Spleen volume varies with colony size and parasite load in a colonial bird

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Comparisons across bird species have indicated that those more exposed to parasites and pathogens invest more in immunological defence, as measured by spleen size. We investigated how spleen volume varied with colony size, parasite load and an individual's colony-size history in the cliff swallow, *Petrochelidon pyrrhonota*, a colonial passerine bird of North America. We used a sample of over 1700 birds that had all died during a period of inclement weather in 1996. We experimentally manipulated ectoparasitism by fumigating nests in some colonies prior to the bad weather. Birds from parasite-free colonies had significantly smaller spleens than those from naturally infested sites; spleen volume did not differ between the sexes and did not vary with age. Mean spleen volume increased significantly with the colony size at a site prior to the bad weather in 1996 and at the site in 1995, both measures of colony size being indices of ectoparasitism at a site. An individual's history of breeding-colony size (defined as the average colony size it had occupied in years prior to 1996) had no association with its spleen size. The results are consistent with parasite-induced splenomegaly whenever birds are exposed to large numbers of ectoparasites. The results do not support spleen size as being a signal of differential life-history investment in immunological defence among individuals and thus run counter to interpretations from recent cross-species comparisons.

Keywords: coloniality; immunological defence; parasitism; *Petrochelidon pyrrhonota*; spleen

1. INTRODUCTION

Investment in immunological function is now considered to be a component of most organisms' life histories. Recent comparative work across species indicates that animals allocate this investment differently, depending on their probability of exposure to parasites and pathogens. For example, bird species that migrate to separate breeding and wintering grounds, reuse old nests, live in large colonies or inhabit the tropics show greater immunological responses than other species, presumably because they encounter more kinds of parasites in those areas (Møller & Erritzøe 1996, 1998; Møller 1998; Møller *et al.* 2001). These results have been interpreted to indicate that selection can favour animals that invest more in immunological function in certain situations.

One index of immunological defence that has attracted recent interest and figured prominently in comparative analyses is spleen size. The spleen is thought to have a role in lymphocyte recirculation, antibody synthesis and phagocytosis with the consequent destruction of antigens, although its precise role in the immunological response has not been well characterized, especially in birds (John 1994). Comparative studies across species have demonstrated positive correlations between the extent of parasitism by nematodes and spleen size (John 1995; Morand & Poulin 2000), further indicating a link between spleen function and immunological response to parasitism. However, it is unknown whether larger spleens represent the evolution of greater immunological investment as a life-history strategy or merely reflect splenomegaly as a proximate response to a current exposure to parasites. One possible approach to answering this question is to examine

the variation in spleen size within species, in which exposure to parasites can be experimentally manipulated and in which individuals encounter variable numbers of parasites under natural conditions.

Little is known about intraspecific variation in spleen size, especially in birds. Seasonal variation in size was reported in white-crowned sparrows (*Zonotrichia leucophrys*), pied flycatchers (*Ficedula hypoleuca*) and willow tits (*Parus montanus*), with birds having smaller spleens at the beginning of the breeding season (Oakeson 1953, 1956; Silverin 1981; Fänge & Silverin 1985; Silverin *et al.* 1999). The only previous attempt to relate spleen size with parasite load within a species that we are aware of was the study by Shutler *et al.* (1999) of helminth parasitism in lesser snow geese (*Chen caerulescens*), in which they found little apparent association between parasitism by cestodes, trematodes or nematodes and spleen size.

