Female-biased mortality in experimentally parasitized Alpine Swift *Apus melba* nestlings

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Summary

1. Sex-biased mortality in adult vertebrates is often attributed to lower immunocompetence and higher parasite susceptibility of males. Although sex-specific mortality has also been reported during growth, the importance of sex-specific immunocompetence and parasite susceptibility in explaining male-biased mortality remains ambiguous in growing individuals because of potentially confounding sources of mortality such as sexual dimorphism.

2. Here, we investigated sex-specific susceptibility to the blood-sucking louse fly *Crataerina melbae* and sex differences in cell-mediated immunity in a bird species that is sexually monomorphic both in size and plumage coloration at the nestling stage, the Alpine Swift, *Apus melba*.

3. For this purpose, we manipulated ectoparasite loads by adding or removing flies to randomly chosen nests in two years, and injected nestlings with mitogenic phytohaemagglutinin (PHA) in another year.

4. There were no significant differences between male and female offspring in immune response towards PHA, parasite load, and parasite-induced decrease in growth rate. Secondary sex ratios were however biased toward males in parasitized broods, and this was explained by a greater mortality of females in parasitized than deparasitized broods.

5. Our findings are in contrast to the widely accepted hypothesis that males suffer a greater cost of parasitism. We discuss alternative hypotheses accounting for female-specific mortality.

Key-words: Hippoboscidae, immunocompetence, phytohaematogglutinin, sex-specific mortality, sibling competition

Introduction

Although parity in sex ratios seems to be the norm at conception, differential mortality between the sexes during early stages of development and at adulthood has been widely reported in mammals and birds (review in Clutton-Brock 1991; Moore & Wilson 2002). A major explanation for sex-specific mortality relies on sexual difference in immune defence with, in a large number of species including humans, males possessing smaller immune defence organs (Møller *et al*. 1998) and mounting weaker humoral and cellular immune responses in comparison with females (e.g. Grossman 1985; Moreno *et al*. 2001; Fargallo *et al*. 2002). The 'immunocompetence-handicap' of males is supposed to arise from the circulation of androgenic hormones such as testosterone, immunosuppressive molecules that are necessary for the full expression of male sexual secondary characters (Folstad & Karter 1992; Casto *et al*. 2001; Roberts *et al*. 2004). An alternative hypothesis postulates that immune responses are traded-off against other energetically demanding processes, and the greater male investment in sexual ornaments or in size depresses immune function (Wedekind & Folstad 1994; Lochmiller & Deerenberg 2000; Rolff 2002; Zuk & Stoehr 2002). Thus, sexual dimorphism in such traits may lead to a sex-specific immunocompetence, and thereby account for greater mortality of males, in particular when exposed to pathogens and parasites.

In agreement with the prediction of the immunocompetence handicap hypothesis that males suffer a

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greater cost of parasitism, a recent comparative study in mammals showed that the greater parasite loads found on adult males was associated with male-biased mortality (Moore & Wilson 2002; see also Poulin 1996; Zuk & McKean 1996; Schalk & Forbes 1997; Roberts *et al*. 2004). In contrast, current knowledge of sex-specific effects of parasitism during growth and development is scant, although such information would be important for understanding how resources are allocated to male and female offspring (Trivers & Willard 1973) and how population dynamics is affected as a consequence (Hudson & Dobson 1997; Ferrari *et al*. 2004). To our knowledge, the Great Tit, *Parus major*, is the only species in which the relative effect of parasites on male and female offspring has been experimentally investigated (Tschirren *et al*. 2003). Simultaneous injection of phytohaemagglutinin (PHA) and manipulation of flea loads showed that male nestlings mount a lower T-cell-mediated immune response and were more affected by fleas than female nestlings. Because in the Great Tit males are larger and more colourful than females, males may have allocated more resources to growth and production of colour pigments at the expense of immunocompetence (Tschirren *et al*. 2003). Correlative studies in size monomorphic nestling Pied Flycatchers, *Ficedula hypoleuca*, suggest nonetheless that environmental factors may interact with sex-specific susceptibility to parasites. Indeed, these studies showed that although male nestlings developed shorter tarsi than female siblings in the presence of mites in some years (Potti & Merino 1996), the opposite result was found in other years (Potti 1999; Potti *et al*. 2002). More works on sexual dimorphism in parasite susceptibility are therefore required. To properly test whether the greater male susceptibility to parasites is due to male investment in large body size and ornament or to the circulation of male immunosuppressive hormones, one needs to consider a model organism in which there is no sexual dimorphism in size and ornaments (Sheldon *et al*. 1998). Such a model system is appropriate because if it appears that males are as susceptible to parasites as females, it would provide support to the hypothesis that the observation that males are usually more susceptible is due to the fact that they invest more resources to develop a large body size and ornaments. In contrast, if in such a model system males nevertheless appear to be more susceptible than females, it would indicate that independently of investment in body size and ornaments, male-specific hormones are immunosuppressive.

