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Evolution, Medicine, and Public Health [2024] pp.105–116
https://doi.org/10.1093/emph/eoae011
Advance access date 27 June 2024

ORIGINAL RESEARCH ARTICLE

EVOLUTION, MEDICINE, & PUBLIC HEALTH

Life history traits and cancer prevalence in birds

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Received 27 January 2024; revised version accepted 2 June 2024.

ABSTRACT

Background and objectives: Cancer is a disease that affects nearly all multicellular life, including the broad and diverse taxa of Aves. While little is known about the factors that contribute to cancer risk across Aves, life history trade-offs may explain some of this variability in cancer prevalence. We predict birds with high investment in reproduction may have a higher likelihood of developing cancer. In this study, we tested whether life history traits are associated with cancer prevalence in 108 species of birds.

Methodology: We obtained life history data from published databases and cancer data from 5,729 necropsies from 108 species of birds across 24 taxonomic orders from 25 different zoological facilities. We performed phylogenetically controlled regression analyses between adult body mass, lifespan, incubation length, clutch size, sexually dimorphic traits, and both neoplasia and malignancy prevalence. We also compared the neoplasia and malignancy prevalence of female and male birds.

Results: Providing support for a life history trade-off between somatic maintenance and reproduction, we found a positive relationship between clutch size and cancer prevalence across Aves. There was no significant association with body mass, lifespan, incubation length, sexual dimorphism, and cancer.

Conclusions and implications: Life history theory presents an important framework for understanding differences in cancer defenses across various species. These results suggest a trade-off between reproduction and somatic maintenance, where Aves with small clutch sizes get less cancer.

Lay Summary Life history can help us understand cancer prevalence in birds. We examined potential life-history variables that may explain the variance in cancer prevalence across birds and found that species with larger clutch size, but not sexual dimorphism, larger weight, longer incubation length, or longer lifespan, have higher cancer prevalence.

KEYWORDS: aves; cancer; neoplasia; life history evolution; malignancy; tumors

INTRODUCTION

Nearly all multicellular organisms are susceptible to neoplastic disease [1, 2]. Neoplasia is a disease of uncontrolled cell division and growth, resulting ultimately in the formation of a tumor, as well as invasion or metastasis in the case of malignant neoplasia (a.k.a. cancer) [3]. Over the past few decades, cancer research has focused on identifying different molecular pathways, hallmarks, and control mechanisms of cancer—all with the ultimate aim of improving cancer treatment [4, 5]. Understanding why organisms differ in their ability to suppress cancer, as well as how they respond to neoplastic expansion, is a central question in comparative cancer research.

In general, life history trade-offs govern how organisms allocate time and resources to fitness components such as growth, self (or somatic)-maintenance, and reproduction [6, 7]. Somatic maintenance can include tumor suppression mechanisms such as cell cycle control and DNA damage repair. These trade-offs may help explain the variation in cancer prevalence across species. Larger and longer-lived species, due to their higher number of cells and longer time over which they may acquire mutations, are predicted to have much higher chances of developing cancer. However, paradoxically, this prediction is not supported by observations. Larger and longer-lived mammalian species do not have dramatically higher cancer prevalence or cancer risk than smaller and shorter-lived mammalian species, an observation that has come to be known as Peto's Paradox [8–12]. This may be because large and long-lived species that invest in somatic maintenance over reproduction likely evolved enhanced mechanisms to suppress or evade cancer [13]. Utilizing this life history tradeoff approach can both give us insight into the basic biology and origins of cancer and also provide opportunities to discover either universal or novel mechanisms of cancer suppression that could have clinical applications to humans. For example, previous results across vertebrates have shown that life history traits, such as gestation length [14] and trophic levels [15, 16], but not longevity, litter size, or body mass [14], are significantly correlated with cancer prevalence across vertebrates. Within mammals, litter size [12, 17] and diet [11, 16], are significantly correlated with cancer prevalence or cancer risk, but litter size, gestation length, body mass, life expectancy, and lactation length are not significantly correlated with cancer risk in univariate analyses [18].

Birds (taxonomic class *Aves*) represent a diverse vertebrate taxon with considerable variation in life-history characteristics. This diversity in life history traits and the particular ecologies of birds suggests that they may differ from other vertebrates in the factors that explain why some birds are more or less susceptible to cancer.

Understanding cancer susceptibility among birds is currently an active area of research. For instance, Møller et al. surveyed free-living Eurasian birds post-mortem and found that, when analyzing at least 20 individuals per species, larger body size was correlated with tumor prevalence [19], while neither incubation nor nestling time was correlated with tumor prevalence [19]. These results suggest Peto's observation that bigger species do not get more cancer, is not true in the Aves. Recently, Bulls et al. found that body mass and lifespan were not correlated with neoplasia prevalence in birds, with sample sizes ranging from 5 or 10 bird necropsies [20]. Separate studies have reported neoplasms (benign and malignant tumors combined) in bird species, either free-living or in human care [2, 21–26], but the prevalence of malignancy itself using larger sample sizes has not been measured before across bird species.

Beyond body mass and lifespan, there may also be a tradeoff between reproductive investments and somatic maintenance [27], and therefore, cancer defenses. Birds can invest in reproduction in various formats, such as exaggerated morphological traits (e.g. sexually dimorphic or dichromatic color) [28–31], clutch size, and incubation length. Sexually dimorphic or dichromatic species with extreme phenotypes, such as large and colorful ornaments or weapons, may have an increased risk of cancer [27]. However, there has not been a study investigating the relationship between reproductive or sexually selected traits and cancer prevalence in birds.

