

# Covariation between brain size and immunity in birds: implications for brain size evolution

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## Abstract

Parasitism can negatively affect learning and cognition, setting the scene for coevolution between brain and immunity. Greater susceptibility to parasitism by males may impair their cognitive ability, and relatively greater male investment in immunity could compensate for greater susceptibility to parasites, in particular when males have a relatively large brain. We analysed covariation between relative size of immune defence organs and brain in juvenile and adult birds. The relative size of the bursa of Fabricius and the spleen in adults covaried positively with relative brain size across bird species. The relative size of these two immune defence organs covaried with sex differences in relative size of the brain, indicating that the relationship between immune defence and brain size was stronger for males. In contrast, liver and heart size or sexual size dimorphism in size did not covary with immune defence. Thus, species in which males have relatively large brains also have relatively large immune defence organs.

## Introduction

We propose a novel hypothesis linking immunity, sexual selection and brain size evolution, suggesting that selection for larger brain size has favoured individuals (in particular males) with strong immune responses, because such individuals would suffer the least in terms of impaired learning and cognition because of the negative effects of parasites. This hypothesis is based on the assumptions that (i) learning is facilitated by an absence of parasitism; (ii) the two sexes differ in susceptibility to parasites; and (iii) both immunity and secondary sexual characters are condition dependent. Here, we first present these three assumptions; secondly, we provide four different scenarios linking coevolution between immunity and brain size; and, thirdly, we present some testable predictions that we subsequently evaluate in the Results section of the paper.

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The first assumption is that learning is facilitated by an absence of parasites and disease. Discriminative learning and spatial and nonspatial cognitive performance are impaired in individuals suffering from a range of parasitaemias, and parasitism may thus lead to a reduction in cognitive performance (Kershaw *et al.*, 1959; Stretch *et al.*, 1960; Olson & Rose, 1966; Dolinsky *et al.*, 1981; Kvalsvig, 1988; Kvalsvig *et al.*, 1991; Nokes *et al.*, 1992; Kavaliers *et al.*, 1995; Sakti *et al.*, 1999; Al Serouri *et al.*, 2000; Stolfus *et al.*, 2001; Fiore *et al.*, 2002; Jukes *et al.*, 2002). Given that discriminatory ability may be affected by disease status (Kershaw *et al.*, 1959; Stretch *et al.*, 1960; Olson & Rose, 1966; Dolinsky *et al.*, 1981; Kvalsvig, 1988; Kvalsvig *et al.*, 1991; Nokes *et al.*, 1992; Kavaliers *et al.*, 1995; Sakti *et al.*, 1999; Al Serouri *et al.*, 2000; Stolfus *et al.*, 2001; Fiore *et al.*, 2002; Jukes *et al.*, 2002), mate choice by females and other aspects of sexual selection may be directly affected by parasites through their effects on brain function. Bird song may provide an appropriate example. Repertoires are costly at least in terms of brain space utilization. Bird song is associated with an increase in the number of neurones and greater synaptic and dendritic development in the higher vocal centre of the brain (Nottebohm *et al.*, 1981, 1986;

Canady *et al.*, 1984; review in Garamszegi & Eens, 2004). Annual cycles in the size of the higher vocal centre and the robust nucleus of the archistriatum used for song production suggest that the maintenance of these structures is costly in terms of brain space and/or energy (Nottebohm *et al.*, 1986). As the size of the higher vocal centre is positively related to repertoire size in interspecific studies of birds (DeVoogd *et al.*, 1993; Székely *et al.*, 1996), this implies that the magnitude of the cost increases with repertoire size. Repertoire size may be linked to immune defence through the effects of parasitism and disease on song learning (Catchpole, 1996). A recent experiment in the European starling *Sturnus vulgaris* demonstrated that unpredictable short-term food deprivations after fledging caused suppressed humoral response that strongly affected song production in the next breeding season (Buchanan *et al.*, 2003).

Neuroendocrine and neuroimmune secretions provide links between brain, endocrine system and immune system. The immune system has been termed our sixth sense, given its crucial role in sensing the potentially dangerous entry into the body of alien invaders (Blalock, 1994). The efficiency of the immune system depends to a high degree upon the ability of the individual to learn from past experience. Thus, the ability to avoid particular sites at particular times of the day or the year may considerably reduce the risk of serious parasite infection (Hart, 1997).

The second assumption is that sex differences in susceptibility to parasitism are common (Alexander & Stimson, 1989; Zuk, 1990; Zuk & McKean, 1996; Moore & Wilson, 2002), with males generally being more susceptible than females (Poulin, 1996; Schalk & Forbes, 1997; McCurdy *et al.*, 1998). The cause of sex differences in parasitism is unclear. First, Alexander & Stimson (1989), Zuk (1990) and Zuk & McKean (1996) suggested that sex differences in parasitism may arise from sex differences in susceptibility caused by males suffering more than females from intense competition for access to mates. Secondly, Folstad & Karter (1992) proposed that males suffer from immunosuppression due to the negative effects of androgens on immune function, either as a consequence of elevated androgen levels in reproductive males (Folstad & Karter, 1992), or as an adaptive response to such levels (Møller & Saino, 1994; Wedekind & Folstad, 1994). Thirdly, males and females may be differentially exposed to parasites, with individuals of the more exposed sex suffering more from parasitism. Such sex differences in parasitism may also have important implications for learning and the evolution of cognitive abilities in the two sexes. If males suffer more from parasitism than females, then we should expect males to suffer from impaired learning and cognitive abilities. As sexual selection generally is more intense in males than in females in most taxa (Andersson, 1994), such impaired mental abilities would select for increased size of the brain, but also for increased

immune function to ameliorate or compensate for the negative effects of sex differences in susceptibility.

The third assumption is that immunity and brain function are condition dependent. The immune system is condition dependent with responsiveness being directly associated with body condition and ingestion of protein-rich food and essential nutrients (Chandra & Newberne, 1977; Gershwin *et al.*, 1985; Lochmiller *et al.*, 1993; Saino *et al.*, 1997; Møller *et al.*, 1998c, 2003; Alonso-Alvarez & Tella, 2001). Likewise, condition can affect the normal development and functioning of the brain (e.g. Kershaw *et al.*, 1959; Stretch *et al.*, 1960; Olson & Rose, 1966; Dolinsky *et al.*, 1981; Kvalsvig, 1988; Kvalsvig *et al.*, 1991; Nokes *et al.*, 1992; Kavaliers *et al.*, 1995; Sakti *et al.*, 1999; Al Serouri *et al.*, 2000; Stolzhus *et al.*, 2001; Fiore *et al.*, 2002; Jukes *et al.*, 2002). Why should brain function and immune function be condition dependent? Only individuals in prime condition are able to allocate resources differentially to several different costly functions without sacrificing any of these (Zahavi, 1975; Zahavi & Zahavi, 1997). Hence, characters that differentially affect fitness should demonstrate particularly high levels of condition dependence because condition dependence of such characters would allow individuals in prime condition to fine-tune the expression of these different characters to facilitate their efficient co-functioning. Thus it is not surprising that secondary sexual characters, life history characters, immune function and cognitive function all show high degrees of condition dependence (e.g. Chandra & Newberne, 1977; Gershwin *et al.*, 1985; Price & Schluter, 1991; Lochmiller *et al.*, 1993; Andersson, 1994; Saino *et al.*, 1997; Møller *et al.*, 1998c, 2003; Alonso-Alvarez & Tella, 2001).

We can imagine four different scenarios for the coevolution of brain and immune defence. First, relative brain size initially became enlarged and immune function evolved later. Secondly, immune function improved initially followed by subsequent brain size evolution. Thirdly, both brain size and immunity coevolved simultaneously in response to a third factor. Fourthly, selection due to parasites may directly have caused a decrease in brain size and an increase in size of immune defence organs, giving rise to a negative relationship between relative brain size and immune function. In the first scenario, species in which learning ability is of particular importance have evolved larger brains, and immune function later evolved increased efficiency to overcome problems of parasite-impaired learning deficiency. This should particularly be the case in males as compared with females if sex differences in susceptibility to parasites occur. Alternatively, a given species may initially have evolved a relatively large brain as a means to achieve a behavioural adaptation to a given problem. The behavioural change may have exposed individuals to greater parasite-mediated natural selection, causing brain size and immune defence to covary without cognition and parasitism being causally linked. In the second scenario

increased investment in immune function allowed some individuals to learn more quickly or more efficiently, and this later favoured the evolution of a larger brain. In the third scenario a third factor is responsible for both brain size and immune defence organ evolution. An example of such a third factor could be body condition. In the fourth scenario species subject to intense selection from parasites may be unable to develop a large brain, giving rise to a negative relationship between brain size and immune function.