In the present paper, we used the Alpine Swift, *Apus melba*, to investigate the difference of male and female nestlings in susceptibility to a blood-sucking ectoparasite, the louse fly *Crataerina melbae* (Diptera; Hippoboscidae), and in cell-mediated immune response to mitogenic PHA. The Alpine Swift is a monogamous bird where both sexes invest equally in the reproduction and look similar (Arn 1960). Nestlings are sexually monomorphic in both size (Arn 1960; present study) and plumage coloration (P. Bize, unpublished results based on reflectance spectra), and hence any sex-biased mortality cannot be explained by these two factors (Teather 1987; Anderson *et al*. 1993; Krijgsveld *et al*. 1998). Within colonies all nests are infested by *C. melbae* (prevalence = 100%), and between 1 and 55 parasites are found per nestling (Roulin *et al*. 1998). This louse fly can impair its host development and reproductive success as recently experimentally demonstrated (Bize *et al*. 2003a; Bize *et al*. 2004).

Materials and methods

The Alpine Swift is a 90 g migrant insectivorous apodiform bird that reproduces in colonies of a few to several hundred pairs located in holes of cliffs or tall buildings. One clutch of one to four eggs (mean is 2·6) is produced per year, and both parents incubate it for approximately 20 days (Arn 1960). Parental care ceases at fledging, which occurs at an age of 50–70 days (Arn 1960; Bize *et al*. 2003a). Alpine Swifts are parasitized by the blood-sucking louse fly *C. melbae* that feeds exclusively on this species (Tella *et al*. 1998). This 7 mm long ectoparasite infests nestlings when they become feathered (at about 10 days after hatching), and prefers senior nestlings to junior nestmates (Roulin *et al*. 2003). Fieldwork was carried out between 2000 and 2002 in two colonies located under the roofs of clock towers in Bienne (47°10′ N, 7°12′ E; about 100 breeding pairs) and Solothurn (47°12′ N, 7°32′ E; about 50 pairs), Switzerland.

Cell-mediated immunocompetence of Alpine Swift nestlings was assessed in Bienne and Solothurn in 2002. For this purpose, we injected 0·1 mg of PHA subcutaneously, dissolved in 0·02 ml phosphate-buffered saline, in the left wing web of 25–42-day-old (mean is 28) nestlings. To lessen stress caused by handling on nestlings and reduce measurement errors, we did not inject the right wing web with PBS as a control (Smits *et al*. 1999). The dose of PHA used in this study is similar to that used in numerous other studies of free-living or captive birds (e.g. Smits *et al*. 1999; Tella *et al*. 2001; Fargallo *et al*. 2002; Roulin *et al*. 2003; Tschirren *et al*. 2003). PHA is a mitogen agent leading to local infiltration and proliferations of leucocytes, which represents a special form of cell-mediated immunity (McCorkle *et al*. 1980; Corrier & DeLoach 1990). The thickness of the wing web at the injection site was measured by the same person (PB) four times in sequence with a pressure-sensitive calliper (Mitutoyo micrometer, #2046FB-60, Mitutoyo SA, CH-8902, Urdorf, Switzerland) to the nearest 0·01 mm prior to injection and 24 (range ± 1) h later. The difference between mean wing web thickness before and 24 h later

was used as an index of the T-cell-mediated immune response (Smits *et al.* 1999). The four successive measurements demonstrated that our method of assessing T-cell-mediated immune response is reliable (one-way ANOVA to test for within-individual repeatability: $r = 0{\cdot}96$, $F_{105,318} = 105{\cdot}63$, $P < 0{\cdot}0001$, $n = 106$; Lessells & Boag 1987). We injected PHA in 61 nestlings belonging to 26 broods in Bienne, and in 45 nestlings from 18 broods in Solothurn.

We manipulated ectoparasite load of louse flies in Solothurn in 2000 and 2001. Starting 10 days after hatching, we created two groups of nests differing in ectoparasite load by transferring louse flies from a donor brood (referred to as 'deparasitized brood') to a receiver brood ('parasitized brood'). For this purpose, nests with a similar clutch size (Pearson correlation, $r = 0{\cdot}33$, $n = 39$ pairs of nests, $P = 0{\cdot}04$), hatching date ($r = 1{\cdot}00$, $n = 39$, $P < 0{\cdot}001$), brood size 10 days after hatching ($r = 0{\cdot}52$, $n = 39$, $P < 0{\cdot}001$), and ectoparasite load 10 days after hatching ($r = 0{\cdot}45$, $n = 39$, $P = 0{\cdot}001$) were matched in 20 and 19 pairs of nests in 2000 and 2001, respectively. For each pair of nests, we extracted and counted ectoparasites discovered on the body of all nestlings, and then put all ectoparasites only in the nest that was randomly assigned to the 'parasitized' treatment, the other nest being kept 'deparasitized'. Because deparasitized nests were frequently reinfested by mobile *C. melbae*, the ectoparasite load was manipulated every 5 days until nestlings were 50 days old. After 20 days of age, nestlings start to invade neighbouring families and are frequently adopted by foster families (Arn 1960; Bize *et al.* 2003b). Mean distance between nests is $94 \pm 1{\cdot}7$ cm (range: 15–400 cm). To prevent adoption, all nests were checked daily between 20 and 50 days after hatching, and each nest-switcher was returned to its natal nest immediately after its discovery (Bize *et al.* 2003b). Nestlings were measured (body mass and wing length) each time we manipulated ectoparasite load. We calculated wing loading of 50-day-old nestlings as the ratio of body mass to wing length (Martins 1997; Antonov & Atanasova 2003).

