Unusual pattern of sex-specific mortality in relation to initial brood sex composition in the black-billed magpie *Pica pica*

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In sexually size-dimorphic species, brood sex composition may exert differential effects on sex-specific mortality. We investigated the sex-specific mortality and body condition in relation to brood sex composition in nestlings of the black-billed magpie *Pica pica*. Neither significantly sex-biased production at hatching nor overall sex-biased mortality during the nestling period was found. Sex-specific mortality as a function of brood sex composition, however, differed between female and male nestlings. We found higher mortality for females in male-biased broods and higher mortality for males in female-biased broods, a phenomenon that we call ‘rarer-sex disadvantage’. As a result, fledging sex ratios became more biased in the direction of bias at hatching, a phenomenon that cannot be readily explained by previous hypotheses for sex-specific mortality. Two temporal variables, fledging date and laying date, were also correlated with sex-specific mortality: female nestlings in earlier broods experienced higher mortality than male nestlings whereas male nestlings in later broods experienced higher mortality. We suggest that this unusual pattern of mortality may be explained by adaptive adjustments of brood sex composition by parents, either through the effects of a slight sex difference in offspring dispersal patterns on parental fitness, or owing to sex differences as regards the benefits of early fledging.

A number of studies suggest that birds can adjust their brood sex ratios in an adaptive manner (reviewed by Hardy 2002, West and Sheldon 2002), and since the advent of molecular sexing for birds (e.g. Griffiths et al. 1998), researchers have made rigorous attempts to find adaptive bias in brood sex ratios, especially at laying and hatching (reviewed in Hasselquist and Kempenaers 2002). The assumption that adaptive sex biases will be manifested at laying or hatching stems from the expectation that if there is a mechanism for adaptive sex ratio adjustment it should act in the early stages of parental investment (Hasselquist and Kempenaers 2002). Compared with the effort to find the adaptive feature of primary sex ratio bias, relatively little effort has been made to find adaptive features in sex-specific mortality occurring between ovulation and independence of the offspring (except for Dijkstra et al. 1990, Husby et al. 2006).

Sex-specific mortality has been best studied in sexually size-dimorphic (SSD) bird species. Two mutually exclusive and non-adaptive mechanisms producing sex-biased mortality in species with SSD have been found: vulnerability of the larger sex owing to higher food demand and greater susceptibility to adverse conditions (Clutton-Brock et al. 1985, Roskaft and Slagsvold 1985, Olsen and Cockburn 1991, Weatherhead and Teather 1991, Wiebe and Bortolotti 1992, Torres and Drummond 1997, Arnold and Griffiths 2003, Martins 2004), and vulnerability of the smaller sex owing to size dominance (Lack 1954, Anderson et al. 1993, Oddie 2000, Fargallo et al. 2003, 2006, Råberg et al. 2005). These two mechanisms assume that sex-specific mortality occurs in a purely mechanistic way. A mechanism for adaptive adjustment of sex-specific mortality of offspring has also been suggested (Trivers and Willard 1973, Clutton-Brock et al. 1985, Brown 2001). The Trivers-Willard hypothesis (TWH, Trivers and Willard 1973) predicts that mothers in good condition may induce mortality of offspring of the sex that has lower variance in reproductive success. However, evidence for the adaptive adjustment of offspring mortality, rather than of primary sex ratio, is scant.

Adaptive adjustment of offspring mortality is most likely to be observed in species where an adaptive bias in brood sex ratio at laying (or at hatching) is difficult to achieve, for example when there is random segregation of sex chromosomes (Williams 1979, Charnov 1982). Although recent comparative studies refute the premise that chromosomal sex determination necessarily prevents adaptive biases in offspring sex ratio (West and Sheldon 2002, Cassey et al. 2006), some species may lack mechanisms for biasing primary sex ratios. For instance, only some of the species under strong pressure to bias their sex ratios (e.g. cooperative breeders with sex-biased helping behaviour;
Griffin et al. 2005 and references therein) appear to have developed the ability to control the sex of offspring in the early stages of parental investment. For a sex-biasing mechanism to evolve, the cost of developing and maintaining such a mechanism needs to be overcome by the benefit that accrues from manipulating offspring sex. In species that have difficulty biasing the offspring sex ratio in the early stage of parental investment, the parents may induce differential mortality of female and male offspring and thus bias brood sex ratios adaptively during the later stages of parental care. This relates to the fact that, contrary to what is pursued in the field of sex ratio research, some of the theoretical models define ‘sex allocation behaviour’ as behaviour affecting the sex ratio at the end of parental care, not at the initial stage of parental care (Pen and Weissing 2002, Wild and West 2007).

