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# Life history, immunity, Peto's paradox and tumours in birds

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## Abstract

Cancer and tumours may evolve in response to life-history trade-offs between growth and duration of development on one hand, and between growth and maintenance of immune function on the other. Here, we tested whether (i) bird species with slow developmental rates for their body size experience low incidence of tumours because slow development allows for detection of rapid proliferation of cell lineages. We also test whether (ii) species with stronger immune response during development are more efficient at detecting tumour cells and hence suffer lower incidence of tumours. Finally, we tested Peto's paradox, that there is a positive relationship between tumour incidence and body mass. We used information on developmental rates and body mass from the literature and of tumour incidence (8468 birds) and size of the bursa of Fabricius for 7659 birds brought to a taxidermist in Denmark. We found evidence of the expected negative relationship between incidence of tumours and developmental rates and immunity after controlling for the positive association between tumour incidence and body size. These results suggest that evolution has modified the incidence of tumours in response to life history and that Peto's paradox may be explained by covariation between body mass, developmental rates and immunity.

## Introduction

Cancers are a natural outcome of multicellularity and uncontrolled cell division (Aktipis & Nesse, 2013). Cancers have traditionally been viewed as a natural consequence of the accumulation of mutations leading to uncontrolled cell replication. Moreover, the large number of evolved suppression mechanisms and their intricate design testify to the intensity of selection imposed by cancers (De-Gregori, 2011). Cancers are also viewed as the consequence of intra-individual somatic selection of cell lineages that outcompete and hence outnumber opponent cell lineages (Møller & Pagel, 1998). Such spread of cell lineages may occur even without increased mutation rates or growth rates by simply reducing death rates with somatic cellular selection promoting the spread (Tomlinson & Bodmer, 1997). Outcompeting somatic cells has negative consequences for fitness-related traits in animals, and thus, at the individual level, cancers arise and evolve due to selection to prevent or postpone deaths due to cancer [i.e. cancer selection (Leroi *et al.*, 2003)]. Although many cancers mainly have nonfitness consequences by killing post-reproductive individuals, interspecific interactions such as those between predators and prey or parasites and hosts may differentially affect individuals with tumours (Møller *et al.*, 2013; Vittecoq *et al.*, 2013). Thus, negative fitness consequences of cancers even occur during the reproductive stage of life. Indeed, there is a significant link between the incidence of tumours and the mortality rate of different species of birds at Chernobyl (Møller *et al.*, 2013). Thus, cancer incidences and characteristics of animals that favour tumour cells (i.e. mutations, number of cell divisions, particular environments) and tumour detection (i.e. immunity) should be related under field conditions (Møller *et al.*, 2013).

Many kinds of tumours are common in domestic animals (Meuten, 2016) and humans (National Agency for Research on Cancer; IARC GLOBOCAN project, year

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2008, http://globocan.iarc.fr/). There is an extensive literature reporting frequency and diversity of tumours in animals from zoological gardens because autopsies allow for correct identification of the cause of death (Ratcliffe, 1933; Lombard & Witte, 1959; Snyder & Ratcliffe, 1966; Stewart, 1966; Effron *et al.*, 1977; Wadsworth *et al.*, 1985; Reavill, 2004). Unfortunately, this research has not had an ecological or evolutionary focus, thus preventing evolutionary functional questions to be addressed. The relevance of such data from captivity may also be questionable because protected conditions in captivity may result in mortality caused by cancer late in life (Andervont & Dunn, 1962), and this incidence of cancer may even vary among species. For example, although it is well described that tumours are more common in mammals than in birds (Ratcliffe, 1933; Lombard & Witte, 1959; Snyder & Ratcliffe, 1966; Effron *et al.*, 1977; Wadsworth *et al.*, 1985), even the most basic information on prevalence and sample size in the wild have rarely been reported. Thus, together with the limited diversity of species in zoos, the special characteristics of captive populations and the lack of records on incidence of precancerous lesions, the detected incidence of tumours and cancers prevents analyses of the ecological and evolutionary correlates of cancer incidence. These kinds of questions are also difficult to explore with wild animals because cancers may increase the risk of mortality from biological agents such as predators and parasites, and, thus, cancers and the underlying tumours may often not be recorded in field studies because of premature death (Vittecoq *et al.*, 2013). Here, we take advantage of analyses of 8468 birds killed by hunters, cats and raptors, or dead for other reasons such as collision with cars and wires, that were brought to a taxidermist in Denmark and were meticulously examined (JE), allowing us to explore evolutionary correlates of cancer.

