ($F_{21,109}=12.48$, $r^2=0.71$). Intraclutch egg-size variation thus represents about 29% of the total variation, which is the same as the mean figure for 26 studies reviewed by

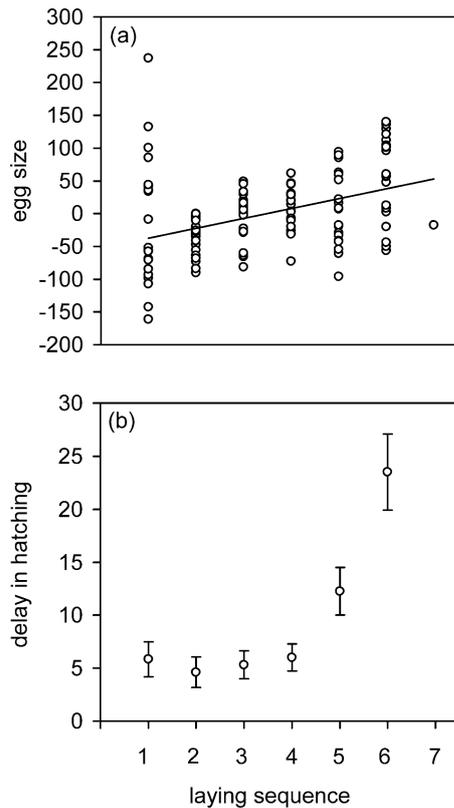


Fig. 1 **a** Egg size as a function of the laying sequence. The regression equation is: egg size (mm^3) = $-52.19 + 14.96$ laying sequence ($F_{1,129} = 24.39$, $P < 0.001$, $r^2 = 0.16$). **b** Time (mean \pm SE; hours) elapsed between hatching of the first-hatched egg in a clutch and the egg at a particular position in the laying sequence of the same clutch in six-egg clutches. The time is never zero because in different nests the first-hatched egg was at a different position (usually one, two, three, or four) in the laying sequence

Christians (2002). The largest egg in a clutch was on average (\pm SD) $177.2 \pm 68.7 \text{ mm}^3$ larger than the smallest one (range $79.9\text{--}332.8 \text{ mm}^3$). Expressed as a percent of the smallest egg, this gives $11.6 \pm 4.3\%$ (mean \pm SD) and $5.0\text{--}19.4\%$ (range). The difference between the largest and the smallest mean egg volume of a clutch was 431.0 mm^3 , which is 31.2% of the smaller mean egg volume. Egg size increased with laying sequence (Fig. 1a). In six-egg clutches, hatching order reflected laying order in such a way that penultimate and ultimate eggs hatched on average about 7 and 18 h later than preceding eggs, respectively (Fig. 1b). Extra-pair paternity was detected in ten of the 22 nests (45%); extra-pair offspring comprised 30 of 128 nestlings/eggs (23.4%). Sex ratio was male-biased: 80 males/48 females ($r = 0.63$). Hatchability was unrelated to egg size (hatched eggs, $-0.10 \pm 5.89 \text{ mm}^3$ (mean \pm SE), $n = 121$; unhatched eggs, $1.15 \pm 20.50 \text{ mm}^3$, $n = 10$; $t = -0.06$, $P = 0.95$).

Egg size did not affect probability of recruitment, nestling survival, fledgling tarsus length and fledgling condition (Table 1). Nestling mass was initially strongly affected by egg size. However, this effect declined steadily as nestlings grew and was not statistically significant after the brood was >9 days old (Table 1, Figs. 2a, b, 3). Weaker

correlation between the mass of 1-day-old nestlings and egg size is probably a methodological artefact caused by the fact that the mass was measured only to the nearest 0.25 g. Therefore, the same mass was assigned to many 1-day-old nestlings although in fact they differed in mass. From a brood age of 3 days onwards, this level of precision was fully adequate as nestlings were much heavier.

In contrast to egg size, hatching asynchrony had a strong effect on offspring performance. Late-hatching young were smaller and had poorer survival than early-hatching young (Table 1). On the other hand, the young from later eggs in the laying sequence were larger than the young from earlier eggs (Table 1). Time to return of offspring to the nest negatively affected offspring survival probability (Table 1). At the time of fledging, extra-pair young tended to be in better condition than young sired by social mates, and sons were in better condition than daughters (Table 1). As it was impossible to assess the effect of paternity on offspring survival while controlling for the effects of other predictors in the generalised linear model (see Materials and methods), we computed at least the probability that extra-pair and pair offspring differ in their survival by Fisher's exact test. Extra-pair young tended to survive better than young sired by social mates ($n = 101$, $P = 0.067$).