A blood sample was taken from nestlings 10–20 days after hatching, and stored in EDTA buffer at −20 °C before analysis. The sex of nestlings was determined from blood cell DNA, using polymerase chain reaction (PCR) amplification of the *CHD* genes (Ellegren 1996; Griffiths *et al.* 1998). All nestlings injected with PHA were successfully sexed. In the experiment of ectoparasite load manipulation, 28 of the 31 (90%) parasitized nestlings and 20 of the 23 (87%) deparasitized nestlings that died between 10 and 50 days after hatching were sexed. All fledglings were successfully sexed.

Difference between the sexes in cell-mediated immunocompetence was analysed with a mixed-model ANOVA, where sex was included as a fixed factor, nest as a random factor, and body condition (calculated as the residuals of a linear regression of log-transformed body mass on log-transformed wing length) as a covariate. We controlled for body condition in this analysis because this trait is often associated with the immune response towards PHA in a number of species (review in Alonso-Alvarez & Tella 2001). We analysed effects of our parasite load manipulation on nestling ectoparasite load, body size and mortality using mean brood values. Because broods raised by the same parents in different years are not independent values, we solved this statistical problem (i.e. pseudo-replication; Hurlbert 1984) by including in our analyses only the first breeding attempt for pairs that were manipulated in more than 1 year. This reduced our sample size from 78 to 65 experimental broods. Effects of our experiment on ectoparasite load and the size of male and female offspring were analysed with mixed-model nested ANOVAs. Treatment and sex were included as fixed factors, and nest was included as a random factor nested within treatment. Hatching rank of nestlings was also included as a fixed factor since, within nests, the last-hatched nestling (assigned to the category 'junior') was already shown to bear fewer ectoparasites than its first-hatched nestmates (category 'senior') (Roulin *et al.* 2003). The number of louse flies found on nestlings between days 10 and 50 after hatching was averaged and then log transformed to fit a normal distribution. The latter value was used in the statistical tests. Although pair-wise comparisons between males and females born in the same nest would have allowed the use of more powerful statistical tests (e.g. repeated-measures ANOVA), we preferred the use of mixed-model and nested ANOVAs because many broods contained nestlings of one sex only (27 out of 65 broods). However, the use of pair-wise comparisons using a subsample of the nests gave similar results; and for the sake of clarity we report only results of the mixed-model and nested ANOVAs. Analyses of variances were carried out with the statistical software JMP IN 4·0 (Sall & Lehman 1996).

Effects of ectoparasite load manipulation on sex ratios at fledging and mortality rates were investigated using logistic regression analyses with binomial errors and a logit link function, using the statistical software GLMStat (Beath 1997). Sex ratios at fledging were analysed taking the number of sons in a brood as the dependent variable and number of sexed nestlings (i.e. brood size 10 days after hatching) as the binomial denominator. Nestling mortality was analysed separately for each sex taking the number of male or female nestlings that died during the course of the experiment as the dependent variable and brood size 10 days after hatching as the binomial denominator. Because sex-specific mortality may be influenced by brood sex composition, we examined

male and female mortality rates by separately considering 10-day-old broods containing only males (n = 12), only females (n = 15) and both sexes (n = 38, hereafter denoted as 'mixed-broods'). We also examined the effect of hatching date and brood size 10 days after hatching on male and female mortality rates.

Our ectoparasite load manipulation affected the body size and mortality rate of nestlings in years 2000 and 2001 similarly (treatment by year interactions, all P-values > 0·12) and, as a consequence, data of 2 years were pooled for statistical analyses. Mean values are quoted ± 1 SE, statistical tests are two-tailed, and P-values smaller than 0·05 considered significant.

Our manipulation of ectoparasite load and PHA injections were performed under the legal authorisation of the veterinary services of the Canton Solothurn and Bern (licence nos 27023 and 54/02). Although our experiment was to add parasites to nests already naturally infested, the number of ectoparasites per parasitized nestling remained within the natural range of infestation, as observed in previous years in Solothurn and other Swiss colonies (Roulin *et al*. 1998; Bize *et al*. 2003a; P. Bize, personal observation). In the Alpine Swift, nestling mortality can be extremely high, since Arn (1960) observed mortality rates ranging between 5 and 98% in Solothurn. In 2000 and 2001, the mortality induced by our experiment was similar to those observed in other Swiss colonies in the same years (Swiss Ornithological Station, personal communication).