The first aim of this study was to test for covariation between relative brain size and relative size of immune defence organs, after accounting for the effects of allometry, using birds as a model system. We did this for the bursa of Fabricius, which is an immune defence organ where B-cells differentiate in juvenile birds. In contrast, the spleen is an important storage organ for B-lymphocyte differentiation and proliferation of B- and T-cells in both juveniles and adults. Therefore, we conducted separate tests of covariation between relative size of the brain and immune defence organs in two different age classes to determine whether the selection pressures that have resulted in covariation between brain size and immune defence organs acted differently among juveniles and adults. The second aim of this study was to investigate sexual size dimorphism in relative brain mass and mass of immune defence organs. If selection pressures acting on the immune system and the brain differ between the sexes, but have acted in a consistent way on these two types of organs, then we should expect covariation between sexual size dimorphism in brain size and size of immune defence organs. Again, we investigated this both in juveniles and in adults to determine to what extent age-specific selection pressures have been involved. As a control, we investigated to what extent the relative size of the heart and the liver covaried with brain size. These two organs play crucial roles in circulation, digestion and assimilation, potentially giving them an important role in a sexual selection context if the more strenuous activity of reproductive males has selected for more efficient circulation and digestion.

The bursa of Fabricius synthesizes antibodies in juvenile birds (Glick, 1983, 1994; Toivanen & Toivanen, 1987), but regresses before sexual maturity (Rose, 1981; Glick, 1983; Toivanen & Toivanen, 1987). The bursa is responsible for differentiation of the repertoire of B-cells in birds. The spleen is an immune defence organ of the peripheral lymphoid tissue, acting as the main site of lymphocyte differentiation (B-cells) and proliferation (B- and T-cells), producing cells involved in the production of humoral and cell-mediated immune responses (reviews in Arvy, 1965; Rose, 1981; Keymer, 1982; Molyneux *et al.*, 1983; John, 1994). We assume that a larger bursa of Fabricius or spleen can provide better immune defence than a smaller organ for a bird of a given body size. More than 75% of the volume of these two immune defence organs is composed of lymphocytes

(Rose, 1981; Alberts *et al.*, 1983; Toivanen & Toivanen, 1987; John, 1994). Spleen size is recommended as a standard measure of immunocompetence in ecotoxicology studies (National Research Council, 1992). An intraspecific study has suggested a link between spleen mass and helminth infection (Shutler *et al.*, 1999), while an interspecific study has revealed a positive association between nematode species richness and relative spleen mass in birds (John, 1995; Morand & Poulin, 2000). Individual birds in better body condition generally have a larger spleen, even when controlling for disease status (Møller *et al.*, 1998c). In addition, individual birds killed by mammalian predators consistently had smaller average spleens than individuals that died from other causes (Møller & Erritzøe, 2000). Finally, bird species with relatively large spleens suffer more from parasite-induced mortality (Møller & Erritzøe, 2002). Therefore, the size of the spleen has implications for survival prospects of individual birds.

## Material and methods

### Data sources and tests for bias in data

The mass of brain, bursa of Fabricius, spleen, liver and heart were recorded by J.E. to the nearest milligram on a precision balance from post-mortem examination of dead birds. This was carried out blindly with respect to the hypothesis under test. Total body mass was also recorded on a balance to the nearest gram. Danish taxidermists are required by law to record the cause of death of all specimens in a log-book, and more than 95% of all specimens in the present data set were found dead, with the remaining being shot by hunters. Birds were frozen when received by J.E., but any effects of storage on measurements should only cause noise in the data set. In addition, there is no reason to expect sex differences in such effects.

We tested for one potential kind of bias in the present data set. Sampling date might influence size estimates of immune defence organs, as the brain, bursa of Fabricius and spleen have sometimes been demonstrated to show annual fluctuations in size (Nottebohm *et al.*, 1986; Toivanen & Toivanen, 1987; John, 1994; Møller *et al.*, 2003). We tested whether date of sampling differed among species. We found a significant difference for median sampling date among species for each of the three organs (Kruskall-Wallis ANOVAS, brain:  $\chi^2_{246} = 370.23$ ,  $P < 0.001$ ; bursa of Fabricius:  $\chi^2_{55} = 98.31$ ,  $P < 0.001$ ; spleen:  $\chi^2_{124} = 273.95$ ,  $P < 0.001$ ). However, we did not find indications for consistent bias in relative organ size caused by species-specific seasonal effects. In species for which data were available for all seasons, two-way ANOVAS revealed nonsignificant interaction terms between species and a four-scale seasonal variable (brain:  $F = 1.282$ , d.f. = 36, 26, n.s.; bursa of Fabricius:  $F = 0.420$ , d.f. = 33, 30, n.s.; spleen:  $F = 0.971$ , d.f. = 36,

28, n.s.). When we dropped species-season interaction terms from the models, only species as main effects explained significant amounts of variance in relative organ size, while main effects for seasons were not significant (brain: species,  $F = 1476.06$ , d.f. = 12, 12,  $P < 0.001$ ; seasons,  $F = 0.777$ , d.f. = 3, 3, n.s.; bursa of Fabricius: species,  $F = 1091.52$ , d.f. = 11, 11,  $P < 0.001$ ; seasons,  $F = 0.978$ , d.f. = 3, 3, n.s.; spleen: species,  $F = 56.31$ , d.f. = 12, 12,  $P < 0.001$ ; seasons,  $F = 1.81$ , d.f. = 3, 3, n.s.). Therefore, we assumed that the results would not be confounded by potential seasonal effects, and the main pattern we intended to explain here was the consistent interspecific variation in relative brain size independent of seasonal variation. All data are provided in Appendices 1–2.

### Comparative analyses

We controlled for allometric effects by using residuals from the phylogenetically adjusted linear regression of  $\log_{10}$ -transformed mass of organ on  $\log_{10}$ -transformed body size for each sex. We expressed absolute brain size dimorphism as  $\log_{10}(\text{absolute female brain size/absolute male brain size})$ . We avoided calculating brain size dimorphism based on sex-dependent relative brain sizes, because these variables are residuals from the relevant regression lines causing them to scale with the independent variable used (sex-dependent body size). Therefore, combining residuals from two regression lines may introduce bias. As the numerator and the denominator of the absolute brain size dimorphism measured as the  $\log_{10}$ -transformed ratio of absolute female and male brain size scale similarly, absolute brain size dimorphism larger than zero indicates that females have relatively larger brains than males, whereas values smaller than zero reflect the opposite trend. However, absolute brain size dimorphism may result from absolute body size dimorphism due to allometric effects. Thus the  $\log_{10}$ -transformed ratio of absolute female and male brain size should be corrected for the similar ratio in body size. This correction was based on the phylogenetically independent regression of  $\log_{10}(\text{absolute female brain size/absolute male brain size})$  on  $\log_{10}(\text{absolute female body size/absolute male body size})$  (slope: 0.18, intercept =  $-0.02$ , the corresponding phylogenetic model:  $\kappa = 0.24$ ,  $\lambda = 0.63$ , likelihood ratio (LR) = 7.56, d.f. = 1,  $P < 0.001$ ,  $n = 127$ ). Note that absolute brain and body size dimorphism were not residuals by definition, allowing them to be combined in a single regression. Residuals from this regression were subsequently termed relative brain size dimorphism and used in the subsequent analyses. Positive values for relative brain size dimorphism thus indicate that females have relatively larger brains when allometric effects were held constant.