In this study, we examined sex-specific mortality of offspring as a function of initial brood sex ratio in a sexually size-dimorphic species, the black-billed magpie Pica pica. We tracked sex-specific mortality and consequent changes in brood sex ratio for each specific initial brood sex ratio. This was because a confounding effect of brood sex ratio on sex-specific mortality was indicated in several studies (Nager et al. 2000, Becker and Wink 2003, Laaksonen et al. 2004, Fargallo et al. 2006). Recently, sex-specific effects of maternally-transmitted hormones, antibodies and antioxidants on offspring growth, survival and susceptibility to parasites have been discovered (e.g. Schwabl et al. 1997, Saino et al. 2003 and 2006, Muller et al. 2005, Rubolini et al. 2006, Rutkowski and Cichon 2006, De Neve et al. 2008). However, the consensus has not been reached on which sex benefits more through maternal effects and how far-reaching is the influence of maternal effects. Moreover, a possibility that both sex-specific maternal effects and brood sex ratio determine sex-specific growth or mortality of offspring has rarely been tested. Thus, currently it is difficult to predict how the maternal effects shape sex-specific mortality of offspring as a function of brood sex ratio without fully understanding the patterns of sex-specific mortality.

Black-billed magpies are large, sexually size-dimorphic passerines. Male fledglings are approximately 10% heavier than the females and they require more food from the parents (Husby 1991). Previously, Husby (1991) found no evidence for sex-biased mortality in a Norwegian population. However, sex-biased mortality may not be detected if other factors, such as initial brood sex ratio, confound the pattern in the overall population. Here we describe ‘rarer sex disadvantage’ among nestlings of the black-billed magpie.

Methods

Field procedures and sex determination

Our study population is located on the campus of Seoul National University in Korea where more than 50 pairs of magpies attempt to breed annually. We collected data on nestling sex and survivorship from the first successful clutches of all accessible breeding pairs in the study population in 2000 and 2004. Magpies in our study area fledged 30 to 38 d after hatching (36.9 d ± 3.6 SD), which is similar to the time of fledging for the European populations (33 d ± 2.5 SD; Husby and Slagsvold 1992) but substantially longer than that of American magpies (27.2 d ± 2.2 SD; Buitron 1988).

We observed the nesting behaviour of breeding pairs from the pre-laying period. When only one bird of the breeding pair was observed, we visited the nest two to four times at intervals of two to three days until we found eggs in the nest. The laying date was recorded as the date when the nest contained the first egg. After egg-laying was recorded, we visited the nest four to eight times during incubation and the nestling period. We predicted hatching dates by egg-candling and visited nests within five days after hatching. Since the nestlings in this stage show clear size differences according to hatching date, we were able to estimate hatching order from the relative sizes of the hatchlings. Nestlings of similar size within a brood were assigned the same hatching order. We marked the nestlings with nail markers and re-marked the nestlings within each five-day period until we were able to band the nestlings (at 14–16 d post-hatch). Nestlings that disappeared between visits were counted as having died. Tarsus length and body mass were measured with digital calipers (to accuracies of ± 0.01 cm) and electronic balance (to accuracies of ± 0.01 g) respectively at three to five days post-hatch to estimate nestling body condition shortly after hatching (see ‘Statistical analyses’ for estimation methods). Fledging date was recorded as the date when at least one of the chicks left the nest.

The nestlings’ sex was determined using molecular methods. We took a few blood drops from their brachial veins and either dropped them onto a piece of Whatman filter paper without any treatment or placed them in 30 μl of STE buffer. To prevent cross-contamination, the portions of filter paper containing blood were cut out and placed into sterile 1.5 ml tubes immediately and kept frozen at −20°C. We extracted genomic DNA from the blood samples with a DNeasy Tissue Kit (QIAGEN) and manufacturer-supplied protocols. Sex determination was conducted with the PCR-based technique (P2-P8 primers) described by Griffiths et al. (1998).