Results

The inflammatory response towards PHA was similar in male and female nestlings (mean PHA response of males *vs* females: 1·16 mm ± 0·09 mm *vs* 1·07 mm ± 0·11 mm; mixed-model four-way ANOVA, sex: $F_{1,58}$ = 0·28, P = 0·60), and in junior and senior nestmates (rank: $F_{1,58}$ = 0·18, P = 0·67; rank × sex: $F_{1,58}$ = 0·005, P = 0·95). PHA response increased with higher body condition ($F_{1,60}$ = 4·05, P = 0·05), and differed significantly among nests ($F_{43,58}$ = 1·73, P = 0·03). There was no relationship between the age of nestlings and their response towards PHA (r = −0·16, n = 44, P = 0·29), and thus this factor was excluded from our final model.

Parasite prevalence was 100% in both treatments, but the number of louse flies was significantly lower in deparasitized than parasitized broods (3·9 ± 0·6 *vs* 13·0 ± 1·0 louse flies per nestling; mixed-model nested four-way ANOVA, treatment: $F_{1,52}$ = 30·06, P < 0·0001). A significant part of the variance was explained by the factor 'nest' ($F_{52,54}$ = 6·42, P < 0·0001). Ectoparasites were evenly distributed on male and female nestlings (8·2 ± 1·1 louse flies per male nestling *vs* 7·1 ± 1·0 louse flies per female nestling; sex: $F_{1,54}$ = 0·34, P = 0·56; treatment × sex: $F_{1,54}$ = 0·43, P = 0·51). As demonstrated elsewhere (Roulin *et al*. 2003), senior nestlings bore more ectoparasites than junior nestmates ($F_{1,54}$ = 10·69, P = 0·002).

Table 1 shows that, at the start of the experiment, there was no difference between treatments and sexes in nestling body mass and wing length. Forty days later, male and female nestlings were still similar in size in both parasitized and deparasitized broods, although our manipulation of ectoparasite loads significantly decreased wing growth rate. Thus, louse flies affected wing length and body mass to a similar extent in males and females, as suggested by the absence of a significant treatment by sex interaction. Parasitized nestlings had on average 3% shorter wings than deparasitized ones at 50 days after hatching.

Fifty days after hatching, the proportion of male fledglings per brood was significantly different between the two treatments ($F_{1,52}$ = 4·07, P = 0·049), with 1·7 times more males at fledging in parasitized compared with deparasitized broods (Fig. 1). Secondary sex ratios were biased toward males in parasitized broods (G-test, G = 4·82, P = 0·045; Fig. 1), but did not deviate significantly from 1:1 sex ratio in deparasitized broods (G = 1·56, P = 0·32; Fig. 1).

Effect of treatments on secondary sex ratios is explained by a greater female mortality in parasitized than deparasitized broods (Table 2; Fig. 2a). Interestingly, in both treatments female nestlings died more often in broods containing at least one male sibling compared with broods containing only female siblings (Table 2; Fig. 2a). Male nestlings died as often in broods containing only males as in mixed-broods (Table 2; Fig. 2b). Male mortality was higher in larger broods, whereas no such effect of brood size could be detected in female mortality (Table 2). Broods hatching late in the season suffered a stronger mortality than those hatching earlier (Table 2). Junior nestlings were more likely to die than their senior nestmates (logistic regression, rank: Wald χ^2 = 8·57, P = 0·003). Juniors died as frequently in parasitized as in deparasitized broods (logistic regression, treatment: Wald χ^2 = 0·65, P = 0·42) and in both treatments their mortality rate did not differ between the sexes (sex: Wald χ^2 = 0·11, P = 0·74; treatment × rank: Wald χ^2 = 1·78, P = 0·18).

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Table 1. Male and female nestling body mass and wing length at 10 and 50 days after hatching (start and end of the experiment, respectively) in relation to the manipulation of ectoparasite load. Mean ± 1 SE are presented together with sample size in brackets; *F*-ratios are presented together with degrees of freedom

		Deparasitized broods		Parasitized broods			
Variable	Age (days)	Female	Male	Female	Male	Treat	Treat × Sex
Body mass (g)	10	43·0 ± 1·3 (30)	43·0 ± 1·4 (25)	43·4 ± 1·6 (23)	43·1 ± 1·4 (25)		0·24 (1,92)
	50	93·7 ± 1·8 (25)	96·8 ± 2·1 (19)	94·3 ± 3·1 (12)	97·3 ± 2·9 (19)		0·09 (1,55)
Wing length (mm)	10	32·3 ± 1·0 (30)	32·3 ± 1·2 (25)	32·2 ± 1·2 (23)	32·4 ± 0·9 (25)		0·06 (1,92)
	50	204·4 ± 2·2 (25)	207·9 ± 2·3 (19)	202·1 ± 3·0 (12)	198·3 ± 3·0 (19)		1·53 (1,55)
Wing loading (g mm^{-1})	50	0·46 ± 0·01 (25)	0·47 ± 0·01 (19)	0·47 ± 0·01 (12)	0·50 ± 0·01 (19)		1·80 (1,55)

Note: *F* ratios are calculated from mixed model nested M[illegible]M with treatment and sex as fixed factors, and the nest nested in treatment as random factor. Only *F*-ratios for fixed factors are presented here.
*$P < 0·05$.