Here we used data for 59 species for bursa of Fabricius with information on liver and heart size for 55 and 50 species, respectively. Data on spleen was available for 127

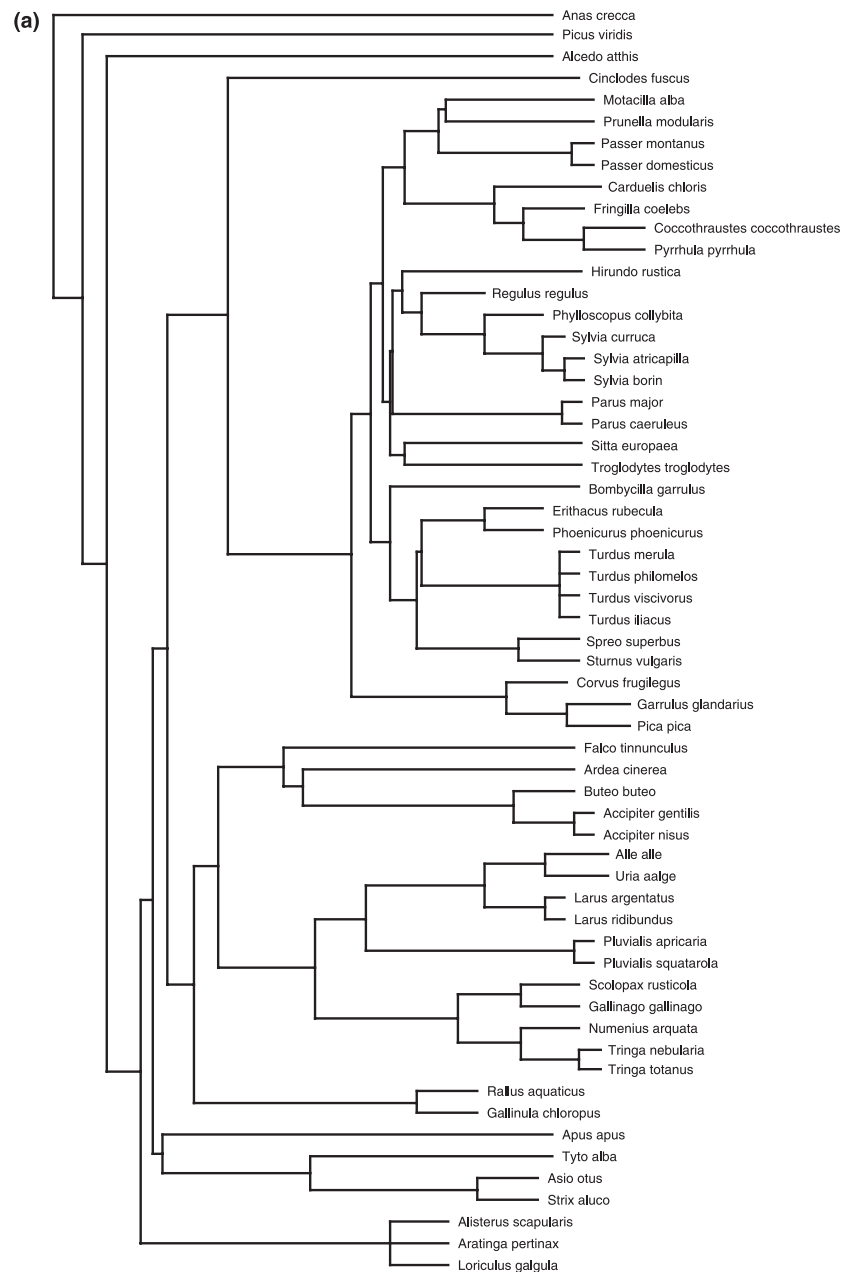
species, and among these species we had data on liver and heart for 111 and 80 species, respectively. These comprised all the species, for which we had data for both males and females. We calculated spleen mass for adult birds and the mass of bursa of Fabricius for juvenile birds, and we tested our predictions with age-specific (adult or juvenile) brain sizes. In both sets of analyses we also estimated the phylogenetic correlations between brain size and liver size and heart size to estimate the importance of coevolution of organs due to metabolic constraints. The correlation between adult spleen mass and mass of bursa of Fabricius in juveniles was weakly positive, but only accounted for 8% of the variance (linear regression of  $\log_{10}$ -transformed data based on species:  $F = 24.09$ , d.f. = 1,47,  $P < 0.05$ ,  $r^2 = 0.08$ ). Hence, the two series of tests were largely independent.

We applied the general method of comparative analysis for continuous variables based on generalized least squares (GLS) models using the statistical software Continuous (Pagel, 1997, 1999). The GLS approach tests phylogenetic hypotheses based on likelihood ratio statistics. This compares the log-likelihood of the model corresponding to a null hypothesis ( $H_0$ ) over the model for an alternative hypothesis ( $H_1$ ), where the likelihood ratio =  $-2 \log_e[H_0/H_1]$ . The likelihood ratio statistic is asymptotically distributed as a chi-squared variate with degrees of freedom equal to the difference in the number of parameters between the two models. Models contain three scaling parameters that can be used to scale branch lengths in the tree ( $\kappa$ ), scale total (root to tip) path in the tree ( $\delta$ ), and to assess the contribution of phylogeny ( $\lambda$ ). We first assessed the contribution of scaling parameters,  $\kappa$ : branch length scaling factor, and  $\lambda$  phylogeny scaling factor [recent simulations showed that the estimation of  $\delta$ : overall path length scaling factor is biased (Freckleton *et al.*, 2002), and therefore we avoided estimating this parameter]. Scaling parameters were fitted sequentially. Once an appropriate model with adjusted scaling parameters had been selected, we studied correlated evolution of traits of interest by comparing the goodness of fit of model  $H_0$  fitted to the data by allowing only independent evolution with that alternative  $H_1$  model that permits correlated evolution of the characters. The appropriate scaling parameters and the log-likelihood ratio statistics testing for correlated trait evolution are presented. When we controlled for potentially confounding factors we entered these variables together with the variables of interest in the same model, and tested for correlated trait evolution. If the model offering the best fit with the data allowed correlation among traits, we calculated partial phylogenetic correlation for the relationship in question.

Allometric effects were controlled by calculating residuals from the regression of the  $\log_{10}$ -transformed dependent variable on  $\log_{10}$ -transformed body mass, using Continuous. Based on this equation residuals were calculated on the raw species data (see also Purvis & Rambaut, 1995).

Phylogenetic information for our comparative analyses originated from a number of sources using molecular techniques. We constructed a composite phylogenetic hypothesis mainly based on information in Sibley & Ahlquist (1990) derived from extensive studies of DNA-DNA hybridization. This phylogeny for higher taxa was supplemented with information from Sheldon *et al.* (1992), Leisler *et al.* (1997), Cibois & Pasquet (1999) and Grapputo *et al.* (2001) to resolve relationships in taxa

with many species. We applied branch lengths from the tapestry tree of Sibley & Ahlquist (1990) for higher taxonomic levels. Within families the distance between genera was set to  $3.4\Delta T_{50H}$  units, and between species within genera to  $1.1\Delta T_{50H}$  units (see also Sibley & Ahlquist, 1990; Bennett & Owens, 2002). The two composite phylogenies used in the phylogenetic analyses of young and adult birds are shown without branch length transformation in Fig. 1.



**Fig. 1** Composite phylogenies of bird species for the comparative analysis of covariation between relative brain mass and (a) relative spleen mass and (b) relative mass of bursa of Fabricius. The sources are given in Material and methods. The scale is given at the bottom left.