In this study, we examine how spleen size varies with colony size and ectoparasite load in the colonially nesting cliff swallow *Petrochelidon pyrrhonota*. We experimentally removed parasites from some nests and used these parasite-free nests as a way of controlling the effect of the ectoparasites. This allowed us to compare directly whether spleen size varied among birds that were exposed versus not exposed to parasites within the same species. We also examined the effect of ectoparasites on the birds' immunological response by correlating spleen size with colony size; cliff swallow colony size at a site is an index of the extent of parasitism by ectoparasites and viruses in this species (Brown & Brown 1996; Brown *et al.* 2001). Finally, we investigated whether there was evidence that cliff swallows, who are more often exposed to parasites through their perennial occupancy of large colonies (for which there is a genetic basis; Brown & Brown 2000), had larger spleens, possibly reflecting a greater investment in

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immunological defence as an evolved adaptation to a highly social lifestyle. The results bear on the unresolved question of whether spleen size is an accurate index of overall investment in immunological defence or whether it mostly reflects current exposure to parasites. Our data-set was a sample of over 1700 birds that were killed by a bad-weather event in our study area in 1996 (Brown & Brown 1998) and for which spleen measurements were taken and colony-use information was available.

2. MATERIAL AND METHODS

(a) *Study animals*

Cliff swallows are highly colonial passerines that breed throughout much of western North America (Brown & Brown 1995). They build gourd-shaped mud nests and attach them to the vertical faces of cliff walls, rock outcrops or artificial sites, such as the eaves of buildings or bridges. Their nests tend to be stacked closely together, often sharing walls. Cliff swallows are migratory, wintering in southern South America, and have a relatively short breeding season in North America. They began to arrive at our study site in late April or early May and departed by late July. They generally raise only one brood.

Cliff swallows are associated with a variety of ectoparasites, endoparasites and viruses (Monath *et al.* 1980; Scott *et al.* 1984; Brown & Brown 1995; Brown *et al.* 2001). In our study area, the most common ectoparasites are a bird flea (Siphonaptera: Ceratophyllidae: *Ceratophyllus celsus*) and the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*). The haematophagous bug has the greatest effect on cliff swallows, causing nestling mortality, having a negative impact on the survival rate of both adult and juvenile birds, influencing colony-site choice and nest use, and serving as a vector for alphaviruses transmitted to the birds (Brown & Brown 1986, 1992, 1996; Chapman & George 1991; Loye & Carroll 1991; Brown *et al.* 2001). Infestations in some of the larger colonies can reach 2600 bugs per nest, and the per capita extent of bug parasitism varies directly with the cliff swallow colony size (Brown & Brown 1996; Brown *et al.* 2001). Swallow bugs are nest-based ectoparasites that over-winter in the cliff swallow nests or in cracks and crevices of the nesting substrate near the nests, and thus the number of bugs found at a site in one year will partly determine the number there the following year. The wingless bugs disperse between colonies only when carried by cliff swallows.

(b) *Study site*

Our study site is centred at the Cedar Point Biological Station (41°13′ N, 101°39′ W) near Ogallala, in Keith County, along the North and South Platte Rivers, and also includes portions of Deuel, Garden and Lincoln counties, southwestern Nebraska. We have studied cliff swallows there since 1982. There are *ca.* 160 cliff swallow colony sites in our 150 km × 50 km study area, with about one-third of these not used in a given year. The colony size at a site varies widely; in our study area, it ranges from 2 to 3700 nests, with some birds nesting solitarily. Over a 20-year period, the mean (± s.e.) colony size (*n* = 1363) was 363 (± 16) nests. Each colony site tends to be separated from the nearest site by 1–10 km but, in a few cases, by ≥ 20 km. In our study area, the birds nest on both natural cliff faces and artificial structures such as bridges, buildings and highway culverts. The study site is described in detail by Brown & Brown (1996).

(c) *Fumigating and designating colony sizes*

We removed swallow bugs and other ectoparasites by applying a dilute solution of Dibrom (naled) as a light mist to the outsides of the nests. This insecticide is highly effective against most arthropods, and we have used it extensively in our previous work (e.g. Brown & Brown 1996). The nests are lightly sprayed once a week to remove any bugs immigrating into the colony on arriving birds. The two fumigated colonies used in this study were fumigated in their entirety, with the process beginning in early May each year as the first cliff swallow arrived. Prior to the bad-weather event (§ 2d), these two colonies consisted of 200 and 1500 nests, from which we collected data on spleen size from 10 and 307 individuals in 1996, respectively. Fumigation has no detectable effects on cliff swallows (Brown & Brown 1996).