Statistical analyses

To determine if and how brood sex ratios at hatching differentially affected sex-specific nestling mortality during brood reduction, we used generalised linear mixed modelling and searched for the minimal models via stepwise backward elimination (Crawley 1993). Starting with maximal models that contained all the relevant explanatory variables up to two-way interactions, we proceeded by deleting variables until only significant effects were left in the model (Crawley 1993, Wilson and Hardy 2002). The validity of variables in the minimal model was checked by the increase in deviance when these variables were deleted.

We used SAS PROC GENMOD (SAS ver. 8.0.1, SAS Inst. 2000) with the logit link function for the generalised linear mixed models. The response variable was the nestling’s fate (survival or death). Explanatory variables included: year of investigation; brood size; hatching sex.
ratio; relative rank of laying date or fledging date of a focal nest (depending on the model); body condition shortly after hatching and relative hatching order within a nest of a focal nestling. Laying date was included in the model as an indicator of parental quality, because, in many bird species, laying date is known to be a good predictor of parental quality (Arnold et al. 2004 and references therein, Birkhead 1991 for magpies). Fledging date was included as a predictor of the survival of the fledglings (Birkhead 1991 for magpies, Siikamäki 1998, Verboven and Visser 1998).

Since we were not able to record the fledging dates of some nests, we conducted model searching with two different sets of data; one including laying dates (number of nests = 44), and the other including fledging date (number of nests = 34). Predicted likelihood of mortality of a focal nestling was generated by PROC GENMOD, based on the algorithm of Empirical Best Linear Unbiased Prediction (EBLUP). Relative ranks of laying and fledging dates of a focal nest were calculated as the rank of the nest divided by the maximum rank of a corresponding year. Body condition shortly after hatching and near fledging was estimated as the residuals from a linear regression of body mass on tarsus length (both were natural-logged). Residuals were calculated with SAS PROC MIXED. Relative hatching order was expressed by dividing the absolute hatching order by brood size (the resulting variable thus ranged between zero and one). In all statistical procedures including calculations of residual body mass, individual nest was considered as a block for analyses. Values are presented as mean ± standard error.

**Results**

Among the 51 nests (29 in 2000 and 22 in 2004) observed, mortality occurred in 26 nests (16 in 2000 and 10 in 2004, or 55.2% and 45.5% of nests, respectively). In 2000, there were 41 male nestlings and 48 female nestlings, which reduced to 31 (75.6% survived), and 33 (68.8%) around the time of hatching. In 2004, there were initially 41 male and 46 female nestlings, which reduced to 35 (85.4%) and 39 (84.8%) respectively. In general, weather condition during nestling period was similar in two years. The slopes of temperature increase during nestling period of the earliest and the latest nests were 0.17 ($r^2=0.38$) and 0.27 ($r^2=0.67$) in 2000, and 0.19 ($r^2=0.27$) and 0.24 ($r^2=0.62$) in 2004 respectively. In both years, temperature increase was less consistent in earlier season.

Seven nests, which contained a total of 30 nestlings, failed to produce any fledglings. These nests were excluded from statistical analyses, because total nest failure can be caused by ecological factors other than sex-specific mortality according to brood sex composition. Before brood reduction, there were a total of 181 nestlings (brood size: 2–8); among these, molecular sexing was not successful with five samples. A total of 138 nestlings survived until the time around fledging.

Nestling mortality can occur before hatching. Hatching failure in our study population amounted to $34.2±26.1\%$ of eggs (37 nests). Correlation between hatching failure (in proportion) and hatching sex ratio was low and not significant ($r = -0.170, n = 37$ nests, $z = -0.100, P = 0.316$). We do not have full clutch size data on seven nests (that contained 32 hatchlings in total), and three of them were very early nests (within 10 percentile of laying date). Statistical analysis on partial dataset shows that mortality of nestling was not influenced by the proportion of hatching failure ($n = 144$ nestlings, $\chi^2 = 1.96, P = 0.16$).

Since putting hatching failure into the model and running statistical procedure not only reduces the sample size but, more importantly, can create bias in the results by excluding early nests, we did not conduct further analyses with regard to hatching failure.