Fig. 1. Mean (SE) proportion of males per brood at 50 days after hatching in relation to the manipulation of ectoparasite load.

Fig. 2. Mean (SE) mortality in percentage of female (a) and male (b) nestlings in broods containing either a single sex or both female and male nestlings in relation to the manipulation of ectoparasite load. Open bars represent deparasitized broods, and closed bars parasitized broods. Sample sizes are indicated above the bars.

Discussion

Although intersexual difference in immune defence has been frequently reported in adult organisms (e.g.

Table 2. Proportion of dead female and male nestlings in a brood in relation to the manipulation of ectoparasite load, brood sex composition, brood size and hatching date. Results are from logistic regression analyses with binomial errors and a logit link function. All interactions were non-significant (all $P > 0{\cdot}30$), and are thus not reported in the table

	df	*F*-ratio	*P*	Difference
Female mortality				
Treatment	1,48	4·98	0·03	Greater mortality in parasitized broods
Brood sex composition	1,48	7·96	0·008	Greater mortality in mixed-sex broods
Brood size	1,48	0·47	0·50	
Hatching date	1,48	5·48	0·02	Greater mortality in late hatched broods
Male mortality				
Treatment	1,45	0·01	0·92	
Brood sex composition	1,45	0·00	0·95	
Brood size	1,45	6·24	0·02	Greater mortality in large broods
Hatching date	1,45	6·02	0·02	Greater mortality in late hatched broods

Grossman 1985; Møller *et al*. 1998; Moreno *et al*. 2001), we found no difference between the sexes in the cell-mediated immunity of growing Alpine Swifts. Similar results were found in the cell-mediated immunity of nestlings in American Kestrel *Falco sparverius* (Tella *et al*. 2000), Barn Swallow *Hirundo rustica* (Saino *et al*. 2002), White Stork *Ciconia ciconia* (Jovani *et al*. 2005) and Magellanic Penguin *Spheniscus magellanicus* (Tella *et al*. 2001), and in cell and humoral immunity of Barn Owl *Tyto alba* nestlings (Roulin *et al*. 2000; A. Roulin, unpublished results). To the best of our knowledge, sex differences in immune defence at the nestling stage have been reported in broods of Eurasian Kestrels *F. tinnunculus*, Black-Headed Gulls *Larus ridibundus* and Great Tits, with male offspring mounting a lower cell-mediated immune response than females (Fargallo *et al*. 2002; Müller *et al*. 2003; Tschirren *et al*. 2003). In Alpine Swift, the absence of sex-related differences in cell-mediated immune response at the nestling stage could be due to the fact that offspring have not yet developed a fully matured immune system, hiding potential differences between the sexes (Apanius 1998). Alternatively, the immune system can be affected by environmental conditions (Christe *et al*. 2000) and, although immunocompetence did not differ between sexes in 2002, it cannot be excluded that heavy infestations differentially affected the ability of male and female offspring to raise an immune response in 2000 and 2001. We found, however, no difference in the number of louse flies per male and female nestlings in 2000 and 2001. Finally, difference between sexes in immune responsiveness could arise only at adulthood because sexual dimorphism in circulating hormones occurs during sexual and reproductive activities or because the two sexes face different trade-offs between immune defence and costly sexual and reproductive activities (Zuk & Stoehr 2002). Alpine Swifts do not differ during growth in size and plumage coloration between the sexes (present study; P. Bize, unpublished results), and adults are socially monogamous (Arn 1960). Thus, males and females may have similar resources allocation in immune defence and, as a consequence, have similar immunocompetence. The similar distribution of louse flies between the sexes in nestling (present study) and adult (Tella & Jovani 2000) swifts supports this hypothesis.

Sex difference in parasite intensity is common at adulthood, with males being usually more infested than females (Poulin 1996; Zuk & McKean 1996; Schalk & Forbes 1997; Moore & Wilson 2002). In the present study, we compared the ectoparasite load between the sexes at the nestling stage, and found no difference of infestation between male and female Alpine Swift nestlings. Along the same line, a similar number of ectoparasites was reported on the body of male and female offspring in the Barn Owl, and ectoparasites were also similarly fecund when collected on male and female nestmates (Roulin *et al*. 2001). At least two hypotheses can explain a lack of sex-biased infestation. First, ectoparasites do not attack one sex preferentially because male and female nestlings display a similar immune response, as found in Alpine Swifts (present study) and Barn Owls (Roulin *et al*. 2000). Second, the within-brood distribution of ectoparasites is governed by other factors than the immunocompetence of their hosts (Roulin *et al*. 2003; Valera *et al*. 2004). For instance, the blood-sucking louse fly *C. melbae* attacks senior nestlings more often than junior ones, and avoids newly hatched and non-feathered nestling hosts (Roulin *et al*. 2003). Thus, one hypothesis accounting for the within-brood distribution of louse flies is that host variation in feather development plays a more important role than host variation in immune defence, with hosts becoming similarly infested as they become similarly feathered (Roulin *et al*. 2003).