The objectives of this study of wild birds are threefold. First, we analyse the incidence of tumours in relation to developmental rates as reflected by relative duration of the incubation and the nestling periods, which by definition implies rapid cell division and cell proliferation due to rapid growth with consequences for tumours and cancer (Leroi *et al.*, 2003). Life-history traits in general affect the relative investment in reproduction and self-maintenance (e.g. Roff, 1992; Stearns, 1992). In particular, species with slow development have been hypothesized to develop a more capable acquired immune response (Ricklefs, 1992) mainly because of the trade-off between immunity and growth (Soler *et al.*, 2003). This trade-off occurs at the intraspecific level because investment in immunity cannot be allocated to somatic growth, but also at the interspecific level because for a given relative investment in immunity there should be a reduction in the relative level of somatic growth (Ricklefs, 1992). Thus, because species with higher growth rates will be those with higher rates of cell division and a weaker immune system, they should also be those with higher incidence of tumours everything else being equal (Jacqueline *et al.*, 2017). These arguments suggest that trade-offs among life-history traits may play a crucial role in the evolution of cancers (Jacqueline *et al.*, 2017).

Second, we explore associations between tumour incidence and immune response. Immune function plays a significant role in host–parasite interactions (e.g. Wakelin, 1996; Møller & Saino, 2004) and in monitoring any uncontrolled proliferation of cells as in tumours and cancers (Caulin & Maley, 2011). Therefore, we predicted a negative relationship between the strength of immune responses and the incidence of tumours, particularly during the phase of rapid development. We test the above prediction using the size of the bursa of Fabricius (the origin of the B-cell repertoire) as a proxy of immune response during early development.

Third, the risk of acquiring a cancer should depend on body size because large species have more cells, longer cell lifespan and a large number of cell divisions. However, no such relationship has so far been found at the interspecific level (hence Peto's, 2016 paradox). The lack of such a relationship between incidence of tumours and body size bears testimony to the evolution of mechanisms that protect against cancer (Leroi *et al.*, 2003; Caulin & Maley, 2011; Nunney, 2013; Aktipis *et al.*, 2015; Brown *et al.*, 2015; Ducasse *et al.*, 2015; Noble *et al.*, 2015). The two previous points suggest that these mechanisms are likely related to life-history characteristics and their trade-offs (Jacqueline *et al.*, 2017), and, thus, we tested whether there is a positive relationship between incidence of cancers and body size in birds after controlling for interspecific covariation with developmental rates and immune responses.

## Materials and methods

### Study area and anatomical information

JE received fresh specimens in prime condition. JE is a professional taxidermist for more than 50 years. In Denmark, all taxidermists need an official licence for their work. In addition, all specimens received by taxidermists are provided with an individually numbered band and registered in an official registry, where information on species identity, date and cause of death must be recorded. Hence, all specimens included in this study were obtained in accordance with Danish legislation. Although most specimens were frozen, there is no reason to believe that this procedure will have caused any consistent bias with respect to the variables under investigation and the hypotheses being tested here. Upon receipt, all specimens were identified to species, date and locality were recorded, and, whenever possible, sex, age (juvenile or adult, mainly according to

Svensson, 1984) and a large number of morphological characters were measured before the specimen was prepared and stored. More than 98% of all specimens were found in the southern part of Jutland, Denmark. All specimens were opened and the skin removed. All organs and other body parts (brain, lungs, liver, gizzard, intestine, kidney, bursa of Fabricius, testes, ovary, oviduct and all major muscles) were carefully inspected for tumours that were recorded in a database together with all other information (sex, age, size and weight of brain, lungs, liver, gizzard, intestine, kidney, bursa of Fabricius, testes, ovary, oviduct, all major muscles, bones and skin). The entire skeleton was kept for the collection, and hence, it was inspected closely for any signs of possible tumours. This post-mortem examination lasted approximately 1 h per specimen. Subsequently, all records were entered into a data file. Tumours were identified as clearly visible lumps of hard tissue (with a diameter of more than 1 mm) on the outside of or within the body relying on standard procedures (Farrow, 2008; Mayer & Donnelly, 2012). A 4× magnifying light glass was used when working with small bird species, which facilitates the detection of tumours. Detection of tumour did not depend on observer as both JE and APM record the presence or the absence of tumours in 50 specimens, of which 42 had one or more tumours. All of these were correctly diagnosed and their location assessed by both JE and APM. In total, 8468 individual birds belonging to 238 species from Europe were analysed here. A total of 52 of these birds had one or more tumours.