At the population level, we did not find any evidence that brood sex ratios deviated significantly from the binomial expectation either at hatching ($0.453±0.04$, $\chi^2=0.24$, $P=0.265$), or at fledging ($0.464±0.05$, $\chi^2=0.48$, $P=0.499$). The relative rank of laying date of a focal nest in a given year was neither significantly correlated with its brood size ($r=0.175, P=0.257$), nor with sex ratio at hatching ($r=-0.053, P=0.710$). The result was similar for relative rank of fledging date ($r=0.066, P=0.747$ for brood size; $r=0.196, P=0.318$ for sex ratio at hatching). Relative ranks of laying date and fledging date were highly correlated ($r=0.770, P<0.001$). In our study population, clutch size declined with laying date (in 2000, $n=39$ nests, $r=-0.374, P<0.05$; in 2004, $n=36$ nests, $r=-0.407, P<0.05$). Sex-specific body condition at hatching did not correlate with relative rank of laying date ($F_{1,174}=0.01, P=0.93$), nor that of fledging date ($F_{1,107}=0.07, P=0.80$).

Brood sex ratios of each nest at hatching and fledging were highly correlated even when we excluded all-female and all-male broods ($n=38$ nests, $r=0.824, Z=6.923$, $P<0.001$; Fig. 1). When we compared the observed fledging sex ratio and the expected fledging sex ratio assuming that no sex ratio change occurred between hatching and fledging, the former was significantly steeper than the latter. The result was similar for both dates ($F_{1,37}=26.1, P=0.001$).

![Figure 1. Changes in brood sex ratio between hatching and fledging. The fitted line (solid) is shown along with the 1:1 line where we assumed no change of brood sex ratio between hatching and fledging (dotted). Arrows indicate the direction of changes in brood sex ratio between hatching and fledging. All-female or all-male broods were excluded from this analysis.](image-url)
than the latter (two-tailed t-test, \( t = 2.040, P = 0.048 \)). This indicates that originally female-biased broods became even more female-biased at fledging, and originally male-biased ones became more male-biased (arrows in Fig. 1).

### Differential effects of brood sex composition on nestling mortality

The minimal model which was found from the initial model containing relative rank of laying date retained a significant interaction term between hatching sex ratio and sex but not relative rank of laying date (Table 1a). The predicted relationship between hatching sex ratio and sex-specific mortality was that male nestlings had higher rates of mortality in female-biased nests whereas female nestlings had higher mortality in male-biased nests (Fig. 2a). The interaction term between laying date and sex did not reach the conventional significance level (\( \chi^2 = 3.12, P = 0.078 \)), and was removed during the course of model reduction. Although the effect of laying date on sex-specific mortality was marginally non-significant, the predicted pattern was that female nestlings died more in earlier nests and male nestlings died more in later nests (similar to Fig. 2b, see below). We also found a significant effect of hatching order on nestling mortality (Table 1), which suggests that the runts (last-hatched and smallest young) were more likely to die. Relative laying date, year, brood size and residual body mass at hatching did not significantly correlate with nestling mortality and were deleted during the course of model reduction.

With the initial model containing fledging date, we found a significant interaction between fledging date and

Table 1. Two minimal models of the mortality of magpie nestlingsa. (a) Without any temporal correlates in initial model, and (b) when relative fledging date was added in initial model.

<table>
<thead>
<tr>
<th>Source</th>
<th>( \Delta ) Deviance when deleted</th>
<th>Minimal model</th>
<th>( df )</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching sex ratio × sex</td>
<td>5.997</td>
<td>1</td>
<td>4.21*</td>
<td></td>
</tr>
<tr>
<td>Hatching sex ratio</td>
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<td>0.26</td>
<td></td>
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<tr>
<td>Sex</td>
<td>1.055</td>
<td>1</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>Hatching order</td>
<td>9.123</td>
<td>1</td>
<td>7.16**</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>( \Delta ) Deviance when deleted</th>
<th>Minimal model</th>
<th>( df )</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fledging date × sex</td>
<td>5.682</td>
<td>1</td>
<td>4.33*</td>
<td></td>
</tr>
<tr>
<td>Hatching sex ratio × sex</td>
<td>5.974</td>
<td>1</td>
<td>5.43*</td>
<td></td>
</tr>
<tr>
<td>Fledging date</td>
<td>0.860</td>
<td>1</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>Hatching sex ratio</td>
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<td>1</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.848</td>
<td>1</td>
<td>4.06</td>
<td></td>
</tr>
<tr>
<td>Hatching order</td>
<td>8.370</td>
<td>1</td>
<td>8.07**</td>
<td></td>
</tr>
</tbody>
</table>

a The initial model contained main effects and two-way interactions of year, brood size, sex, hatching sex ratio, residual body condition at hatching and relative hatching order within a brood. For (b), relative fledging date (early or late in the season) was also added. Residual deviance for null model for (a) it was 183.63 (df = 175), and for (b) it was 112.33 (df = 108). Significance levels were denoted as * for \( P < 0.05 \), and ** for \( P < 0.01 \).