Cliff swallows colony size is defined as the maximum number of nests to have housed one or more eggs. Active nests were counted at some sites by periodically checking the nest contents with a dental mirror and flashlight, whereas the colony size at other sites was estimated by counting the number of nests in active sections of the colony. Full details on these methods of determining colony sizes are given in Brown & Brown (1996). In 1996, we also used estimates of colony size at sites prior to the bad-weather event (§ 2d), which was a period before all birds had laid eggs. These colony-size estimates were derived from observations of the number of cliff swallows present at the colonies in the week preceding the bad weather, primarily at dusk when the birds return to the colony site to roost (a time when accurate estimates of colony size can be made (Brown & Brown 1996; Brown 1998)). Previous histories of breeding-colony size were calculated for all birds in the sample that had been banded in our study area prior to 1996; this was done by averaging the sizes of all breeding colonies that a given individual had been known to occupy in 1995 and earlier. This varied from one to seven colonies per bird, depending in part on the longevity of the individual bird, on when we first caught it and on the extent to which we had caught it in each previous year (some individuals were missed in some years).

(d) *The bad-weather event and the collection and measurement of birds*

The cliff swallows for which spleen measurements were taken were those found to have died during a rare climatic event in the study area in 1996 (Brown & Brown 1998). This period of unusually cold and wet weather occurred during 24–29 May after most cliff swallows had arrived and settled in colonies. Mortality was extensive, with the population reduced by at least 53% during this 6-day period (Brown & Brown 1998). We salvaged 1856 specimens. These were birds found dead beneath the nests at colony sites and, in a few cases, found inside the nests. We visited most colony sites in the area immediately after the bad weather and collected all the specimens we could find. The external morphology of the specimens was measured (Brown & Brown 1998), all were prepared as skins, the carcasses were saved, and later all spleens (if specimen condition allowed; *n* = 1723) were measured by a single person (M.B.B.). At the time of measurement, no hypotheses had been developed, and thus measurements were unlikely to have been biased in any way by expectations. The length and width of each spleen was taken in millimetres with calipers. The spleens were ellipsoidal in shape, and the volume (in mm³), *V*, was calculated using the formula

$$V = \frac{\pi \times \text{length} \times \text{width}^2}{6}. \tag{2.1}$$

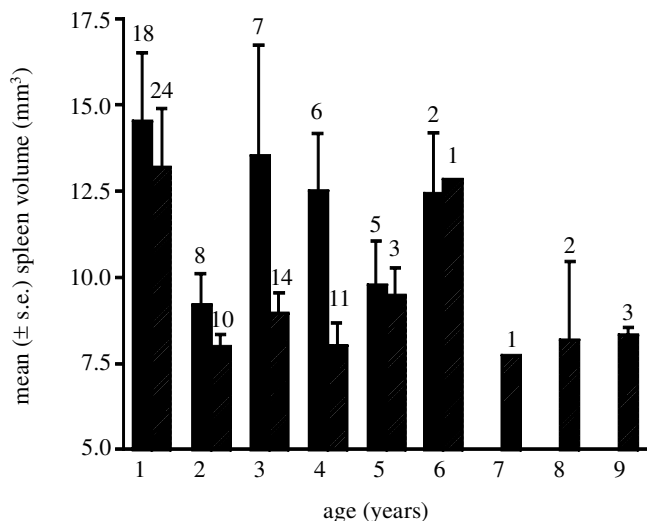Figure 1. Mean (± s.e.) spleen volume values in millimetres cubed in relation to the age of cliff swallows from both fumigated (hatched bars) and non-fumigated (solid bars) colonies in 1996. Spleen volume did not vary significantly with age for either fumigated (Kruskal–Wallis ANOVA, $\chi^2_8 = 6.15$, $p = 0.63$) or non-fumigated colonies ($\chi^2_5 = 2.39$, $p = 0.79$). The sample sizes (number of birds) are shown above the bars.