Potti & Merino (1996) have proposed that 'parasites may interact with both host hormones and the host

immune system to cause sexual size dimorphism in young animals'. To back up this proposition, they observed that, under natural situation, male Pied Flycatchers grew shorter tarsi than females in the presence of mites (Potti & Merino 1996). Similarly, in Great Tits, experimental manipulation of ectoparasite load had a stronger effect on the development of male than female offspring (Tschirren *et al*. 2003). Here, we found that, although experimentally parasitized Alpine Swift nestlings had shorter wings at 50 days after hatching, the detrimental effect of louse flies was similar in the two sexes. Discrepancy between the effect of parasites on Great Tit and Alpine Swift nestlings may arise because sexual dimorphism in size and coloration is present in Great Tit nestlings but not Alpine Swifts. At the nestling stage, male Great Tits are already larger and more colourful than females (Tschirren *et al*. 2003) and, as consequence, may suffer a higher cost of parasitism due to higher energy requirements associated with a large body size (Teather 1987; Anderson *et al*. 1993; Krijgsveld *et al*. 1998), or to the utilization of sex-specific metabolites (Martins 2004) in the expression of plumage traits.

The immunocompetence handicap hypothesis (Folstad & Karter 1992) predicts a greater mortality of parasitized male than female organisms. In contrast to this hypothesis, our results show that in Alpine Swift females suffered a greater mortality in comparison with males when raised in parasitized broods, biasing sex ratios at fledging toward males. The proportion of males per brood at fledging was 1·7 times greater in parasitized than deparasitized nests. At least three mechanisms can account for this female-biased mortality in parasitized broods.

First, females may be physiologically less resistant to parasites than males because of sex differences in immunity or nutrient requirements (Martins 2004), and in turn suffer to a higher extent the detrimental effects of parasites. We found no support for this hypothesis because females were not more intensely attacked than males by louse flies and, more importantly, did not reach a smaller size in comparison to males in parasitized than deparasitized broods. There was also no difference in cell-mediated immunity between the sexes (note, however, that the assessment of immunocompetence was carried out in a different year from the ones when we assessed the effect of parasites on sex-specific mortality).

Second, in birds, females are heterogametic (ZW), and the possession of heterologous sex chromosomes may predispose them to higher mortality rates, because of their higher exposure to deleterious sex-linked recessive alleles (Trivers & Willard 1973; Myers 1978). Although such alleles are probably not involved in parasite resistance, their effects might be particularly harmful in poor environments, predicting an increase in female mortality rates under harsh rearing conditions such as heavy infestation (Trivers & Willard 1973; Myers 1978). Even if deleterious sex-linked recessive alleles are relatively rare (Crow 1997; Giannelli & Green 2000), we cannot exclude that heterogameity contributed to the female-biased mortality of Alpine Swift offspring when faced with a high degree of ectoparasite infestation (see also De Kogel 1997).

Finally, male and female offspring may differ in the amount of food they obtain through sibling competition, or are given by parents. Parasites are known to alter sibling competition (Christe *et al*. 1996) and reproductive effort of parents (Christe *et al*. 1996; Bize *et al*. 2004), which in turn can change the allocation of resources towards male offspring if, for instance, sons are more aggressive and out-compete their sister when competition is intense, or if parents favour male nestlings when conditions deteriorate (Martins 2004). Accordingly, we found that female nestlings had a very low mortality rate between day 10 and 50 after hatching (6%) in deparasitized broods containing females only, whereas they suffered a very high mortality rate (53%) when competing for food with brothers in parasitized broods (Fig. 2a). It suggests that female-biased mortality in parasitized Alpine Swift nestlings might be mediated by sibling competition and / or parental favouritism. Future experiments should focus upon sex-specific differences in behaviour and competitiveness of Alpine Swift offspring, as well as upon how parental and offspring behaviour interact in their effect on secondary sex ratio (see also Martins 2004).

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References

Alonso-Alvarez, C. & Tella, J.L. (2001) Effects of experimental food restriction and body-mass changes on avian T-cell mediated immune response. *Canadian Journal of Zoology* **79**, 101–105.

Anderson, D.J., Reeve, J., Martinez, G.J.E., Weathers, W.W., Hutson, S., Cunningham, H.V. & Bird, D.M. (1993) Sexual size dimorphism and food requirements of nestling birds. *Canadian Journal of Zoology* **71**, 2541–2545.

Antonov, A. & Atanasova, D. (2003) Chick conditions in first and second broods in the pallid swift *Apus pallidus*. *Ardea* **91**, 197–204.

Apanius, V. (1998) Ontogeny of immune function. *Avian Growth and Development: Evolution Within the Atricial–Precocial Spectrum* (J.M. Starck & R.E. Ricklefs, eds), pp. 203–222.Oxford University Press, Oxford.

Arn, H. (1960) *Biologische Studien Am Alpensegler*. Verlag Vogt-Schild AG, Solothurn.

Beath, K.J. (1997) *Glmstat User Manual*, Version 5·2·1. K.J. Beath, Sydney, Australia.