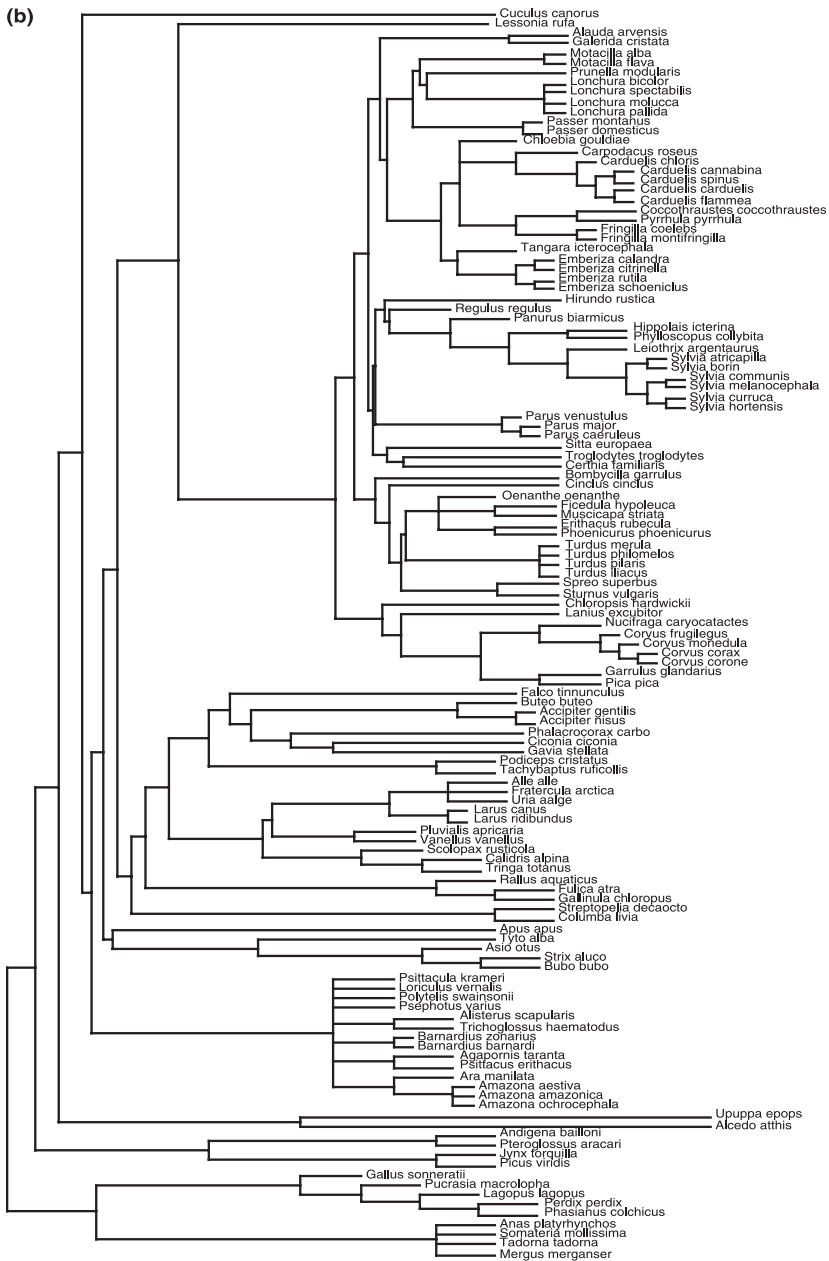


Fig. 1 Continued

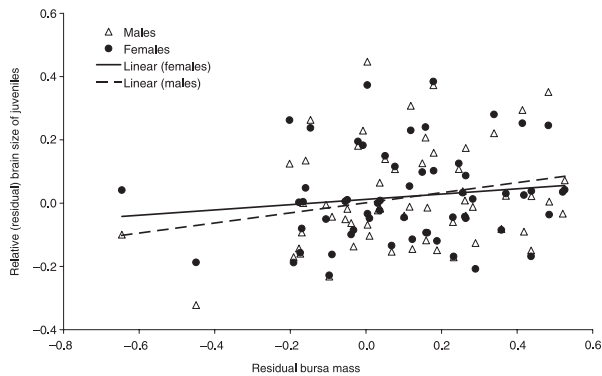
**Results**

**Mass of bursa of Fabricius and mass of brain in juvenile birds**

First we investigated the relationship between the mass of bursa of Fabricius and brain mass of juvenile birds in their first year of life. We made this restriction to ensure that the data on mass of bursa and mass of brain originated from individuals of similar age. For females we did not find a significant relationship between the two

variables (Fig. 2). The phylogenetic correlation was 0.15 (Table 1), and with a sample size of 59 species this test was not powerful if the true effect size is small or intermediate (Cohen, 1988). For males the phylogenetic correlation was 0.28 and significant (Fig. 2; Table 1).

An analysis of sex differences in mass of bursa of Fabricius and mass of the brain for 59 species revealed a phylogenetic correlation of -0.46, which was highly significant (Table 1). Relative brain size in juvenile birds was not related significantly to the mass of the heart and the liver in females (Table 1). However, in males there



**Fig. 2** Covariation between relative brain mass of juvenile birds and relative mass of bursa of Fabricius in different bird species. Relative mass was calculated as residuals from a phylogenetically corrected regression of  $\log_{10}$ -transformed organ mass on  $\log_{10}$ -transformed body mass. The lines are the linear regression lines for males and females, respectively. The corresponding phylogenetic correlations are reported in Table 1, where brain size dimorphism represents the interaction between male and female brain size.

was a significant negative association between brain size and liver size (Table 1). We found no significant evidence for bursa size being related phylogenetically to liver size or to heart size (Table 1). Controlling for the confounding effects due to covariation with liver and heart size we found a stronger association between brain size and bursa size in both sexes (partial phylogenetic correlations: females,  $r = 0.28$ ,  $P = 0.03$ ,  $n = 50$ ; male,  $r = 0.41$ ,  $P < 0.001$ ,  $n = 50$ ).

### Mass of spleen and mass of brain in adult birds

In this analysis of spleen mass and brain mass we restricted the data to adult individuals to allow a test that was

independent of the test based on bursa of Fabricius. For females we found a nonsignificant positive phylogenetic correlation between spleen size and brain size of 0.16, based on a sample of 127 species (Fig. 3; Table 2). For males the phylogenetic correlation was 0.21 and significant (Fig. 3; Table 2). Relative brain size was not significantly related to the mass of the heart or the liver in either sex (Table 2). We found a tendency for spleen mass to be positively related to liver mass but not to heart mass (Table 2). These relationships weakened previous associations between sex-dependent brain size and spleen size in males (partial phylogenetic correlations: females,  $r = 0.19$ ,  $P = 0.08$ ,  $n = 80$ ; male,  $r = 0.14$ ,  $P = 0.21$ ,  $n = 80$ ).

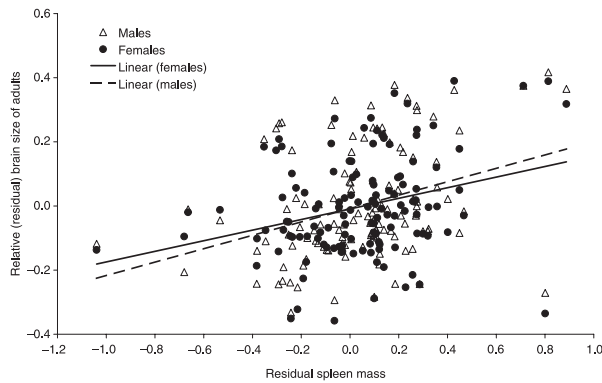
We analysed the sex difference in relationship between spleen mass and brain mass in adult birds for 127 species. The phylogenetic correlation was  $-0.16$  (Table 1).

A global test of the two data sets revealed a phylogenetic correlation  $r = 0.16$ ,  $P = 0.04$ ,  $n = 186$  for females. A test of heterogeneity showed no evidence of statistical significance ( $P = 0.95$ ). For males the phylogenetic correlation was  $r = 0.23$ ,  $P = 0.002$ ,  $n = 186$ . Again, a test of heterogeneity was not statistically significant ( $P = 0.62$ ). Based on fixed effects, the sex difference in the relationship had a phylogenetic correlation  $r = 0.26$ , which was highly significant ( $P < 0.001$ ,  $n = 186$ ). However, a test of heterogeneity was statistically significant ( $P < 0.05$ ). If calculations were based on random effect sizes, the phylogenetic correlation for sex difference was  $r = 0.31$ , at  $P = 0.12$ . If we used partial phylogenetic correlation coefficients to control for covariation between sex-dependent brain size and relative mass of liver and heart, the overall effect sizes were positive, significant and homogenous (females:  $r = 0.22$ ,  $n = 130$ ,  $P < 0.51$ ,  $P$  for heterogeneity = 0.62;

**Table 1** Phylogenetic correlations between sex-dependent relative mass of brain, bursa of Fabricius, liver and heart in juvenile birds.