Due to the fact that our primary sample of birds were ones that had died during unusual conditions, spleens of a smaller sample of 15 birds that had died due to other causes (mostly roadkills) in a later year (2000) were measured in the same way by the same person, to allow comparison of spleen size in cliff swallows during more normal conditions.

Statistical analyses were done with SAS (SAS Institute, Inc. 1989). As spleen volume was not normally distributed (Shapiro–Wilk's test on the entire dataset; $W = 0.67$, $p < 0.0001$, $n = 1723$), we used non-parametric tests whenever an individual bird was the unit of analysis (e.g. those analyses involving sex, age, or comparisons between birds from fumigated and non-fumigated sites).

3. RESULTS

(a) Effects of parasite load, sex and age

Birds from the two parasite-free (fumigated) colonies had significantly smaller spleen volumes than those from the 32 non-fumigated sites; for the sexes combined, the mean (± s.e., n) for the fumigated sites was 9.39 mm³ (± 0.21, 317), compared with 11.61 mm³ (± 0.17, 1406) for the non-fumigated colonies (Wilcoxon test, $Z = -8.28$, $p < 0.0001$). Sex had no effect on spleen size: males from fumigated and non-fumigated colonies had mean spleen volumes of 9.87 mm³ (± 0.39, 141) and 11.79 mm³ (± 0.24, 730), respectively, compared with 9.00 mm³ (± 0.21, 176) and 11.40 mm³ (± 0.24, 676) for females, respectively. The intersexual difference was nonsignificant for both fumigated (Wilcoxon test, $Z = 0.97$, $p = 0.33$) and non-fumigated sites ($Z = -0.51$, $p = 0.61$). To determine the effect of age, we only used birds that had been banded as nestlings or juveniles in an earlier year and thus for whom we knew exact ages. Age had no effect on spleen volume among birds from either fumigated or non-fumigated colonies (figure 1).
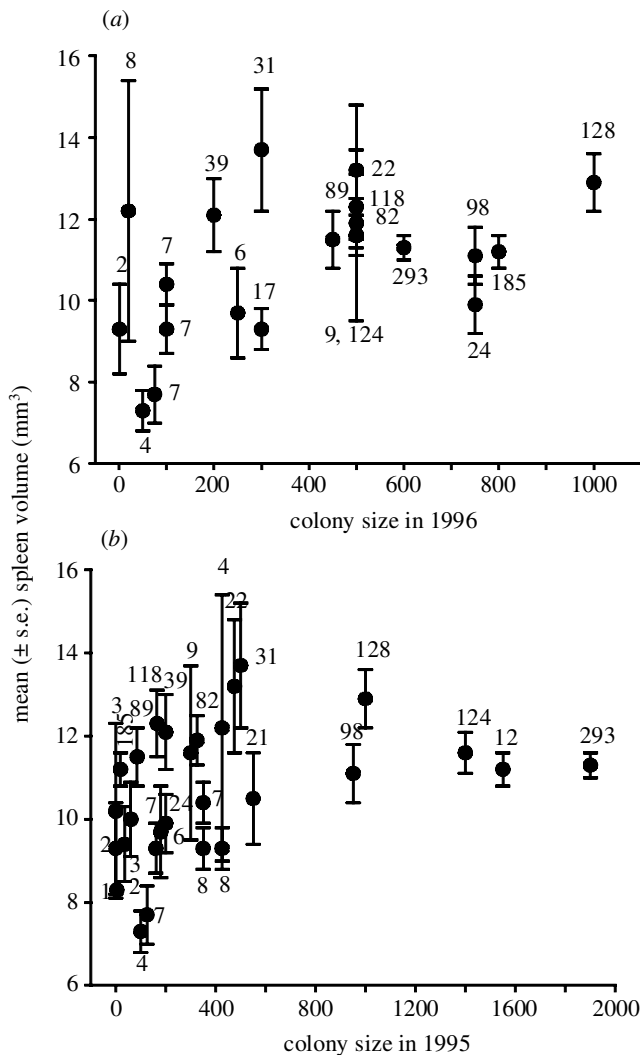