### Life-history variables

Information on the mean duration of the incubation period and the nestling period was derived using Cramp & Perrins (1977–1994) as a source.

### Immunity

The size of the bursa of Fabricius was obtained from post-mortem examinations of dead birds brought to JE, who weighed the immune defence organs to the nearest mg on a precision balance, blindly with respect to the hypothesis under test. Birds were frozen when received by JE, but any effects of storage on measurements should only cause noise in the data set. We tested for two potential kinds of bias. First, we tested whether the variance in bursa size among species was significantly larger than the variance within species. There was larger variance among than within species as shown by one-way analyses of variance of relative organ size measured as the residuals from a linear regression of $\log_{10}$-transformed bursa mass on $\log_{10}$-transformed body mass ($F = 18.057$, d.f. = 185, 894, $P < 0.0001$). This implies that for even a small sample of individuals, the average value will provide reliable information on the relative size of a given organ for a given species. Second, sampling date might influence size estimates of the bursa of Fabricius as the bursa regresses following development of the B-cell repertoire. We tested whether date of sampling differed among species, but did not find any significant difference (Kruskal–Wallis ANOVA, $P > 0.40$). Thus, there is no reason to believe that date of sampling and hence relative regression of bursa will differ among species.

The bursa of Fabricius is the most important immune organ in juvenile birds, as it synthesizes antibody, and it is responsible for differentiation of the repertoire of B cells (Glick, 1983, 1994; Toivanen & Toivanen, 1987). The relative size of this immune defence organ in birds may reflect the ability to respond to an infection (Rose, 1981; Glick, 1983; John, 1994). For example, selection for increased and decreased immune response to an immune challenge by sheep red blood cells resulted in a correlated response to selection for size of the bursa of Fabricius (Parmentier *et al.*, 1995).

Body mass was recorded for the specimens investigated using a precision balance to the nearest mg. All data were not available for all specimens, and hence, sample sizes vary among characters. Bursa of Fabricius is only present in juveniles, which reduced sample size in analyses of this immune defence organ. A list of all data is provided in the Table S1.

### Statistical analyses

Before analyses, $\log_{10}$ transformation was applied to the mass of bursa of Fabricius, body mass and to the duration of the incubation and nestling periods. Frequencies of sampled individuals of each species with and without tumours were included in the models as multiresponse variables. Probability of detecting tumours was therefore used as a binary response by assuming multinomial distribution of our statistical models (see below).

Sample size in species in which tumours were detected was on average lower [log *n*-tumour (SD) = 2.41 (1.49)] than that of species with no tumours detected [log *n*-tumour (SD) = 3.55 (1.08); $t = 5.63$, d.f. = 236, $P < 0.0001$]. Thus, it is worth discussing the possibility of bias affecting our data set and thus inferences of our analyses. Several lines of evidence suggest that this was not the case. First, we used bivariate models based on binomial information for each individual sampled because this approach statistically accounts for differences in sample size (weight). Second, sample sizes positively covaried with an abundance of species in Europe using Burfield & van Bommel (2004) as a source ($R = 0.489$, $t = 8.40$, $N = 232$, $P < 0.00001$), which suggests that the data set does not suffer from bias due to variation in sampling effort among species. Third, the association between sample size and frequency of tumours did not reach statistical significance (dependent variable: arcsine tumour rates,

independent variable log-transformed sample sizes, $R = 0.099$, $F = 2.81$, d.f. = 1, 236, $P = 0.132$). Moreover, except for the incubation period, after controlling for the effect of abundance of populations, none of the independent variables used in our statistical models was significantly associated with sample size (log-spleen size: $F = 0.002$, d.f. = 1, 176, $P = 0.96$; log-body mass: $F = 2.99$, d.f. = 1, 229, $P = 0.085$; log-incubation period: $F = 4.73$, d.f. = 1, 229, $P = 0.031$; log-nestling period: $F = 2.37$, d.f. = 1, 220, $P = 0.125$) and, thus, it is unlikely that the detected associations were artefacts of variation in sample sizes between species with and without detected tumours. Four, the results do not change if we limit sample size to a maximum of 50 randomly selected individuals per species (see Table S2). Finally, restricting the considered species in our analysis to those with a minimum of 20 individuals sampled provided qualitatively identical results except for the effects of nestling period (see Results and Table S3). However, given the positive association between sample size and species abundance, removing from the analyses species with a small number of individuals sampled produces a bias towards more abundant species. Thus, we are confident about the appropriateness of our data set for the hypothesis tested, and report results from models that did or did not include species with < 20 individuals sampled.