	$\kappa$	$\lambda$	Phylogenetic correlation	LR	d.f.	$P$	$N$
Bursa of Fabricius							
Female brain	0.62	0.89	0.15	0.65	1	0.25	59
Male brain	0.54	0.85	0.28	2.44	1	0.03	59
Brain dimorphism	0.00	0.10	-0.46	7.08	1	<0.001	59
Liver	0.22	0.53	0.14	0.54	1	0.30	55
Heart	0.00	0.25	<0.001	0.00	1	0.98	50
Female brain							
Liver	1.00	1.00	-0.21	1.28	1	0.11	55
Heart	1.00	0.83	-0.21	1.18	1	0.13	50
Male brain							
Liver	1.00	1.00	-0.28	2.28	1	0.03	55
Heart	1.00	0.80	-0.13	0.43	1	0.36	50
Brain dimorphism							
Liver	0.54	0.47	0.02	0.02	1	0.86	55
Heart	0.00	0.18	-0.03	0.02	1	0.86	50

Relative values were controlled for effects of allometry, using residuals from phylogenetically controlled linear regressions. See Results for further details.



**Fig. 3** Covariation between relative brain mass of adult birds and relative mass of the spleen in adults of different bird species. Relative mass was calculated as residuals from a phylogenetically corrected regression of  $\log_{10}$ -transformed organ mass on  $\log_{10}$ -transformed body mass. The lines are the linear regression lines for males and females, respectively. The corresponding phylogenetic correlations are reported in Table 2, where brain size dimorphism represents the interaction between male and female brain size.

males:  $r = 0.25$ ,  $n = 130$ ,  $P < 0.01$ ,  $P$  for heterogeneity = 0.11).

## Discussion

The main findings of this comparative study were that (1) the relative mass of two important immune defence organs covaried positively with the relative mass of the brain. That was the case for juvenile birds for which the relative mass of the bursa of Fabricius covaried with the relative mass of the brain. Likewise, in adults we found positive covariation between relative mass of the spleen

and relative mass of the brain. (2) The relative mass of immune defence organs covaried with sex differences in relative mass of the brain. We found little evidence of significant covariation between brain size and size of liver and heart, both when analysed for the two sexes separately, and when investigating sexual size dimorphism. Thus the patterns reported for immune defence organs are not common to all organs of physiological importance, suggesting a specific role for immune function in brain size evolution. These patterns are all the more striking given the inherent heterogeneity in the data sets and resulting conservative nature of our statistical tests. This study provides the first empirical evidence suggesting that the impact of parasites through the evolution of the immune system may have a direct role in the evolution of the nervous system. The patterns reported here are particularly striking given that nothing similar has been derived from the enormous research effort in neurosciences during the last decades. We briefly discuss each of our main findings in the following paragraphs.

Positive covariation between the relative mass of immune defence organs and relative mass of the brain was present in two data sets on birds (Figs 2 and 3). As this effect was present already in juveniles, we can infer that the selection pressures that have resulted in such covariation occur already among young age classes before sexual maturation. The relative mass of both bursa of Fabricius and spleen is positively correlated with sexual dichromatism, repertoire size, extra-pair paternity, a tropical distribution, bird migration, coloniality, hole nesting, scavenging, and predation risk (Møller & Erritzøe, 1996, 1998, 2000; Møller, 1997, 1998; Møller *et al.*, 1998a, 2000; Garamszegi *et al.*, 2003). Thus, a whole suite of ecological factors has acted on the evolution of relative size of these two immune defence organs.

**Table 2** Phylogenetic correlations between sex-dependent, relative mass of brain, spleen, liver and heart in adult birds.

	$\kappa$	$\lambda$	Phylogenetic correlation	LR	d.f.	$P$	$N$
<b>Spleen</b>							
Female brain	0.48	0.81	0.16	1.60	1	0.07	127
Male brain	0.60	0.87	0.21	2.72	1	0.02	127
Brain dimorphism	0.20	0.32	-0.16	1.63	1	0.07	127
Liver	0.00	0.53	0.17	1.68	1	0.07	111
Heart	0.00	0.53	-0.12	0.62	1	0.26	80
<b>Female brain</b>							
Liver	0.53	0.87	0.04	0.08	1	0.70	111
Heart	0.65	0.92	-0.13	0.68	1	0.24	80
<b>Male brain</b>							
Liver	0.70	0.93	0.08	0.36	1	0.40	111
Heart	1.00	0.97	0.15	0.93	1	0.17	80
<b>Brain dimorphism</b>							
Liver	0.21	0.22	0.07	0.25	1	0.48	111
Heart	0.00	0.00	0.12	0.55	1	0.29	80

Relative values were controlled for effects of allometry, using residuals from phylogenetically controlled linear regressions. See Results for further details.



In addition, the relative mass of the spleen is positively correlated with the magnitude of parasite-induced mortality among nestling altricial birds (Møller & Erritzøe, 2002). Thus species of birds with relatively large spleens are currently subject to greater mortality caused by parasitism. These findings suggest that the positive relationship between relative size of immune defence organs and the relative size of the brain has arisen from a range of different selection pressures associated with parasitism. This is consistent with our hypothesis that hosts from a very early age invest in immune defence to mitigate negative effects of parasitism on neural tissue.

There was significant positive covariation between the mass of two immune defence organs and sex difference in relative mass of the brain, implying that the relationship between immune defence mechanisms and brain size is stronger for males than for females (Figs 2 and 3). That was not the case for the liver or the heart, which are important organs involved in digestion, assimilation and circulation. Sexual differences in immune function is common in birds, as revealed by consistent sex difference in the size of immune defence organs, which is greatest in species with the highest frequency of extra-pair paternity, implying that males of such species have disproportionately small spleens for their body size (Møller *et al.*, 1998b). Sex differences in impact of parasites on their hosts are also common in many different taxa (Alexander & Stimson, 1989; Zuk, 1990; Poulin, 1996; Schalk & Forbes, 1997). While the two sexes of juvenile birds differ in the size of bursa of Fabricius, the sex difference in size of the spleen first appears at sexual maturity (Møller *et al.*, 1998b). Positive covariation between sex difference in brain size and the size of immune defence organs would be consistent with an hypothesis suggesting that parasite-mediated selection has played a role in the evolution of sex differences in brain size. Our empirical findings are consistent with the hypothesis that sex differences in brain function have evolved as a consequence of sex differences in susceptibility to parasitism. Selection acting on the immune system at large may through increased susceptibility of males to parasitism impact negatively on cognitive performance including learning. Therefore, different components of the immune system (bursa and spleen) may have evolved to mitigate this negative impact of parasites on brain function. Obviously, we cannot make claims about causation based on our correlations. However, the assumptions and predictions presented here are open to experimental tests in particular species or even to experimental tests in different species allowing for tests of assumptions and predictions in a comparative framework.

The results presented here also have implications for the evolution of brain size and sex differences in brain size. Sexual size dimorphism in brains of birds covaries

with sex differences in complexity of vocalizations (L.Z. Garamszegi, M. Eens, J. Erritzøe & A.P. Møller, unpublished), suggesting that sexual selection has played a role in the evolution of sexual size dimorphism of brains. This hypothesis was corroborated by a second study showing that sexual size dimorphism in brain mass covaried negatively with the extent of extra-pair paternity (L.Z. Garamszegi, M. Eens, J. Erritzøe & A.P. Møller, unpublished). Thus, female birds have evolved relatively larger brains than males in species with frequent extra-pair paternity. Thus behaviour and cognitive tasks associated with extra-pair paternity and paternity assurance have been significant selective factors acting on the evolution of brain size in the two sexes. The present study suggests that sex differences in susceptibility to parasites may also have played a role in the evolution of sexually size dimorphic brains in birds.

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**Appendix 1** Sex dependent body mass (g), brain mass (g), sample sizes of adult birds and mass of spleen (g) liver (g) and heart (g) with the corresponding body mass averaged across sexes.