To assess the validity of the statistically non-significant results concerning egg size, we computed 95% confidence

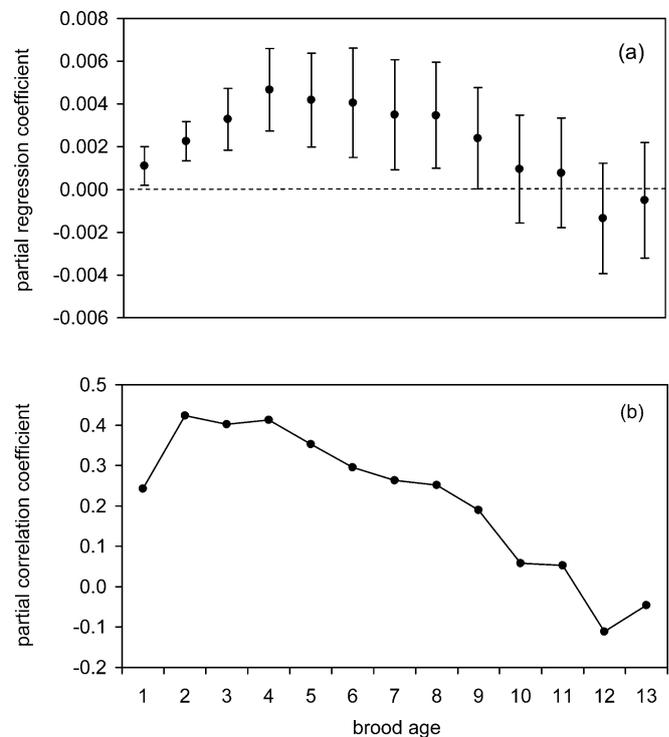


Fig. 2, **a** Nestling mass as a function of the relative egg size in the course of the nestling period. Displayed are **a** partial regression ($\pm 95\%$ confidence intervals) and **b** partial correlation coefficients between the two, partialled with respect to hatching asynchrony, laying order, offspring sex, paternity and nest. Brood age is in days. Brood age zero is the day the first egg of a clutch hatched

Table 1 Parameter estimates and type III *F*-tests of fixed effects for recruitment^a, nestling survival^a, mass, fledgling tarsus length and fledgling condition

	Estimate	SE	<i>df</i> ^b	<i>F</i>	<i>P</i>
Recruitment					
Intercept	-2.976	1.198	83		
Relative egg size	-0.00102	0.00703	83	0.02	0.886
Laying sequence	0.1002	0.335	83	0.09	0.766
Hatching asynchrony	0.0250	0.0460	83	0.30	0.589
Sex	-0.375	0.941	83	0.16	0.691
Paternity	0.260	0.944	83	0.08	0.784
Nestling survival					
Intercept	8.07	1.62			
Relative egg size	0.00868	0.00649	91.6	1.79	0.184
Laying sequence	0.203	0.305	91.1	0.44	0.507
Hatching asynchrony	-0.263	0.0522	93.7	25.42	<0.001
Sex	-0.253	0.659	88.4	0.15	0.702
Time to return	-0.479	0.140	90.5	11.77	<0.001
Nestling mass					
Intercept	1.474	0.224			
Brood age	1.173	0.0323	19.2	1,271.53	<0.001
Relative egg size	0.00456	0.00127	1254	12.91	<0.001
Laying sequence	0.0670	0.0364	164	3.39	0.067
Hatching asynchrony	-0.0664	0.00662	150	100.60	<0.001
Sex	-0.115	0.105	160	1.20	0.275
Paternity	0.0918	0.150	188	0.37	0.542
Relative egg size × brood age	-0.00034	0.000164	574	4.32	0.038
Tarsus length					
Intercept	16.53	1.49			
Relative egg size	-0.00111	0.000699	66.6	2.52	0.118
Laying sequence	0.0744	0.0341	68.1	4.76	0.033
Hatching asynchrony	-0.0203	0.00573	74.6	12.58	<0.001
Sex	0.130	0.0990	67.2	1.74	0.191
Paternity	-0.0107	0.135	81.7	0.01	0.937
Mean egg size	0.00192	0.000915	15.1	4.39	0.054
Condition					
Intercept	0.669	0.385			
Relative egg size	0.000175	0.00124	66.4	0.02	0.888
Laying sequence	-0.0644	0.0604	67.3	1.14	0.290
Hatching asynchrony	0.00135	0.0103	70.7	0.02	0.896
Sex	-0.398	0.174	67.6	5.24	0.025
Paternity	0.465	0.253	78.8	3.39	0.069
Year	-1.06	0.495	16.8	4.62	0.046