Size of the bursa and the duration of the incubation and nestling periods are known to be correlated with body mass (Ricklefs, 1993; Møller *et al.*, 2003, 2005), and consequently, body mass was included in all models to control for indirect effects in the expected relationships. Because the expected interspecific associations may have a phylogenetic component, we considered the phylogenetic association among the considered bird species in our analyses. To account for phylogenetic uncertainty, we downloaded 100 phylogenetic trees from http://birdtree.org/ (Jetz *et al.*, 2012) of the species pools with information for all variables in each model (i.e. different groups of phylogenetic trees for different models) and fitted each of our models to each of these trees. Briefly, we fitted our models using Bayesian phylogenetic mixed models from the MCMCglmm package (Hadfield, 2010) as implemented in R (R-Core-Team, 2015) with the appropriate libraries ('MCMCglmm', 'ape' (Paradis *et al.*, 2004) 'MASS' (Paradis *et al.*, 2004) and 'mvtnorm' (Venables & Ripley, 2002) that enables the inclusion of a phylogeny as a design matrix that is considered as a random effect (Genz & Bretz, 2011). We used the prior [list (R = list (V = 1, nu = 0.002), G = list (G1 = list (V = 1, nu = 1, alpha.mu=0, alpha.V = 100)))] and we let the MCMC algorithm run for 43 000 interactions, with a burn-in period of 3000 and a thinning interval of 10. We then combined the 100 resulting model outputs, and report average values and the minimum and maximum values of lower and upper 95% credibility intervals of estimates, respectively. We also reported mean ± 95% CI of pMCMC values of the 100 models.

To facilitate the visualization of the detected patterns, we plotted the association between tumour prevalence (rather than the binomial information of each individual sampled) and size of bursa of Fabricius, and duration of the incubation and nestling periods. Our statistical models testing such relationships also included (log-transformed) body mass as additional independent factors and, thus, for plotting the detected pattern, we used residuals of these (log-transformed) variables after controlling for body mass. Furthermore, because tumour prevalence did not approach a Gaussian distribution even after arcsine transformation, we used ranked values to estimate residuals after controlling for body mass.

## Results

We recorded 8468 specimens belonging to 238 species in our analyses, and 40 individuals (0.47%) had tumours. Most tumours were located in the lungs (24), intestine (19), liver (14), kidney (10) and gizzard (10). Body mass among the species included here ranged from 3.9 to 13 000 g or by a factor 3333.

The duration of the nestling period, but not that of the incubation period, was negatively related to the incidence of tumours after controlling for the positive effect of body mass (Fig. 1; Table 1). However, this association disappeared when considering species with a minimum of 20 individuals sampled (Table S3).

Bird species with a larger bursa of Fabricius had a lower incidence of tumours than bird species with a smaller bursa after controlling for the effect of body mass, which was positively related to the incidence of tumours (Fig. 1; Table 1). This result did not change when using species with 20 or more individuals sampled in the analyses.

When considering the bursa of Fabricius and the nestling period in the same statistical model, only the former was significantly negatively associated with the tumour incidence after controlling for the positive effects of body mass (Table 1). Again, similar results were obtained when considering species with a minimum of 20 individuals sampled (Table S3). These results suggest that the effects of nestling period should be cautiously considered and that it may be due to the covariance shared with the development of the immune system. On the other hand, the positive relationship between the incidence of tumours and body mass was independent of the predictor variables included in the model (Table 1).