Figure 2. Sex-specific mortality in relation to hatching sex ratio (a) and to fledging date (b). Predicted values (solid circles and crosses) were obtained from a generalised linear mixed model. For observed values (bars), nests were pooled according to their hatching sex ratio (a), or fledging date (b). Numbers above the bars denote ‘(the number of dead nestlings)/(all nestlings of the same sex)’. Numbers in parentheses denote the number of broods used in the analysis. A total of 176 nestlings (with known sex) from 44 nests for (a), and 108 nestlings from 34 nests for (b) were used in the analyses.

nesting sex (Table 1b) that remained in the minimal model and there was higher female mortality in early-fledging nests than in late-fledging nests (Fig. 2b). The pattern of sex-specific mortality explained by relative fledging date was similar to the pattern explained by relative laying date.

Checking sex-specific body condition of nestlings around fledging is of importance because body condition around fledging can indicate post-fledging survival of nestlings (Both et al. 1999, Green and Cockburn 2001, Monró et al. 2002). In our results, sex-specific body condition of nestlings near fledging was only affected by brood size at hatching \( (\beta = -0.0139, F_{1,42} = 4.17, P = 0.048) \). Thus, there was no effect of brood sex ratio at hatching on sex-specific body condition of nestlings after brood reduction had occurred. Adjusted means for body mass were 147.18 ± 2.07 (g) for females and 157.54 ± 2.18 (g) for males (mean ± SE). Males were approximately 7% larger than females near fledging.
Discussion

We found that sex-specific mortality of nestlings was differentially correlated with brood sex ratio. Our results suggest that there may be a ‘rare-sex disadvantage (RSD)’: that is, greater mortality of male (the larger sex) nestlings in female-biased broods and greater mortality of females (the smaller sex) in male-biased broods. The latter phenomenon could be explained by the effects of size dominance, which is known for some birds (Oddie 2000, Fargallo et al. 2003, 2006). Since there are more male nestlings that are dominant over female nestlings and may prevent them from receiving parental care, female mortality would be predicted to be greater in male-biased broods. On the other hand, higher mortality of male nestlings in female-biased broods cannot be explained by any previously documented mechanism. Since larger sex vulnerability may result in higher male mortality in male-biased broods owing to potential food shortage, it does not contribute to explain the pattern of RSD.

We propose two hypothetical, mutually non-exclusive, adaptive explanations that are consistent with both effects (on males and on females) detected in our results. Considering that magpies have an adaptive adjustment of brood size via nestlings’ mortality shortly after hatching (Husby 1986), it is likely that they also adjust the brood sex ratio through early sex-biased mortality. The first possibility involves a variant form of condition-dependent sex ratio adjustment by parents owing to slightly sex-biased dispersal. The TWH (Trivers and Willard 1973, Clutton-Brock et al. 1985) predicts that better-quality parents, who lay their clutches early, would produce male-biased broods since this would bring them higher fitness returns. Only better parents can afford to rear male-biased broods since rearing males is costlier than rearing females in magpies (males need more food than females, based on differences in gizzard contents; Husby 1991). If better parents cannot optimally shift the sex ratio at hatching toward a more beneficial male-biased condition (see below on this issue), then these parents may induce more female nestling mortality to produce more male-biased broods at the end of parental care. The reverse argument holds for poor-quality parents. Recently, Wild and West (2007) suggested that sex ratio bias can sometimes be in the opposite direction to the classical prediction by TWH. For instance, if dispersal of males (larger sex) is limited, local resource competition increases among males, which reduces the marginal fitness gain of producing sons, and this may result in sex ratio bias (at the fledgling stage) contrary to predictions from TWH (Wild and West 2007). In black-billed magpies, it is known that males are slightly more philopatric than females (Eden 1987). However, since the sex difference in dispersal distance is only slight (1.19 territories for second-year males, 2.54 territories for second-year females, values are medians; Eden 1987), the effect of parental quality on the sex-specific mortality of nestlings might be only slightly weakened, instead of the pattern being the complete opposite of what TWH predicts. We think that the effect of slight sex-biased dispersal may be the reason why the effect of laying date of the brood, which was used as an indicator of parental quality in our study, on the sex-specific mortality of magpie nestlings was marginally non-significant, although the pattern of sex-specific mortality was congruent with TWH.