^aIn the models for recruitment and nestling survival the probabilities for recruitment/survival are modelled. The model for recruitment does not contain a random factor, all other models contain a random intercept for nest. The variables are coded as follows—sex: male = 0, female = 1; paternity: young sired by social male = 0, by extra-pair male = 1; year: 2002 = 0, 2001 = 1

^bNumerator *df* were always 1

intervals for standardised effect sizes of egg size on fledgling mass, condition and tarsus length (Fig. 4). This method may be preferable to commonly used power analysis because confidence intervals have several advantages as compared to power analysis in evaluating non-significant results (e.g. Steidl et al. 1997; Hoenig and Heisey 2001). Effects were predicted for mean difference between the largest and the smallest egg in the clutch, i.e. 177.2 mm³ (11.6% of the smaller egg). We multiplied this value by the parameter estimate for the effect of egg size on a particular trait and its 95% confidence limits and standardised by dividing them by the SD of the particular trait. Cohen (1988) suggested a convention that the values of standardised effects of 0.2, 0.5 and 0.8 could be treated

as small, medium and large effects, respectively, when two groups are compared. Thus, our data suggest that the difference between the largest and the smallest eggs within clutches could at most cause only small positive effects in fledgling mass and condition (Fig. 4).

Discussion

Egg-size effects

Despite controlling for a number of potentially confounding variables (hatching asynchrony, sex, paternity and laying sequence), we found no effect of within-clutch

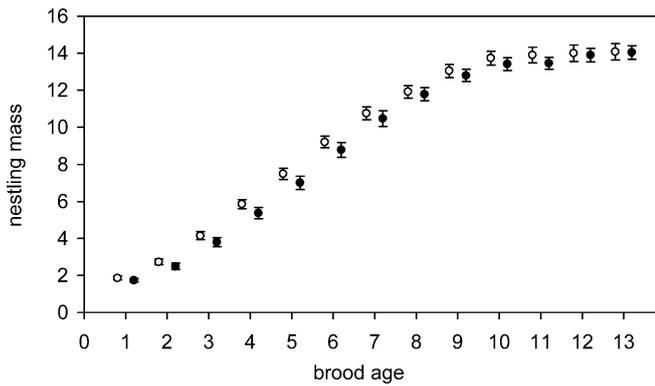


Fig. 3 Nestling mass (mean \pm 2SE, grams) in relation to brood age (days) for nestlings from large (greater than clutch mean) (open circle) and small (smaller than clutch mean) (filled circle) eggs. Brood age zero is the day the first egg of a clutch hatched

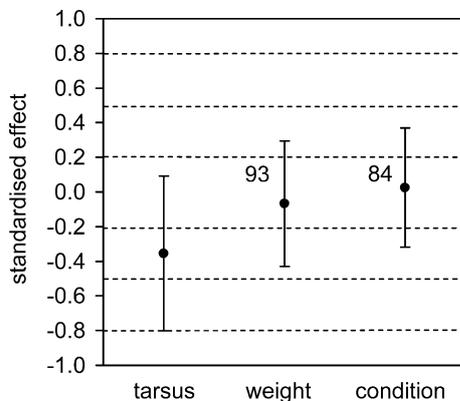


Fig. 4 Standardised effects (estimate \pm 95% confidence interval) caused by the mean difference between the largest and smallest egg in the clutch (177.2 mm³) for fledgling tarsus length, mass and condition. The *dashed lines* refer to small (0.2), medium (0.5) and large (0.8) standardised effects as suggested by Cohen (1988). *Numbers* by the confidence intervals are probabilities (in percent) that the size of the standardised effect is below that indicated by the *dashed line*