## Discussion

The incidence of tumours in free-living birds from Denmark was associated with the nestling period, the size of the bursa of Fabricius and body mass. The incidence of tumours was elevated in species with short

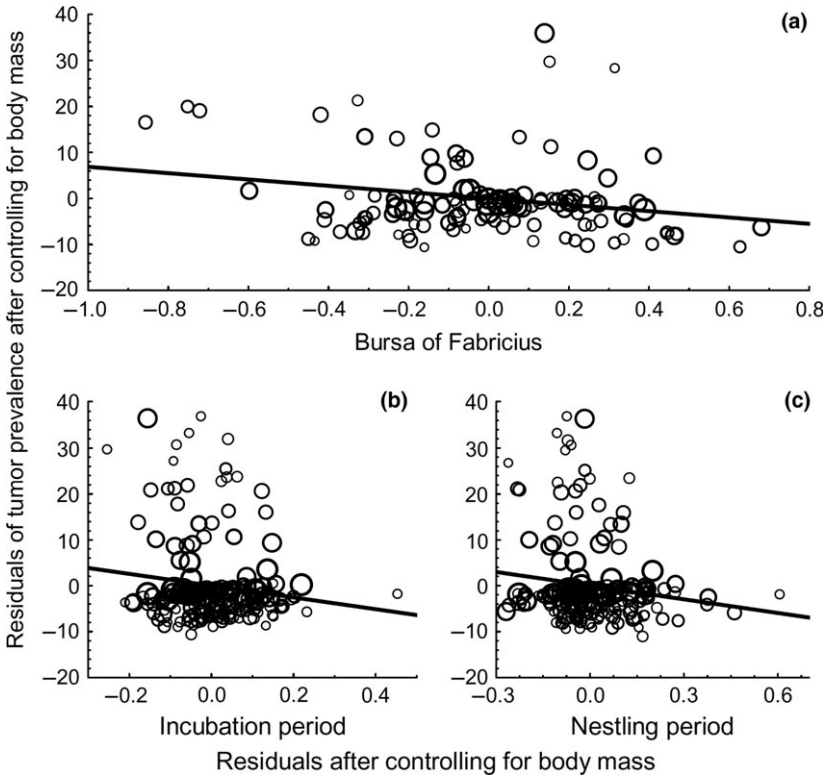


**Fig. 1** Relationships between residuals of ranked values of tumour prevalence in birds after controlling for the effect of body mass and residuals of bursa of Fabricius, and incubation and nestling periods. Residuals and lines are phylogenically corrected from PGLS models of arcsine-transformed tumour prevalence and log-transformed nestling and incubation periods and mass of bursa of Fabricius. Size of points is proportional to $\log_{10}$-transformed sample sizes.

developmental rates as reflected by the duration of the nestling periods after controlling for allometric effects of body mass, but this effect disappeared when restricting the analyses to species with 20 or more individuals sampled. In addition, the incidence of tumours was higher in species with a small bursa of Fabricius after controlling for body mass. The bursa of Fabricius is mainly responsible for immune response during the phase of rapid development of birds. Finally, as predicted by the necessarily large number of cell divisions of bigger species, bird species with a larger body mass had a higher incidence of tumours after controlling for the effects of the duration of the nestling period and the size of the bursa of Fabricius. Below, we first compare the incidence of tumour in our study and those known from birds in zoos or mammals. Then, we discuss the importance of these findings in the context of life history of birds having evolved in response to the incidence of tumours. We pay special attention to the discussion of the hypothesis that life-history characteristics adapted to underlying risks of tumours are responsible for Peto's paradox, which highlights the absence of the expected association between tumour incidence and body mass across species (Caulin & Maley, 2011; Nunney, 2013).

The incidence of tumours reported here may be an underestimate if small lesions go undetected, or because we have missed individuals that are eaten by predators because of their cancer. However, the incidence of tumours may be an overestimate because we could only investigate birds that were dead and subsequently delivered to JE, and such individuals may have died because of their cancer. To summarize, we see no reason why such estimates would show any bias relative to the variables under investigation and the hypotheses being tested. Although precancerous lesions are common in humans and animals (Folkman & Kalluri, 2004; Bissell & Hines, 2011), they were not recorded in our study or in many other studies of cancer (Ratcliffe, 1933; Lombard & Witte, 1959; Snyder & Ratcliffe, 1966; Stewart, 1966; Effron *et al.*, 1977; Wadsworth *et al.*, 1985; Reavill, 2004). We found an incidence of tumours of 0.40% in wild birds, which was significantly lower than published estimates of birds from zoos (Effron *et al.*, 1977: 1.8%; Ratcliffe, 1933: 0.8%; Lombard & Witte, 1959: 1.0% and Snyder & Ratcliffe, 1966: 0.4%; mean = 1.0%, SE = 0.29, $t_3 = 3.37$, $P = 0.043$). These values for captive birds were 4.4-fold smaller than equivalent estimates for captive mammals of 2.8% (Effron *et al.*, 1977), 2.5% (Ratcliffe, 1933), 2.8% (Lombard & Witte, 1959) and 0.7% (Snyder & Ratcliffe, 1966); mean (SE) = 2.2% (0.50), $t_3 = 4.34$, $P = 0.023$). This finding is surprising given the high metabolic rate and turnover of cells in birds compared to mammals (Holmes & Ottinger, 2003), but it suggests that mechanisms to control and reduce the frequency of tumours have an important phylogenetic component, with birds being more efficient controlling the spread of tumoral cells. The phylogenetic component of the models that