Our second hypothesis, which is not mutually exclusive with the first hypothesis, states that early-fledging nests would contain more males since fledging early and joining non-breeding flock early is more important for the social status of juvenile male magpies. The advantage for magpie nestlings to fledge early was suggested by Eden (1987), and Trost and Webb (1997). It is also known that social status is more important for male magpies than females (Birkhead 1991, Trost and Webb 1997). Thus, the male offspring in earlier-fledging broods should obtain higher social status, and ultimately higher reproductive success, than male offspring from later-fledging broods, benefiting parents of early-fledging broods that produce male-biased broods, either by producing more males or by inducing higher mortality of females. Parents of late-fledging nests, conversely, should produce female-biased broods, because late-fledging male offspring are at a disadvantage compared with early-fledging males in competition for social status.

Currently, we do not have data that suggest proximate mechanisms for sex-specific mortality in magpies. A number of studies have found that maternal allocation of hormones, immunity and carotenoids can be sex-specific, and maternal allocation can affect competitive behaviour of nestlings and susceptibility to parasites. Most of the studies suggest that one sex of offspring, than the other sex, gets greater benefit from maternal resource allocation. Thus, it is difficult to presume that RSD would be related to maternal effect on the basis of current knowledge. Although we do not have evidence for sex-specific maternal effect, RSD seems not to be related to sex difference in susceptibility to ectoparasites owing to low occurrence of parasitised nests (8/51 nests). Moreover, a recent study on magpies found no correlation between sex difference in maternally-transmitted antibodies in relation to brood sex ratio in magpies (Pihlaja et al. 2006), which suggests that RSD might result from biased provisioning effort rather than maternal effects.

In our results, we found that sex-specific mortality of nestlings depended on brood sex ratio at the hatching stage and temporal correlates such as laying or fledging date. If RSD during the period of parental investment does result from adaptive adjustment of brood sex composition by parents, why do parents not establish their ideal brood sex ratio by manipulating the primary sex ratio? There are two possibilities. First, if it is difficult to produce sex-biased broods at the early stages of parental care (at egg-laying or hatching), because, for instance, of the constraint of random chromosomal segregation (Williams 1979, Charnov 1982), parents may initially produce only slightly biased broods and induce more mortality of the ‘unwanted’ sex during the period of parental care. It has already been shown in some species that birds may bias their broods towards one sex by biasing their provisioning (Kilner 1998, Lessells 2002) or by controlling sibling competition (Lotem 1998, Tanner et al. 2007). Although some species are reported to have the ability to bias offspring sex ratio at the early stage of parental care, this ability may not be common to all the bird species, especially to those with long periods of parental care.
Alternatively, it may be an adaptive bet-hedging strategy to produce slightly suboptimal brood sex composition initially. Magpies have a relatively long nesting period for passerines. Because environmental conditions during the nesting period are unpredictable, optimal brood sex composition may change (West and Sheldon 2002, Zann and Runciman 2003). By regulating sex-specific mortality after an initial suboptimal brood sex ratio is produced, parents may have opportunities later to ‘fine-tune’ the brood sex ratio in either direction so that it closely matches the optimal value to maximise their own condition or that of their offspring at the end of parental investment (suggested by Martins 2004). Unlike the previous hypothesis, however, this mechanism does not easily explain (but suggested by Martins 2004). Unlike the previous hypothesis, however, this mechanism does not easily explain (but not totally exclude) a systematic bias towards male mortality in female-biased broods and towards female mortality in the male-biased broods because the ‘fine-tuning’ (in either direction) should depend on season-specific ecological circumstances rather than on the initial (at hatching) brood sex ratio. The presented data are not sufficient to evaluate these two hypotheses fully.