Figure 2. Mean (± s.e.) spleen volume values in millimetres cubed in relation to cliff swallow colony size before the bad-weather event in 1996 (*a*) and colony size at the site the preceding year (*b*). The numbers by the dots indicate the sample sizes (number of birds) for each colony. In (*a*), spleen volume increased significantly with colony size ($r = 0.47$, $p = 0.03$, $n = 21$ colonies); in (*b*) spleen volume also increased significantly with colony size ($r = 0.38$, $p = 0.04$, $n = 29$ colonies). Some sites were not included in the analysis for 1996 because we lacked estimates of their colony size prior to the bad weather. Only non-fumigated colonies were used.

As spleen size did not vary significantly with sex or age among the birds in our sample, we disregarded these variables in further analyses (§ 3b, c).

(b) Effects of colony size at a site

Another measure of parasite load at a site is the colony size, given that swallow bug and flea infestations, and viral infection rates, increase in larger colonies (Brown & Brown 1996; Brown *et al.* 2001). Using the colony-size estimates from 1996 prior to the bad weather, we found that the average spleen volume of the colony residents increased significantly with colony size (figure 2a). Thus, there was a relationship between spleen size and the colony size occupied by a bird in the current year. There was a similar result when we compared the spleen volume of

birds at a site with the colony size of that site in the previous year: the average spleen volume (in 1996) increased significantly with the colony size in 1995 (figure 2b). The previous year's colony size reflects the number of over-wintering swallow bugs and fleas (and virus infection frequency) at a site in the current year, because many bugs and fleas can survive from one year to the next. By contrast, we found no significant association between spleen size at a site in 1996 and its colony size two years before that (in 1994; $r = 0.09$, $p = 0.65$, $n = 28$) or three years before that (in 1993; $r = 0.05$, $p = 0.79$, $n = 28$). The two fumigated sites were not included in any of these analyses of colony size.

(c) *Effects of an individual's previous colony-size history*

Using our sample of birds for which their breeding-colony sizes were known in at least one year prior to 1996, we examined whether an individual's colony-use history was associated with its spleen size. We first considered whether a bird's previous use of fumigated versus non-fumigated colonies, irrespective of size, potentially influenced spleen size. For this analysis, an individual was scored as having used a fumigated site if it had used at least one fumigated colony prior to 1996; non-fumigated ones were birds not known to have ever used a fumigated site. For birds that occupied fumigated colonies in 1996, spleen volumes did not differ significantly among those that previously had (9.10 ± 0.25 mm^3, $n = 149$) versus had not (9.67 ± 0.57 mm^3, $n = 25$) used fumigated sites (Wilcoxon test, $Z = 1.46$, $p = 0.14$). For birds occupying non-fumigated colonies in 1996, spleen volumes did not differ significantly among those that previously had (11.94 ± 0.58 mm^3, $n = 127$) versus had not (11.27 ± 0.36 mm^3, $n = 206$) used fumigated sites (Wilcoxon test, $Z = 0.42$, $p = 0.67$). Thus, we disregarded the fumigation status of previous colony use in further analyses.