Species	Female body mass (g)	Female brain mass (g)	N	Male body mass (g)	Male brain mass (g)	N	Spleen mass (g)	Liver mass (g)	Heart mass (g)	Body mass (g)
<i>Accipiter gentilis</i>	1059.0	7.815	4	678.3	7.619	6	0.827	13.62	8.061	851.0
<i>Accipiter nisus</i>	247.3	3.103	24	136.7	2.648	14	0.149	4.18	1.926	200.0
<i>Agapornis taranta</i>	46.2	1.810	1	45.3	1.975	1	0.020	0.95	0.531	43.1
<i>Alauda arvensis</i>	38.0	0.910	1	40.6	0.966	5	0.028	1.25	0.534	36.1
<i>Alcedo atthis</i>	38.9	0.811	5	38.3	0.832	2	0.031	2.09	0.627	36.6
<i>Alisterus scapularis</i>	168.2	3.900	2	202.2	5.054	5	0.076	4.15	1.792	194.0
<i>Alle alle</i>	138.4	1.790	6	156.5	1.897	3	0.118	3.13	1.104	147.0
<i>Amazona aestiva</i>	203.0	6.980	1	327.4	8.290	2	0.560	14.02	3.403	312.0
<i>Amazona amazonica</i>	312.3	7.636	2	350.8	8.680	2	1.510	7.94	2.756	286.0
<i>Amazona ochrocephala</i>	204.5	6.420	2	341.5	8.800	2	0.325	7.85	2.007	320.0
<i>Anas platyrhynchos</i>	1200.0	5.440	1	1228.0	5.920	7	0.740	29.12		1145.0
<i>Andigena bailloni</i>	149.5	2.910	1	178.1	2.900	2	0.197	11.17		161.0
<i>Apus apus</i>	42.8	0.673	4	38.6	0.730	1	0.022	1.55		30.2
<i>Ara manilata</i>	250.0	7.880	2	291.5	8.840	1	1.200	5.64		265.0
<i>Asio otus</i>	298.0	5.590	38	254.9	5.622	16	0.163	6.53	1.928	284.0
<i>Barnardius barnardi</i>	118.6	3.580	2	119.5	4.137	6	0.168			104.0
<i>Barnardius zonarius</i>	129.4	4.100	1	110.6	4.100	2	0.127	7.28	1.362	127.0
<i>Bombycilla garrulous</i>	65.0	1.046	4	61.8	1.159	4	0.055	3.10	0.832	61.1
<i>Bubo bubo</i>	2992.0	17.187	1	2380.0	17.632	1	0.797			1690.0
<i>Buteo buteo</i>	892.2	7.971	26	687.4	7.968	39	0.689	13.91	4.875	761.0

## Appendix 1 Continued

Species body mass (g)	Female body mass (g)	Female brain mass (g)	N	Male body mass (g)	Male brain mass (g)	N	Spleen mass (g)	Liver mass (g)	Heart mass (g)	Body mass (g)
<i>Calidris alpina</i>	37.6	0.880	2	37.4	0.855	6	0.018	1.46		36.9
<i>Carduelis cannabina</i>	19.1	0.580	2	18.6	0.680	10	0.030	0.45	0.289	19.1
<i>Carduelis carduelis</i>	13.3	0.551	9	16.8	0.642	13	0.006	0.35	0.245	15.6
<i>Carduelis chloris</i>	27.3	0.857	11	26.3	0.888	34	0.028	0.95	0.413	26.7
<i>Carduelis flammea</i>	12.7	0.527	1	11.0	0.550	1	0.005	0.33	0.199	18.2
<i>Carduelis spinus</i>	11.4	0.523	4	12.3	0.607	6	0.013	0.42	0.184	12.7
<i>Carpodacus roseus</i>	21.2	1.030	1	21.0	0.955	2	0.025	1.15	0.308	20.3
<i>Certhia familiaris</i>	10.4	0.570	1	8.8	0.450	1	0.016	0.48		8.5
<i>Chloebia gouldiae</i>	12.2	0.400	1	13.6	0.462	4	0.012	0.46		10.4
<i>Chloropsis hardwickii</i>	21.2	0.720	1	26.3	0.800	1	0.058	1.88	0.388	30.6
<i>Ciconia ciconia</i>	3334.0	15.780	2	3350.0	16.240	1	1.439			4000.0
<i>Cinclus cinclus</i>	58.0	1.440	2	72.0	0.920	1	0.030	2.59		58.0
<i>Coccothraustes coccothraustes</i>	56.7	1.492	3	55.5	1.585	15	0.076	1.66	0.814	52.6
<i>Columba livia</i>	247.0	2.285	1	282.0	2.694	3	0.250			304.0
<i>Corvus corax</i>	1350.0	14.520	1	1313.3	15.815	3	1.207			1328.0
<i>Corvus corone</i>	512.9	8.197	11	553.7	8.443	5	0.388	11.58	3.998	467.0
<i>Corvus frugilegus</i>	472.9	7.640	2	574.3	8.248	2	0.390	13.39	5.293	461.0
<i>Corvus monedula</i>	165.3	4.410	3	232.5	4.883	6	0.139	6.29	2.431	155.0
<i>Cuculus canorus</i>	93.2	1.419	2	118.6	1.500	3	0.031	2.08	1.100	82.6
<i>Emberiza calandra</i>	53.3	1.149	1	57.8	1.138	2	0.030	1.53	0.665	53.9
<i>Emberiza citrinella</i>	28.2	0.781	12	28.7	0.785	26	0.040	0.93	0.427	28.5
<i>Emberiza rutila</i>	18.6	0.491	1	16.5	0.635	2	0.020	0.74	0.265	14.5
<i>Emberiza schoeniclus</i>	20.1	0.750	2	20.8	0.645	2	0.032	0.87		20.8
<i>Erithacus rubecula</i>	17.2	0.713	3	18.3	0.629	9	0.028	0.93	0.200	17.0
<i>Falco tinnunculus</i>	227.6	3.842	5	175.2	3.823	12	0.085	6.10	2.080	198.0
<i>Ficedula hypoleuca</i>	14.5	0.510	1	12.7	0.443	3	0.015	0.66		13.6
<i>Fratercula arctica</i>	318.5	4.200	1	383.8	3.725	2	0.469	17.98		394.0
<i>Fringilla coelebs</i>	23.6	0.743	17	24.3	0.761	39	0.032	0.77	0.279	29.9
<i>Fringilla montifringilla</i>	26.7	0.660	1	23.4	0.749	5	0.036	0.57	0.259	22.7
<i>Fulica atra</i>	509.7	2.990	2	527.0	3.435	2	0.811	53.36	5.291	834.0
<i>Galerida cristata</i>	42.6	1.009	2	41.7	1.143	3	0.024	0.87		41.3
<i>Gallinula chloropus</i>	281.8	1.965	5	343.1	2.110	2	0.336	7.99	2.906	246.0
<i>Gallus sonneratii</i>	321.0	2.770	1	915.0	3.615	2	0.624	16.90	2.080	737.0
<i>Garrulus glandarius</i>	161.2	3.864	17	171.4	3.999	17	0.183	3.98	1.386	162.0
<i>Gavia stellata</i>	984.0	5.200	1	1480.0	6.000	1	0.708	48.42		1663.0
<i>Hippolais icterina</i>	14.6	0.483	3	13.1	0.518	3	0.038	0.80		13.4
<i>Hirundo rustica</i>	17.1	0.538	6	16.1	0.584	6	0.029	0.44		17.8
<i>Jynx torquilla</i>	35.5	0.825	2	40.1	0.865	4	0.036	1.57	0.475	34.3
<i>Lagopus lagopus</i>	561.8	2.400	3	600.4	2.690	2	2.190			598.0
<i>Lanius excubitor</i>	64.1	1.580	1	55.2	1.380	1	0.040			64.2
<i>Larus canus</i>	345.3	4.060	3	470.5	4.100	1	0.413	15.14	3.137	336.0
<i>Larus ridibundus</i>	267.8	2.775	6	200.2	2.897	4	0.413	10.14	2.003	228.0
<i>Leiothrix argentauris</i>	27.0	0.932	3	25.4	1.004	5	0.023			17.9
<i>Lessonia rufa</i>	15.6	0.500	1	14.0	0.400	1	0.004	0.58		14.3
<i>Lonchura bicolor</i>	9.7	0.460	1	9.7	0.410	1	0.009	0.48	0.126	8.2
<i>Lonchura molucca</i>	9.7	0.379	2	9.9	0.424	1	0.010	0.40	0.139	10.4
<i>Lonchura pallida</i>	10.8	0.560	1	10.5	0.452	1	0.043	0.46	0.153	10.7
<i>Lonchura spectabilis</i>	7.8	0.470	1	8.0	0.500	1	0.007	1.10	0.113	7.5
<i>Loriculus vernalis</i>	22.2	0.950	1	26.2	1.380	2	0.046	1.05		24.0
<i>Mergus merganser</i>	1285.0	6.325	2	1635.8	7.313	5	0.555	45.36	18.134	1253.0
<i>Motacilla alba</i>	19.0	0.530	2	21.0	0.569	9	0.032	0.78	0.484	19.3
<i>Motacilla flava</i>	16.9	0.471	1	17.7	0.417	5	0.011	0.69		16.7
<i>Muscicapa striata</i>	12.5	0.396	1	16.8	0.531	1	0.023	0.73	0.175	15.5
<i>Nucifraga caryocatactes</i>	183.0	5.580	1	204.5	6.060	1	0.236			180.9
<i>Oenanthe oenanthe</i>	25.1	0.72	4	24.0	0.600	1	0.030	0.58	0.313	27.0
<i>Panurus biarmicus</i>	13.5	0.567	3	15.5	0.577	3	0.040			14.2
<i>Parus caeruleus</i>	10.8	0.640	3	11.0	0.596	10	0.013	0.39	0.152	10.6