Bize, P., Roulin, A., Bersier, L.-F., Pfluger, D. & Richner, H. (2003a) Parasitism and developmental plasticity in Alpine swift nestlings. *Journal of Animal Ecology* **72**, 633–639.

Bize, P., Roulin, A. & Richner, H. (2003b) Adoption as an offspring strategy to reduce ectoparasite exposure. *Proceedings of the Royal Society of London B (Suppl.) Biology Letters* **270**, S114–S116.

Bize, P., Roulin, A., Tella, J.L., Bersier, L.-F. & Richner, H. (2004) Additive effects of ectoparasites over the reproductive attempts in the long-lived Alpine swifts. *Journal of Animal Ecology* **73**, 1080–1088.

Casto, J.M., Nolan, V. & Ketterson, E.D. (2001) Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). *American Naturalist* **157**, 408–420.

Christe, P., Richner, H. & Oppliger, A. (1996) Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. *Behavioral Ecology* **7**, 127–131.

Christe, P., Møller, A.P., Saino, N. & De Lope, F. (2000) Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). *Heredity* **85**, 75–83.

Clutton-Brock, T.H. (1991) *The Evolution of Parental Care*. Princeton University Press, Princeton, NJ.

Corrier, D.E. & Deloach, J.R. (1990) Interdigital skin test for evolution of delayed hypersensitivity and cutaneous basophil hypersensitivity in young chickens. *American Journal of Veterinary Research* **51**, 950–954.

Crow, J.F. (1997) The high spontaneous mutation rate: is it a health risk? *Proceedings of the National Academy of Sciences USA* **94**, 8380–8386.

De Kogel, C.H. (1997) Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. *Journal of Animal Ecology* **66**, 167–178.

Ellegren, H. (1996) First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society of London B* **263**, 1635–1641.

Fargallo, J.A., Laaksonen, T., Pöyri, V. & Korpimäki, E. (2002) Inter-sexual differences in the immune response of Eurasian kestrel nestlings under food shortage. *Ecology Letters* **5**, 95–101.

Ferrari, N., Cattadori, I.M., Nespereira, J., Rizzoli, A. & Hudson, P.J. (2004) The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. *Ecology Letters* **7**, 88–94.

Folstad, I. & Karter, A.J. (1992) Parasites, bright males, and the immunocompetence handicap. *American Naturalist* **139**, 603–622.

Giannelli, F. & Green, P.M. (2000) The X chromosome and the rate of deleterious mutations in humans. *American Journal of Human Genectics* **67**, 515–517.

Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G. (1998) A DNA test to sex most birds. *Molecular Ecology* **7**, 1071–1075.

Grossman, C.J. (1985) Interactions between the gonadal steroids and the immune system. *Science* **227**, 257–261.

Hudson, P.J. & Dobson, A.P. (1997) Host–parasite processes and demographic consequences. *Host–Parasite Evolution: General Principles and Avian Models* (eds D.H. Clayton, & J.M. Moore). Oxford University Press, Oxford.

Hurlbert, S.H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**, 187–211.

Jovani, R., Tella, J.L., Blanco, G. & Bertellotti, M. (2005) Variable inter-annual relationships between T-cell mediated immunity and individual life-history traits in white storks. *Ardeola* in press:.

Krijgsveld, K.L., Dijkstra, C., Visser, G.H. & Daan, S. (1998) Energy requirements for growth in relation to sexual size dimorphism in marsh harrier *Circus aeruginosus* nestlings. *Physiological Zoology* **71**, 693–702.

Lessells, C.M. & Boag, P.T. (1987) Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116–121.

Lochmiller, R.L. & Deerenberg, C. (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87–98.

Martins, T.L.F. (1997) Fledging in the common swift, *Apus apus*: weight-watching with a difference. *Animal Behaviour* **54**, 99–108.

Martins, T.L.F. (2004) Sex-specific growth rates in zebra finch nestlings: a possible mechanism for sex ratio adjustment. *Behavioral Ecology* **15**, 174–180.

McCorkle, F.M., Olah, I. & Glick, B. (1980) The morphology of phytohaemagglutinin-induced cell response in the chicken's wattle. *Poultry Science* **59**, 616–623.

Møller, A.P., Sorci, G. & Erritzoe, J. (1998) Sexual dimorphism in immune defence. *American Naturalist* **152**, 605–619.

Moore, S.L. & Wilson, K. (2002) Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* **297**, 2015–2017.

Moreno, J., Potti, J., Yorio, P. & Borboroglu, P.G. (2001) Sex differences in cell-mediated immunity in the Magellanic penguin *Spheniscus magellanicus*. *Annales Zoologici Fennici* **38**, 111–116.

Müller, W., Dijkstra, C. & Groothuis, T.G.G. (2003) Inter-sexual differences in T-cell-mediated immunity of black-headed gull chicks (*Larus ridibundus*) depend on the hatching order. *Behavioral Ecology and Sociobiology* **55**, 80–86. Myers, J.H. (1978) Sex-ratio adjustment under food stress: maximization of quality or numbers of offspring. *American Naturalist* **112**, 381–388.