There was no significant association between a bird's history of breeding-colony size, measured as its average colony size used in all years prior to 1996, and spleen volume for individuals occupying either fumigated or non-fumigated colonies in 1996 (figure 3). For birds at the two fumigated sites, there was an indication that, if anything, spleen size may have been smaller among individuals known to have used larger colonies prior to 1996 (figure 3b), although the correlation coefficient was nonsignificant at the 0.05 level. In case the differences in spleen volumes among sites in 1996 (figure 2a) might have obscured any relationship between spleen size and colony-size history when individuals from different colonies were combined, we also analysed separately 11 colonies in which we had at least six individuals with known histories. The correlation between spleen volume and colony-size history was positive for the birds at six of these colonies and negative for the remaining five; none was statistically significant ($p \geqslant 0.14$ on all).

(d) *Spleen volumes for birds not killed by the bad-weather event*

The mean spleen volume for the 15 birds killed by other causes in 2000 was 10.44 (± 1.47) mm^3. Nine of these birds had probably occupied fumigated colonies in 2000 and six had used non-fumigated sites. This compares with
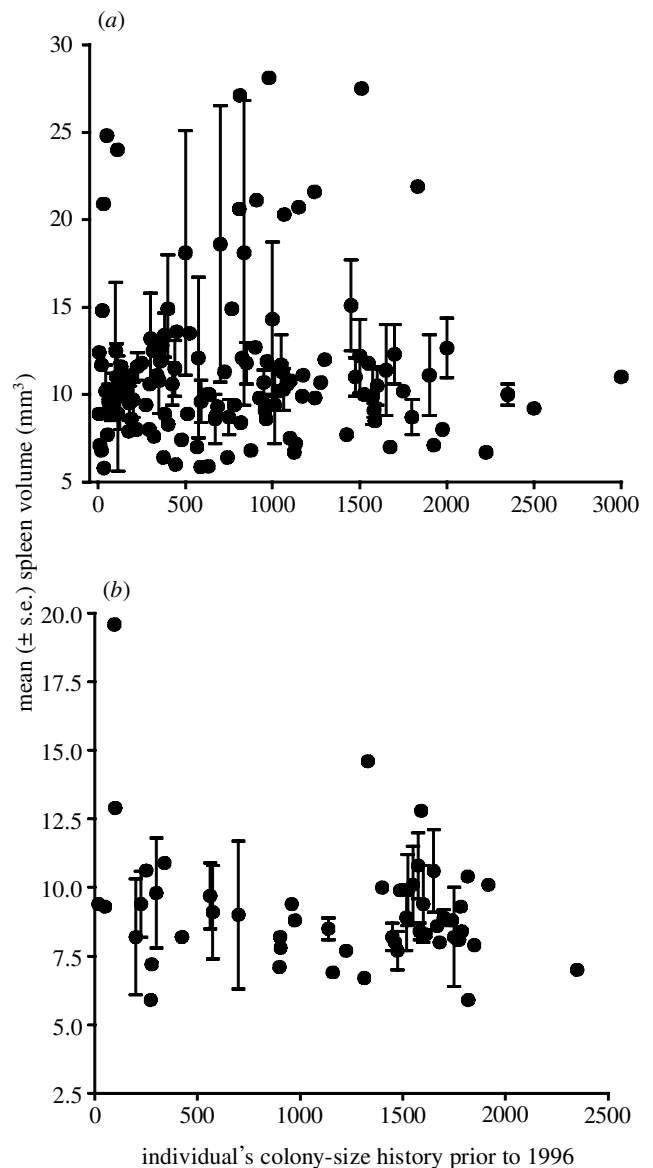

Figure 3. Mean ($\pm$ s.e.) spleen volume values in millimetres cubed in relation to an individual cliff swallow's history of colony-size use, defined as their mean breeding-colony size over all years prior to 1996 for which we knew its history, for birds occupying non-fumigated (a) and fumigated (b) colonies in 1996. Each filled circle represents a single individual, unless s.e. bars are shown, in which case the mean $\pm$ s.e. value for that colony-size history is plotted. In (a), spleen volume did not vary significantly with colony-size history ($r = 0.02$, $p = 0.79$, $n = 131$). The same is true for (b) ($r = -0.23$, $p = 0.09$, $n = 53$).

a mean spleen volume of 11.20 (± 0.14) mm^3 ($n = 1723$) averaged over all birds from both fumigated and non-fumigated colonies in the bad weather of 1996. Thus, spleen sizes among those birds that starved to death in 1996 were similar to those under more benign ('normal') conditions.