**Table 1** MCMCglmm models with probability of detecting tumours as the binary response variable and body mass as a predictor variable in analyses of incubation and nestling periods and mass of bursa of Fabricius as predictors. The random effect of phylogeny was tested for each of the 100 phylogenetic trees considered. For each estimate, we report average values and minimum and maximum values of lower and upper 95% CI, respectively. Finally, we also report 95% CI of pCMCM of values estimated for the 100 models (i.e. one for each of the phylogenetic trees considered). Model 1 included information from 237 species (52 and 8403 individuals with and without tumour detected, respectively). Model 2 included information from 229 species (52 and 8339 individuals with and without tumour detected, respectively). Model 3 included information from 128 species (41 and 7617 individuals with and without tumour detected, respectively). Model 4 included information from 123 species (41 and 7556 individuals with and without tumour detected, respectively).

| Model | Estimate | Lower 95% CI | Upper 95% CI | pCMCM (−95% CI) | pCMCM (+95% CI) |
|---|---|---|---|---|---|
| 1 | | | | | |
| Body mass | 1.832 | 0.900 | 2.784 | < 0.001 | < 0.001 |
| Incubation period | −1.684 | −5.530 | 2.238 | 0.368 | 0.410 |
| Phylogeny | 1.123 | 0.000 | 9.414 | | |
| 2 | | | | | |
| Body mass | 2.573 | 1.467 | 3.732 | < 0.001 | < 0.001 |
| Nestling period | −3.551 | −6.731 | −0.498 | 0.020 | 0.027 |
| Phylogeny | 1.205 | 0.000 | 4.174 | | |
| 3 | | | | | |
| Body mass | 3.330 | 1.924 | 4.813 | < 0.001 | < 0.001 |
| Bursa mass | −1.635 | −2.968 | −0.306 | 0.015 | 0.021 |
| Phylogeny | 0.988 | 0.000 | 4.174 | | |
| 4 | | | | | |
| Body mass | 3.971 | 2.231 | 5.777 | < 0.001 | < 0.001 |
| Nestling period | −2.155 | −5.591 | 1.178 | 0.197 | 0.224 |
| Bursa mass | −1.590 | −2.922 | −0.257 | 0.019 | 0.025 |
| Phylogeny | 1.132 | 0.000 | 3.936 | | |

we tested here with around 200 bird species was small (i.e. the low CIs of the estimates include the zero value, Table 1) suggesting that it may be important at higher taxonomic levels.

We investigated the hypothesis that life-history traits of birds were associated with tumours. Life-history theory predicts how living beings apportion reproduction and self-maintenance to an optimal level under given environmental conditions (e.g. Roff, 1992; Stearns, 1992). Here, we reported that the incidence of tumours decreased with the duration of early developmental periods as reflected by nestling but not incubation period after having accounted for variation due to body size. This result should however be considered cautiously as the effect of nestling period disappeared when including the size of the bursa of Fabricius as an additional independent factor, or when restricting the analyses to species with 20 or more individuals sampled. The negative association between tumours and relative duration of developmental periods was predicted because a slow developmental rate would imply development of a more capable acquired immune response (Soler *et al.*, 2003), a superior ability to detect and eliminate rapidly proliferating cell lineages (e.g. Aktipis *et al.*, 2015; Noble *et al.*, 2015), and a low probability of being affected by mutation due to telomere shortening (e.g. Blasco *et al.*, 1997; Blackburn, 2001) and therefore cancer. Thus, slow development may imply strong investment in anticancer mechanisms. Larger sample sizes of species removed from our restricted analyses (i.e. including species with a minimum of 20 individuals sampled) are necessary to further test the detected pattern.