deviations in egg size on recruitment, nestling survival, fledgling condition and fledgling tarsus length of the collared flycatcher. Similarly, the initially strong effect of the egg size on the nestling mass diminished steadily as young were growing, resulting in fledgling mass being independent of egg size. Moreover, the fact that interactions between egg size and some brood-level factors (year, advancement of breeding season and mean egg volume of the clutch) did not improve the model fit substantially, suggests that the lack of egg-size effects holds true in a wide range of external conditions. Of course, it is possible that within-clutch deviations in egg size affected some component of offspring fitness that we did not measure (e.g. immunocompetence). However, fledgling condition, mass and structural size (indicated by tarsus length) are generally assumed to be the traits with the greatest influence on fitness later in life (Gebhardt-Henrich and Richner 1998). This may be true in the collared flycatcher because tarsus length and condition at fledging were found to be under strong directional selection in this species

(Kruuk et al. 2001; Merilä et al. 2001). Furthermore, the narrow confidence intervals for egg-size effects suggest that the lack of effect was not caused by small sample size, at least in the three measures of offspring size for which such confidence intervals can be determined (as the response variable is distributed normally).

As this is a correlative study, it is impossible to derive definite conclusions about causal relationships. However, we have suggested previously that the within-clutch approach is the most powerful one from the range of non-experimental approaches available for the analysis of egg-size effects (Krist and Remeš 2004). Our conclusions of no egg-size effects could be compromised only when other pre-laying maternal effects affecting offspring performance (e.g. concentration of carotenoids or steroids in egg yolk) are negatively correlated with within-clutch deviations in egg size thus tending to cancel each other out (see Krist and Remeš 2004). Although this problem is solvable only by experimental manipulation of egg size, we believe that such counteractive pre-laying maternal effects are unlikely. Thus although the definite answer to the question of egg-size effects on offspring performance can be derived only from a manipulative study, results of our detailed study suggest that within-clutch variation in egg size is unimportant for offspring performance in the collared flycatcher.

So far only a few studies have examined effects of within-clutch differences in egg size on offspring survival (Leblanc 1987b; Grant 1991; Williams et al. 1993b; Dawson and Clark 1996; Amat et al. 2001) and even fewer on their effects on offspring mass in birds (Howe 1976; Anderson et al. 1997; Erikstad et al. 1998; Magrath et al. 2003). Of the four studies on the effect of intraclutch egg-size variation on offspring mass, egg size was not influential in one study (Magrath et al. 2003), was fully confounded with laying order in another one (Erikstad et al. 1998) and influential in the remaining two studies (Howe 1976; Anderson et al. 1997). Out of the five studies on offspring survival, only one (Amat et al. 2001) found a positive effect of larger eggs. Thus, our finding of no effects of within-clutch deviations in egg size on components of offspring performance is in accordance with most previous studies, which suggests that adaptive explanations of intraclutch egg-size patterns should be invoked with caution.

An alternative to adaptive explanations is that intra-clutch egg-size patterns can be primarily generated by proximate constraints on egg development. For example, in studies that found the effect of embryo sex on egg size, the difference ranged from 1 to 3.5% of the smaller egg (Mead et al. 1987; Anderson et al. 1997; Cordero et al. 2000, 2001; Blanco et al. 2003; Magrath et al. 2003). It seems unlikely to us that the 1.3% difference in egg size in favour of males found in the house sparrow (*Passer domesticus*) (Cordero et al. 2000) would be sufficient to improve offspring fitness significantly when, in the collared flycatcher, a species with similar nestling development and breeding biology, a 11.6% difference was unimportant to the offspring. Instead, the sex of the

embryo could affect in some, yet unknown, way the deposition of albumen into the egg in the oviduct thus causing the slight difference in egg size. Similarly, a number of proximate constraints and processes could generate complex patterns of egg-size variation with laying sequence. These include the depletion of endogenous reserves in capital breeders (Pierotti and Bellrose 1986), gearing up physiologically for egg production (Parsons 1976), changing hormonal levels at the onset of egg laying and incubation (Mead and Morton 1985), changing the ambient temperature before egg laying (Ojanen et al. 1981; Järvinen and Ylimaunu 1986), a time constraint for laying early (Slagsvold and Lifjeld 1989; Nilsson and Svensson 1993) and a changing food supply in income breeders (Perrins 1970). A different degree of importance of individual proximate factors in different species/populations could be responsible for a rich diversity of intraclutch egg-size variation patterns found in birds.