Potti, J. (1999) Environmental factors and sexual differences in mass and condition of nestling pied flycatchers *Ficedula hypoleuca*. *Ecoscience* **6**, 19–24.

Potti, J. & Merino, S. (1996) Parasites and the ontogeny of sexual size dimorphism in a passerine bird. *Proceedings of the Royal Society of London B* **263**, 9–12.

Potti, J., Dávila, J.A., Tella, J.L., Frías, Ó. & Villar, S. (2002) Gender and viability selection on morphology in fledgling pied flycatchers. *Molecular Ecology*. **11**, 1317–1326.

Poulin, R. (1996) Sexual inequalities in helminth infections: a cost of being a male? *American Naturalist* **147**, 287–295.

Roberts, M.L., Buchanan, K.L. & Evans, M.R. (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* **68**, 227–239.

Rolff, J. (2002) Bateman's principle and immunity. *Proceedings of the Royal Society of London B* **269**, 867–872.

Roulin, A., Ducrest, A.-L., Jeanmonod, J. & Broch, L. (1998) Prévalence et intensité parasitaire d'un Hippoboscide (Diptera) chez les jeunes martinets à ventre blanc *Apus melba*. *Nos Oiseaux* **45**, 185–190.

Roulin, A., Jungi, T., Pfister, H. & Dijkstra, C. (2000) Female barn owls (*Tyto alba*) advertise good genes. *Proceedings of the Royal Society of London B* **267**, 937–941.

Roulin, A., Riols, C., Dijkstra, C. & Ducrest, A.-L. (2001) Female plumage spottiness signals parasite resistance in the barn owl (*Tyto alba*). *Behavioral Ecology* **12**, 111–119.

Roulin, A., Brinkhof, M.W.G., Bize, P., Richner, H., Jungi, T.W., Bavoux, C., Boileau, N. & Burneleau, G. (2003) Which chick is tasty to parasites? The importance of host immunology versus parasite life history. *Journal of Animal Ecology* **72**, 75–81.

Saino, N., Ambrosini, R., Martinelli, R., Calza, S., Møller, A.P. & Pilastro, A. (2002) Offspring sexual dimorphism and sex-allocation in relation to parental age and paternal ornamentation in the barn swallow. *Molecular Ecology* **11**, 1533–1544.

Sall, J. & Lehman, A. (1996) JMP start statistics. *A Guide to Statistical and Data Analysis Using JMP and LMP in Software*. Duxbury Press, London.

Schalk, G. & Forbes, M.R. (1997) Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. *Oikos* **78**, 67–74.

Sheldon, B.C., Merilä, J., Lindgren, G. & Ellegren, H. (1998) Gender and environmental sensitivity in nestling collared flycatchers. *Ecology* **79**, 1939–1948.

Smits, J.E., Bortolotti, G.R. & Tella, J.L. (1999) Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13**, 567–572.

Teather, K.L. (1987) Intersexual differences in food consumption by hand-reared great-tailed grackle (*Quiscalus mexicanus*) nestlings. *Auk* **104**, 635–639.

Tella, J.L. & Jovani, R. (2000) Sources of variability in aggregation and sex ratios of *Crataerina melbae* (Diptera: Hippoboscidae) among adult colonial Alpine swifts. *Journal of Parasitology* **86**, 933–938.

Tella, J.L., Gajon, A., Gortazar, C. & Osacar, J.J. (1998) High host specificity of *Crataerina melbae* (Diptera: Hippoboscidae) in a mixed colony of birds. *Journal of Parasitology* **84**, 198–200.

Tella, J.L., Bortolotti, G.R., Dawson, R.D. & Forero, M.G. (2000) The T-cell-mediated immune response and return rate of fledgling American kestrels are positively correlated with parental clutch size. *Proceedings of the Royal Society of London B* **267**, 891–895.

Tella, J.L., Forero, M.G., Bertellotti, M., Donázar, J.A., Blanco, G. & Ceballos, O. (2001) Offspring body condition and immunocompetence are negatively affected by high breeding densities in a colonial seabird: a multiscale approach. *Proceedings of the Royal Society of London B* **268**, 1455–1461.

Trivers, R.L. & Willard, D.E. (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**, 90–92.

Tschirren, B., Fitze, P.S. & Richner, H. (2003) Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings (*Parus major*). *Journal of Animal Ecology* **72**, 839–845.

Valera, F., Hoi, H., Darolova, A. & Kristofik, J. (2004) Size versus health as a cue for host choice: a test of the tasty chick hypothesis. *Parasitology* **129**, 59–68.

Wedekind, C. & Folstad, I. (1994) Adaptive or non-adaptive immunosuppression by sex-hormomes? *American Naturalist* **143**, 936–938.

Zuk, M. & McKean, K.A. (1996) Sex differences in parasite infections: patterns and processes. *International Journal of Parasitology* **26**, 1009–1023.

Zuk, M. & Stoehr, A.M. (2002) Immune defence and host life history. *American Naturalist* **160**, S9–S22.

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