Although it is possible that the correlation between hatching sex ratio and sex-specific mortality continue after fledging, our results on the body condition of fledglings do not support this possibility. Survival during the post-fledging period is known to be influenced by body condition at the time of fledging (Both et al. 1999, Green and Cockburn 2001, Monró et al. 2002) and by the fledging date (Siikamäki 1998, Verboven and Visser 1998). In our results, body condition near fledging was affected by the brood size at hatching, but not by the brood sex ratio at hatching. This suggests that the hypothesis parental adjustment of sex-specific mortality, which was related to initial brood sex ratio, does not extend beyond fledging. Consistent with this suggestion, neither of the two previous studies on the post-fledging survival of magpies found sex-biased mortality (Eden 1987, Husby and Slagsvold 1992).

Recently, a study on house sparrows *Passer domesticus* found sex-specific recruitments that show adaptive patterns. Husby et al. (2006) examined sex-specific recruitment of house sparrow nestlings in complete broods, and found that male survival (probability of recruitment) was positively related to hatch day but female survival was not. Thus, their result could be an evidence of larger sex vulnerability in early nests where environmental conditions are harsh, and in accordance with this, late-breeding house sparrow parents produced more sons. Since only complete broods, which did not go through any mortality before fledging, were used in the analysis, adaptive sex ratio adjustments in house sparrows were made at the early stage of parental investment.

The results of Husby et al. (2006) are different from what we have found in terms of the direction of sex-specific mortality; we found higher female mortality in earlier nests. If early breeders are in harsher conditions, we could expect more male mortality as Husby et al. found with house sparrows. This excludes the possibility of larger sex vulnerability in our results. In addition, male magpie nestlings that were produced by earlier breeders were not particularly in better condition than those produced later in the season and early breeders did not produce more male nestlings than late breeders. Thus, it is difficult to presume that increased female mortality in earlier magpie nests would be due to size dominance by healthy males. Thus, as stated earlier, neither ‘larger sex vulnerability’ nor ‘size dominance’ hypotheses can explain RSD in magpies.

It is also difficult to expect that breeding conditions that may change with season influenced sex-specific mortality of magpie nestlings. In both of our study years, temperature increase was less consistent in earlier season. Considering this, late breeders might be in better condition to raise more sons because of warmer and more consistent weather which would be related to higher and more stable availability of insect prey. In our results, however, earlier nests have experienced greater female mortality and later nests experienced greater male mortality. Thus, it seems less likely that our results could be explained by difference in breeding conditions such as temperature between early and late breeders.

Since RSD reinforces the sex ratio bias that was initially created by the female, we think that RSD could be part of the adaptive strategy of parents to adjust their offspring sex ratio at the end of parental care, possibly in accordance with TWH mechanisms. As far as we know, patterns of sex-specific mortality suggesting RSD that reinforces initial brood sex ratio biases have not been observed in other avian species. Our results raise a number of questions for further studies. First, it would be helpful to know the proximate mechanisms affecting patterns of sex-specific mortality. For instance, parents may bias their feeding efforts toward nestlings of one sex (reviewed in Stamps 1990), or sex-specific mortality could be induced through maternal transmission of hormones, antibodies or nutrients as many recent studies have revealed (examples listed in the introduction). Second, it would be interesting to estimate the cost of sex-biasing mechanisms. Although some studies have been successful in revealing proximate mechanisms for early adjustments of brood sex ratio (e.g. Correa et al. 2005), how costly this mechanism is to evolve and develop in a given species remains unknown. Comparisons of the frequency of facultative adjustments of offspring sex between species with short periods of parental care (such as tits) and those with long periods of parental care (such as corvids and owls) would also shed light on the benefits of sex ratio adjustment as a bet-hedging strategy, although the potential publication bias in sex ratio studies (Cassey et al. 2004) may make this analysis unreliable.

**Acknowledgements** – We wish to thank all the members of our magpie team since 1998 for their help in collecting field data. S.-I. L. is especially grateful to Ben Sheldon for developing ideas and Piotr Jablonski, Joonghwan Jeon, Susan Lappan and Hugh Drummond for valuable comments on earlier manuscripts. We appreciate the improvements in English usage made by Peter Lowther through the Association of Field Ornithologists’ program of editorial assistance. This study was supported by a BK21 Research Fellowship (to Y. H. and to J. L.) and a BK21 Post-doctoral Fellowship (to S.-I. L.) from the Ministry of
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