Moreover, the relative importance of individual proximate factors may change systematically with body size, breeding habitat or latitude. In an influential study, Slagsvold et al. (1984) showed that large bird species lay small last eggs (brood-reduction strategy) and small species lay big last eggs (brood-survival strategy). They argued that this pattern is adaptive because large birds are less vulnerable to nest predation than small species. However, the alternative explanation could be that this pattern is driven by proximate constraints. Large species are often capital breeders, whereas small species are income breeders (see Meijer and Drent 1999). Since endogenous reserves may be depleted during laying, large species could lay relatively small last eggs. In contrast, small species must forage for all nutrients that they deposit into eggs and since food supply increases at the time of laying from day-to-day (Perrins 1970), they may gather more resources at the end of laying resulting in relatively large last eggs. This hypothesis seems to explain the observed pattern, i.e. a gradual change in egg size with laying sequence (Howe 1976; Wiggins 1990; Cichoń 1997; Hillström 1999; this study), better than the adaptive hypothesis, which predicts that only the last eggs hatching asynchronously should be larger/smaller (Howe 1976; Slagsvold et al. 1984).

Hatching asynchrony, laying sequence and paternity

As in many other studies (reviewed in Magrath 1990), hatching asynchrony was an important determinant of both nestling size and survival. This strong effect as compared to no effect of egg size was probably caused by much greater mass differences in nestling hierarchies, generated by hatching asynchrony, than by egg-size differences, as was found in several other species (e.g. Magrath 1992; Viñuela 1996). Nestlings hatching from ultimate, and to a lesser extent penultimate, eggs hatched usually later (Fig. 1) and thus were disadvantaged. On the other hand, laying sequence per se tended to affect positively both

nestling mass and fledgling tarsus length suggesting that egg composition could be responsible. The concentration of several egg components can change with laying sequence, including antibodies and carotenoids (Saino et al. 2002a, b), or steroid hormones (e.g. Schwabl 1993). Steroid hormones are the most probable candidates causing the larger size of nestlings from later-laid eggs. First, their concentration has recently been found to increase in laying sequence in a number of species (see Whittingham and Schwabl 2002). Second, steroids enhance the development of muscles important for begging (Lipar 2001) and nestling growth (Schwabl 1996).

There was a marginally non-significant effect of paternity on fledgling condition with extra-pair young tending to be in better condition than young sired by the social mate (Table 1), which is in agreement with an earlier study on the same species by Sheldon et al. (1997). This finding supports the hypothesis that extra-pair mates are genetically better than social mates (Sheldon 2000; but see Colegrave 2001) which would have important implications for the theory of sexual selection (Griffith et al. 2002). However, it is still not clear whether a difference in offspring performance in relation to paternity is directly caused by good genes inherited from extra-pair sires or female favouritism of extra-pair young (Gil et al. 1999; Cunningham and Russell 2000). Nevertheless, our study is the first in which some potential pathways of female favouritism (i.e. egg size, hatching asynchrony) were controlled on the within-clutch level. Recently two other studies have demonstrated that, on the between-female level, genetic benefits of mate choice (Parker 2003) and polyandry (Kozzińska et al. 2004) exist even after controlling for female favouritism. Although extra-pair young tended to survive better in this study it is even more unclear whether this tendency is due to any genetic effect, because in this test no other factors were controlled for. It may be that this tendency was driven by the effect of hatching asynchrony, since extra-pair young hatch earlier than their half-sibs in this species (M. Krist, P. Nádvorník, L. Uvířová, S. Bureš, unpublished data).

Conclusions

It has been often claimed that females exploit intraclutch egg-size variation adaptively in relation to offspring sex and laying order. However, the assumption of increasing offspring performance with increasing egg size has been rarely properly tested. We found no influence of intraclutch egg-size variation on offspring performance. Although a definite conclusion can be made only after manipulative studies are performed, our results strongly suggest that the assumption does not hold in the collared flycatcher, and this finding is in agreement with most previous studies. Consequently, there seems to be limited potential for female birds in many species to exploit within-clutch variation in egg size adaptively. Instead, we suggest that intraclutch egg-size variation most often has

no adaptive significance and is caused by proximate constraints on laying females.

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