## Appendix 1 Continued

Species body mass (g)	Female body mass (g)	Female brain mass (g)	N	Male body mass (g)	Male brain mass (g)	N	Spleen mass (g)	Liver mass (g)	Heart mass (g)	Body mass (g)
<i>Parus major</i>	17.3	0.793	7	17.8	0.880	15	0.027	0.59	0.202	16.9
<i>Parus venustulus</i>	10.6	0.510	1	10.2	0.552	2	0.014	0.35	0.124	10.9
<i>Passer domesticus</i>	28.3	0.892	10	28.5	0.886	33	0.054	1.03	0.399	29.3
<i>Passer montanus</i>	23.0	0.779	6	22.2	0.770	9	0.031	0.84	0.295	21.4
<i>Perdix perdix</i>	400.8	1.895	4	402.0	1.876	9	0.137	9.08	2.560	371.0
<i>Phalacrocorax carbo</i>	1932.8	10.065	5	2283.0	8.380	1	1.386	59.30		2074.0
<i>Phasianus colchicus</i>	1047.0	3.294	3	1411.4	4.075	46	0.564	25.76	6.360	1353.0
<i>Phoenicurus phoenicurus</i>	15.8	0.350	1	13.3	0.454	7	0.036	0.71	0.175	16.4
<i>Phylloscopus collybita</i>	7.3	0.320	3	9.0	0.292	2	0.007			8.1
<i>Pica pica</i>	200.7	5.036	15	229.2	5.640	13	0.332	6.26	2.294	205.0
<i>Picus viridis</i>	155.5	3.800	1	184.3	4.625	2	0.167			176.0
<i>Pluvialis apricaria</i>	199.3	2.253	4	199.2	2.360	1	0.085	7.70		180.0
<i>Podiceps cristatus</i>	952.8	3.657	3	1024.3	3.505	2	0.577	43.70	7.909	859.0
<i>Polytelis swainsonii</i>	141.9	3.171	2	130.2	3.210	1	0.075	6.33	1.664	81.5
<i>Prunella modularis</i>	21.5	0.623	6	20.3	0.690	10	0.019	0.95	0.222	20.3
<i>Psephotus varius</i>	46.1	1.780	2	45.2	2.100	1	0.136			47.2
<i>Psittacula krameri</i>	111.2	2.968	1	95.0	3.200	1	0.037	5.55		82.2
<i>Psittacus erithacus</i>	240.3	7.450	1	344.9	8.790	2	1.248	14.44	3.117	378.0
<i>Pteroglossus aracari</i>	272.8	3.375	2	256.0	3.600	1	0.432	18.68		275.0
<i>Pucrasia macrolopha</i>	932.0	3.338	1	940.5	3.580	1	0.317	21.48		1009.3
<i>Pyrrhula pyrrhula</i>	24.0	0.834	8	26.5	0.869	30	0.035	0.73	0.321	23.7
<i>Rallus aquaticus</i>	121.9	1.580	1	117.3	1.573	3	0.095			124.0
<i>Regulus regulus</i>	5.5	0.343	6	5.9	0.380	11	0.010	0.31	0.079	5.6
<i>Scolopax rusticola</i>	331.2	2.542	8	330.8	2.476	16	0.181	8.51	3.022	441.0
<i>Sitta europaea</i>	24.1	1.010	2	24.3	1.110	1	0.029	0.60		22.6
<i>Somateria mollissima</i>	1740.0	8.040	1	2244.7	7.909	18	0.703	73.13	12.004	2197.0
<i>Spreo superbus</i>	56.6	1.760	1	51.7	1.804	5	0.111	2.61	0.531	48.1
<i>Streptopelia decaocto</i>	134.4	1.450	1	202.7	1.586	3	0.045	4.24	2.364	134.0
<i>Strix aluco</i>	515.5	9.249	21	426.5	8.903	17	0.244	10.51	2.517	458.0
<i>Sturnus vulgaris</i>	75.5	1.720	3	80.9	1.837	7	0.099	3.16	1.059	69.4
<i>Sylvia atricapilla</i>	18.7	0.594	3	18.6	0.628	10	0.036	0.88	0.215	19.6
<i>Sylvia borin</i>	19.2	0.520	2	18.4	0.657	6	0.020	0.96	0.287	18.4
<i>Sylvia communis</i>	15.2	0.540	4	13.6	0.518	5	0.024	0.54		14.4
<i>Sylvia curruca</i>	13.6	0.470	3	11.5	0.482	5	0.035	0.53	0.150	12.8
<i>Sylvia hortensis</i>	20.9	0.680	1	23.6	0.900	1	0.035	0.78		22.3
<i>Sylvia melanocephala</i>	10.7	0.488	4	11.0	0.567	3	0.020	0.40		10.3
<i>Tachybaptus ruficollis</i>	144.2	1.694	1	143.5	1.751	1	0.010	6.85	1.615	135.5
<i>Tadorna tadorna</i>	676.0	4.880	1	1410.0	5.160	1	0.236	24.07		819.0
<i>Tangara icterocephala</i>	17.3	0.544	1	12.7	0.629	1	0.039	1.28	0.157	15.8
<i>Trichoglossus haematodus</i>	106.1	3.333	3	120.6	3.645	4	0.117			106.0
<i>Tringa totanus</i>	139.2	1.820	1	98.5	1.406	2	0.051	3.32		102.0
<i>Troglodytes troglodytes</i>	8.7	0.445	2	9.8	0.520	7	0.019	0.49	0.124	10.4
<i>Turdus iliacus</i>	62.3	1.076	1	68.1	1.354	1	0.077	2.89	0.783	67.3
<i>Turdus merula</i>	101.9	1.765	22	95.8	1.773	34	0.251	3.92	0.923	98.6
<i>Turdus philomelos</i>	70.4	1.429	14	69.4	1.542	19	0.122	2.46	0.798	69.1
<i>Turdus pilaris</i>	100.5	1.760	2	92.1	1.762	3	0.127	5.19		104.0
<i>Tyto alba</i>	263.8	5.369	7	260.2	5.758	20	0.106	5.55	1.887	308.0
<i>Upupa epops</i>	34.5	1.370	1	38.8	1.180	2	0.069	1.37		34.6
<i>Uria aalge</i>	1092.3	4.965	2	633.0	4.360	1	0.560			735.0
<i>Vanellus vanellus</i>	211.6	2.136	8	240.4	2.000	2	0.166	8.27	2.205	162.0

**Appendix 2** Sex-dependent mass of body (g) and brain (g), sample sizes of juvenile birds and mass of bursa of Fabricius (g), liver (g) and heart (g) with the corresponding body mass averaged across sexes.