4. DISCUSSION

To our knowledge, this is only the second intraspecific study of how spleen size varies with parasite load in birds and the first experimental investigation in free-living birds.

In contrast to the snow goose study by Shutler *et al.* (1999), which found little association between spleen size and parasitism, we found that spleens among cliff swallows parasitized by haematophagous bugs and fleas were significantly larger (by more than 20% on average) than among birds occupying parasite-free colonies. Our results were not potentially confounded by seasonal variation in spleen size because all of our samples were taken at one point in time (on 29 May when the birds succumbed to the cold weather). The increase in spleen size for birds using larger colonies is also one of the few predictive ecological relationships involving the spleen reported within a species to date.

The positive correlation between spleen size and colony size in cliff swallows is consistent with comparative studies showing larger spleens in species routinely exposed to more parasites (Møller 1998; Møller & Erritzøe 1998); colonial species specifically were found to have larger spleens than paired congenors that nested solitarily (Møller & Erritzøe 1996). This was interpreted in the context of life-history theory to mean that species encountering more parasitism invest relatively more in immunological defence (e.g. Møller *et al.* 1998a, 2001). However, larger spleens can also reflect splenomegaly as a response to current levels of parasitism and immunological challenge, and consequently spleen size *per se* might have little to do with evolved life-history allocations of energetic or physiological investment. The correlations in spleen size seen between species might simply reflect the fact that individuals of some species are more often parasitized and the resulting splenomegaly is more likely to be found in these individuals at any given time. This might particularly be the case if diseased or heavily parasitized individuals with enlarged spleens are more likely to die.

For two reasons, our data indicate that spleen size in the cliff swallow mostly reflects a response to current levels of ectoparasitism and is unlikely to signal differential investment in immunological function between individuals. First, the experimental removal of ectoparasites via fumigation clearly showed that birds parasitized by bugs and fleas had larger spleens than birds unexposed to parasites sampled at the same time and from the same population. Second, colony-use history had no effect on spleen size. As cliff swallows show heritable variation in colony-size use, with birds tending to occupy breeding colonies similar in size to those of their parents (Brown & Brown 2000), some individuals are routinely exposed to high levels of parasitism and others to low levels throughout their lives. This should select for differential investment in immunological defence, following the rationale developed in the comparative work (Møller & Erritzøe 1996, 1998; Møller 1998; Møller *et al.* 1998a, 2001). However, colony-size history had no significant association with spleen size, indicating that only current exposure to ectoparasites is relevant. The correlations between spleen size and the current and previous year's colony size at that site—both measures of present parasite load and virus infection frequency—are consistent with larger spleens mostly reflecting parasite-induced splenomegaly. Although we do not rule out the possibility that cliff swallows, using different colony sizes, may invest differentially in immunological defence or show different reactions to parasitism, this study indicates that spleen size *per se* is unlikely to be a

meaningful index of any such differences among individuals.

Our intraspecific results differ in another way from those obtained across species: we found no difference in spleen size between males and females. A comparative study of 368 species of birds (albeit many with very small sample sizes, often $n < 5$) found significantly larger spleens in females than males after adjusting for body size (Møller *et al.* 1998b). (Such an adjustment is unnecessary in monomorphic cliff swallows because males and females are the same size.) It was suggested that the pattern across species resulted from males having reduced immunological function, possibly mediated through sexual selection, implying that males are investing more in behaviour or physiology related to mate attraction than in their immune systems. In support of this interpretation, Møller *et al.* (1998b) reported that the frequency of extra-pair paternity varied inversely with spleen size among males. We found no support for such a scenario in cliff swallows: spleens were larger among birds in larger colonies where, if anything, sexual selection is more intense and the frequency of extra-pair copulations greater than in small colonies (Brown & Brown 1996). The similarity in spleen size among males and females in the cliff swallows is further evidence that the spleen reflects primarily the current exposure to parasites (which will be the same for males and females within a colony) and not differential investment in immunological defence.