Immunity was predicted to be associated with the incidence of tumours because of its role in detecting and clearing either tumour cells (see above) or pathogenic microorganisms that may directly or indirectly influence neoplasm growth and thus tumour proliferation (Aktipis & Nesse, 2013). Here, we have shown that bird species with a relatively larger bursa of Fabricius for their body size had a lower incidence of tumours. Immune responses against potential tumour cells (i.e. mutants) should be differentially important for periods of high developmental rates and, thus, of rapid telomere shortening (Blasco *et al.*, 1997; Blackburn, 2001), which is a likely reason explaining the detected association. One possible explanation is that parasitism could directly or indirectly influence cancer (see examples in Aktipis & Nesse, 2013). We know that species with a strong immune response also experience strong selection pressure due to parasites as revealed by positive associations between parasite-induced mortality and the relative size of immune defence organs (Martin *et al.*, 2001; Møller & Saino, 2004). As the parasites are the main force selecting for strong immune responses, our results could be interpreted as suggesting a role of parasites in determining tumour incidence in birds. It may also depend on the specific components of immunity that are activated during bacterial, viral or parasite infection and the components of immunity that are involved in controlling cancer.

Peto's paradox is based on the surprising finding that the incidence of cancer does not increase with body size or other measures of the number of targets of cancer, when such an association is expected (Peto, 2016). Here, we demonstrate across a large number of bird species a positive association after controlling for the effects of life-history characteristics. It can be argued that the detected positive association between tumour and body mass was due to tumours being more easily detectable in species of large size. However, we think this possibility is unlikely because all birds were equally and carefully analysed, all organs were isolated and individually checked for tumours and a 4× magnifying light glass was used when working with small birds. We are unaware of any other study of free-living organisms testing for Peto's paradox. The lack of a positive relationship between incidence of cancers and body size has

traditionally been explained by the evolution of protective anticancer mechanisms (Leroi *et al.*, 2003; Caulin & Maley, 2011; Nunney, 2013; Aktipis *et al.*, 2015; Ducasse *et al.*, 2015; Noble *et al.*, 2015). Anticancer mechanisms are costly (Aktipis & Nesse, 2013) and thus should be adjusted to the probability of developing cancers and to the expected negative fitness consequences. The expected adjustment is therefore the cause of not detecting the expected positive association between tumours and body size across species (Caulin & Maley, 2011; Nunney, 2013). If that was true, the expected association between body mass and tumour incidence should emerge after statistically controlling for variables directly or indirectly related to such adjustment. Here, we made such an analysis by including a number of potentially confounding variables (such as developmental rates and immunity) in our model testing the expected relationship. We detected a positive relationship between the incidence of tumours and body mass after controlling for supposedly important life-history characteristics. These findings may suggest that these traits are necessary for explaining Peto's paradox.

The expected association between body mass and tumour incidence was even detected in models that did not include information on growth rate or immunity (MCMCglmm, $P < 0.001$), which may suggest that Peto's paradox may not apply to birds. The range in body mass from 3.9 to 13 000 g is sufficiently large to assure that this was not the cause of the effect. However, we know that immunity and body mass are closely related across avian taxa (e.g. Chandra & Newberne, 1977; Møller *et al.*, 1998), which obscures interpretation of this correlation. We urge collection of data for other classes of organisms to address whether this finding extends beyond birds.

We have provided a preliminary investigation for potential predictors of the incidence of tumours across a class of free-living animals (i.e. birds). The incidence of tumours was linked to life history and immunity. These findings have implications for studies of the origin, the evolution and the consequences of tumours and cancer. In particular, the observations are consistent with a significant role of tumours and cancer in ecology and evolution of birds. In general, the incidence of tumours was low in species with high investment in self-maintenance, as expected if ecological and evolutionary processes have played a role in the evolution of tumours and cancer.

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## Author contributions

APM and JJS designed research; APM and JE performed measurements; APM and JJS analysed data; and APM and JJS wrote the paper.

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## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
**Table S1** Information on tumor prevalence, body mass, mass of bursa of Fabricius and duration of incubation and nestling periods of the 248 species of bird considered.
**Table S2** Results of ten replicates of MCMCglmm models with probability of detecting tumors as the binary response variable and body mass as a predictor variable in analyses of incubation and nestling periods and mass of bursa of Fabricius as predictors.
**Table S3** MCMCglmm models with probability of detecting tumors as the binary response variable and body mass as a predictor variable in analyses of incubation and nestling periods and mass of bursa of Fabricius as predictors.

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