Species body mass (g)	Female body mass (g)	Female brain mass (g)	N	Male body mass (g)	Male brain mass (g)	N	Bursa mass (g)	Liver mass (g)	Heart mass (g)	Body mass (g)
<i>Accipiter gentilis</i>	977.4	7.811	4	699.0	7.370	8	0.332	17.61	5.535	851.0
<i>Accipiter nisus</i>	240.4	2.953	40	135.0	2.617	22	0.165	4.94	1.631	200.0
<i>Alcedo atthis</i>	38.7	0.798	6	36.0	0.784	5	0.046	2.03	0.597	36.6
<i>Alisterus scapularis</i>	190.2	4.980	1	204.6	3.750	1	0.093	6.43	1.225	194.0
<i>Alle alle</i>	109.1	1.987	4	175.3	1.864	1	0.079	6.05	1.063	147.0
<i>Anas crecca</i>	316.7	2.806	1	336.6	2.625	3	0.324	8.00	3.278	364.0
<i>Apus apus</i>	30.5	0.616	2	23.1	0.420	1	0.012	0.69	0.476	30.2
<i>Aratinga pertinax</i>	63.6	3.420	1	63.3	4.220	1	0.064	1.94	0.900	66.8
<i>Ardea cinerea</i>	1074.0	7.604	3	1637.5	8.270	4	1.663	27.31	9.565	1794.0
<i>Asio otus</i>	292.0	6.026	32	275.4	6.042	7	0.143	6.51	1.995	284.0
<i>Bombycilla garrulus</i>	60.5	1.169	16	60.0	1.184	14	0.040	3.51	0.909	61.1
<i>Buteo buteo</i>	787.7	7.814	42	680.4	7.689	20	0.663	15.11	4.352	761.0
<i>Carduelis chloris</i>	28.9	0.818	8	27.9	0.964	10	0.024	0.94	0.386	26.7
<i>Cinclodes fuscus</i>	27.8	0.910	1	33.1	0.950	1	0.050			58.0
<i>Coccothraustes coccothraustes</i>	56.4	1.905	1	52.9	1.888	7	0.059	1.65	0.722	52.6
<i>Corvus frugilegus</i>	403.0	7.503	2	476.0	8.677	2	0.764	14.25	3.225	461.0
<i>Erithacus rubecula</i>	17.2	0.613	3	16.2	0.667	22	0.027	0.81	0.173	17.0
<i>Falco tinnunculus</i>	192.0	3.654	26	201.3	3.574	3	0.265	5.09	1.967	198.0
<i>Fringilla coelebs</i>	23.5	0.815	10	22.8	0.831	10	0.036	0.91	0.288	29.9
<i>Gallinago gallinago</i>	113.8	1.410	4	96.0	1.302	5	0.060	2.96	1.168	103.0
<i>Gallinula chloropus</i>	308.1	2.125	2	337.3	2.150	4	0.143	12.74	2.744	246.0
<i>Garrulus glandarius</i>	165.0	4.782	10	174.3	4.299	7	0.280	4.48	1.536	162.0
<i>Hirundo rustica</i>	16.6	0.538	8	19.4	0.612	15	0.032	0.86	0.242	17.8
<i>Larus argentatus</i>	561.5	5.385	2	1252.0	5.989	1	0.862	30.14	7.270	530.0
<i>Larus ridibundus</i>	225.1	2.758	5	224.1	2.984	3	0.514	9.72	2.147	228.0
<i>Loriculus galgulus</i>	30.0	1.470	1	25.3	1.404	1	0.032	1.89	0.330	30.0
<i>Motacilla alba</i>	15.5	0.528	3	18.7	0.653	3	0.054	0.47		19.3
<i>Numenius arquata</i>	833.5	3.951	2	570.8	3.630	3	0.772	34.45	6.533	669.0
<i>Parus caeruleus</i>	11.1	0.648	2	11.1	0.890	1	0.027	0.42	0.150	10.6
<i>Parus major</i>	15.2	0.796	2	17.7	1.022	3	0.030	0.58	0.198	16.9
<i>Passer domesticus</i>	26.7	0.993	13	27.3	0.940	18	0.043	1.30	0.310	29.3
<i>Passer montanus</i>	20.4	0.767	11	20.2	0.788	19	0.028	0.95	0.258	21.4
<i>Phoenicurus phoenicurus</i>	16.3	0.525	2	16.1	0.630	5	0.019	0.74	0.216	16.4
<i>Phylloscopus collybita</i>	7.9	0.357	3	8.4	0.377	3	0.011			8.1
<i>Pica pica</i>	170.8	4.503	3	207.2	6.358	6	0.470	6.15	1.599	205.0
<i>Picus viridis</i>	149.2	4.117	2	188.5	4.341	4	0.197	6.25	2.132	176.0
<i>Pluvialis apricaria</i>	187.2	2.048	5	183.2	1.883	4	0.215	8.71	2.152	180.0
<i>Pluvialis squatarola</i>	184.7	2.157	3	174.2	2.500	1	0.217			196.0
<i>Prunella modularis</i>	18.4	0.642	2	19.9	0.831	3	0.045	1.12	0.221	20.3
<i>Pyrrhula pyrrhula</i>	26.3	0.880	5	26.8	0.954	9	0.019	0.74	0.336	23.7
<i>Rallus aquaticus</i>	89.7	1.580	1	128.5	1.733	2	0.106	3.95	0.826	124.0
<i>Regulus regulus</i>	5.4	0.385	9	6.0	0.343	5	0.002	0.28	0.076	5.6
<i>Scolopax rusticola</i>	324.1	2.400	9	323.8	2.425	7	0.183	9.05	2.946	441.0
<i>Sitta europaea</i>	21.7	1.015	2	22.6	1.118	5	0.032	0.74		22.6
<i>Spreo superbus</i>	44.5	1.795	6	31.6	1.765	2	0.048	2.75		48.1
<i>Strix aluco</i>	484.7	11.305	5	395.8	9.430	5	0.442	10.79	2.094	458.0
<i>Sturnus vulgaris</i>	70.4	1.656	8	73.3	1.778	6	0.118	3.81	0.710	69.4
<i>Sylvia atricapilla</i>	21.4	0.692	4	18.5	0.715	5	0.044	1.15	0.228	19.6
<i>Sylvia borin</i>	14.8	0.579	2	19.3	0.686	5	0.023	1.06	0.220	18.4
<i>Sylvia curruca</i>	14.1	0.549	1	13.3	0.574	5	0.029	0.79	0.146	12.8
<i>Tringa nebularia</i>	149.7	1.607	7	153.5	1.628	4	0.207			151.0
<i>Tringa totanus</i>	102.7	1.295	6	96.7	1.338	4	0.243	2.25		102.0
<i>Troglodytes troglodytes</i>	8.8	0.501	8	10.1	0.525	2	0.048	0.50	0.114	10.4
<i>Turdus iliacus</i>	66.4	1.520	4	66.9	1.489	5	0.057	3.42	0.710	67.3
<i>Turdus merula</i>	99.3	2.043	39	99.1	2.014	58	0.237	4.98	0.846	98.6
<i>Turdus philomelos</i>	72.2	1.671	16	67.5	1.645	23	0.153	3.46	0.673	69.1

**Appendix 2** Continued.

Species body mass (g)	Female body mass (g)	Female brain mass (g)	<i>N</i>	Male body mass (g)	Male brain mass (g)	<i>N</i>	Bursa mass (g)	Liver mass (g)	Heart mass (g)	Body mass (g)
<i>Turdus viscivorus</i>	108.1	2.165	4	103.6	2.313	4	0.296	4.26		101.0
<i>Tyto alba</i>	289.8	5.890	20	236.4	6.169	12	0.281	6.24	1.755	308.0
<i>Uria aalge</i>	843.5	4.420	2	608.5	4.548	3	0.347	27.28	5.258	735.0