Our data indicate that the spleen is involved in the immunological response of cliff swallows to haematophagous arthropods. Most work on the spleen in both birds and mammals has investigated how it responds to malarial protozoans or to endoparasites such as helminths (e.g. Vincent & Ash 1978; Molyneux *et al.* 1983; Ali & Behnke 1985; Garside *et al.* 1989; Watkins *et al.* 1991; John 1994, 1995; Shutler *et al.* 1999; Morand & Poulin 2000). Even for these parasites, the spleen's specific role in immunological resistance is not well known, especially in birds. To date, little to no attention has been given to how the avian spleen responds to challenge from ectoparasites such as ticks, fleas or cimicid bugs. In mammals, immunological responses to ectoparasitism by ticks include antibody production, and lymphocytic and basophilic hypersensitivity; peripheral blood lymphocytes appear to be stimulated by saliva from the feeding tick (Willadsen 1980; Wakelin 1996). Assuming that the avian immune system responds similarly to bites from ectoparasites such as bugs and fleas, the increase in spleen size among more heavily parasitized cliff swallows is consistent with the spleen's presumed role in production of T and B lymphocytes and its trapping of antibody-complexed antigens (John 1994). Overall, avian and mammalian spleens appear to be broadly similar in structure and function, and thus presumably they respond to immunological challenge in similar ways.

The physiological costs of mounting an immunological response have seldom been quantified for any taxa. It has been assumed that investment in the immune system (e.g. spleen size) is costly and reduces the energy available for other activities such as mate attraction or nestling growth (Møller *et al.* 1998b, 2001). Whether this assumption is true in general is unknown, but if immunological defence is costly, our results illustrate yet another parasite-related disadvantage of living in large colonies for cliff swallows.

Birds in the largest colonies exhibit spleen volumes that are up to 75% larger than those of birds in some of the smaller colonies (figure 2). Cliff swallows in the larger colonies are paying both the direct survival costs of ectoparasitism, in terms of its negative effect on nestling survivorship (Brown & Brown 1986, 1996), and the more indirect energetic costs of immunological defence. The costs associated with higher levels of immunological defence may be one contributing factor to the reduced survival probability for parasitized adults (Brown *et al.* 1995).

Due to the fact that our data were taken from individuals that had succumbed in a rare climatic event (Brown & Brown 1998), it is probable that our sample of birds was not truly random. We know that the birds that died were smaller and had greater levels of asymmetry in wings and tails than those that survived. Thus, our reported spleen volumes may not be representative for the population as a whole, especially if more heavily parasitized individuals were less likely to survive the severe weather. However, our conclusions should be unaffected by this for two reasons. First, limited data indicated that spleen size among those killed in the bad weather was not markedly different from that of birds killed due to other causes in a later year. Second, and more importantly, all of our comparisons (e.g. fumigated versus non-fumigated, large versus small colonies) were relative ones carried out among birds that all died at the same time and thus presumably had similar attributes at least with respect to their ability to survive inclement weather.

Our results complement the comparative work on spleen size across species, being among the first studies within a species to address the same questions. However, our experimental approach and the temporally controlled nature of our sampling are in contrast with the more opportunistic collection of data for the cross-species comparisons. We found no evidence to support spleen size as an index of differential investment in immunological defence among different individuals, suggesting caution for comparative studies in interpreting larger spleens as anything other than parasite-induced splenomegaly. Our results indicate that comparative analyses across species should focus on spleen size only in demonstrably uninfected individuals of all taxa.

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