To investigate the relationship between life history and cancer risk in birds, we expanded on previous life history studies in birds by including a wider range of life history traits from trait-rich life-history databases and compared these traits to cancer prevalence data from veterinary records of 108 bird species under managed care. This represents the second-largest study of cancer prevalence across bird species [19]. We hypothesized that the incredible diversity of life-history strategies observed across the class Aves can explain taxonomic differences in cancer

risk in birds, due to the evolutionary trade-offs between growth, reproduction, and somatic maintenance. Specifically, we tested whether malignancy prevalence or neoplasia prevalence is correlated with other avian traits such as incubation length, clutch size, and degree of sexual dimorphism and dichromatism.

Lastly, species with the homogametic sex (e.g. XX females in mammals and ZZ males in birds) tend to live longer [32] and it has been proposed that the existence of two X chromosomes may offer some cancer protection to humans [33]. Therefore, we also tested whether male birds (ZZ sex chromosomes) have lower cancer prevalence than female birds (ZW sex chromosomes).

METHODS

Cancer and life-history data

We obtained 5729 individual adult necropsy records for 108 bird species (representing 24 orders) under human care from 25 different institutions over 25 years. Necropsies are typically performed by a veterinarian or veterinary pathologist who reviews each organ system. Through this process representative samples of each organ or representative samples of any abnormality found are submitted for histopathology. Through this process of necropsy and histopathology, the majority of neoplasms would be found. The necropsy records in our dataset were from Association of Zoos and Aquariums (AZA) institutions [34], where animals that die are required to be necropsied. We extracted the following data from these records: age at death (1287 individuals, 51 species, 17 orders), malignancies and neoplasias reported (5729 individuals, 108 species, 24 orders), and sex [34]. We measured malignancy (i.e. cancer) prevalence and neoplasia prevalence (benign and malignant tumor) for each species by dividing the total number of necropsies reporting malignancies (or neoplasms) by the total number of necropsies available for that species [35]; a measurement also used in previous studies [12, 16].

We assembled life-history variables from multiple published resources, including AnAge [36] and the Amniote Life History Database [37]. The collected life-history variables included species averages of adult body mass (g), lifespan (months), incubation length (months), clutch size (number of offspring per brood) [36, 37], presence and degree of sexual plumage dichromatism (plumage brightness and plumage hue) [38], and sexual size dimorphism (mass and tail size) [39].

Data filtering

We only included bird species for which we had at least 20 necropsies in our analysis. For analyses comparing female and male malignancy prevalence or neoplasia prevalence, as well as sex bias regressions, we used species with at least 10 necropsy records per sex. We present the neoplasia and malignancy prevalence of 108 bird species [35]. We excluded chickens (*Gallus gallus*) from the analyses because as a largely domesticated agricultural species, they have been selected for egg-laying and frequently develop ovarian cancer [40]. We only included chickens (*Gallus gallus*) in Supplementary Table S1 and in the Supplementary Fig. 10 illustration of normalized frequency of the species' age at death as a percentage of the species lifespan.

We excluded all infant data from our dataset because (i) the low prevalence of age-related diseases, such as cancer, in infants would likely bias the neoplasia prevalence data towards lower values and (ii) cancers in infants are medically different than adult cancers [41]. We defined infancy as a record's age that is smaller or equal to that species' age of infancy (or the average of male and female maturity). In cases of no records of infancy age, the record was considered an infant if it contained any of the following words: infant, juvenile, immature, adolescent, hatchling, subadult, neonate, newborn, offspring, and fledgling. We performed correlations between clutch size and neoplasia or cancer prevalence with and without removing domesticated and semi-domesticated species [42–51] (dataset [35]:). When comparing female and male malignancy prevalence and neoplasia prevalence, we removed all cases of reproductive cancer in order to minimize any effects of controlled reproduction in managed environments on our results.

Ancestral state reconstruction phylogenies

Bird neoplasia and cancer prevalence may be driven by various different evolutionary forces. In order to find which evolutionary force is predominant in shaping these traits, we reconstructed ancestral state phylogenies. Utilizing TimeTree.org, and inputting the species of our dataset, we built a phylogeny that details the lineages of our bird species. With this, we used the Geiger and Phytools packages in R to create two ancestral state reconstruction phylogenies. These reconstructions provided estimates of neoplasia and malignancy prevalence across our bird species as well as their ancestors. In our reconstructions, we utilized a Markov Chain Monte Carlo (MCMC) to fit our data and estimate the best model of evolution. In order to determine the model of drift/selection that best fits our data, we compared Ornstein–Uhlenbeck (OU) to Brownian motion, to Early Burst models of evolution using Akaike information criterion comparisons to determine the best fit.

Statistical analyses

We performed all statistical analyses in R version 4.0.5 [52]. We prepared figures using the data visualization software ggplot2

[53] and generated summary statistics in dplyr [54]. We tested whether the *P*-values passed the false discovery rate (FDR) correction in each of these 25 analyses (Supplementary Table S2A). To evaluate the robustness of our results, we carried out a subsampling analysis (Supplementary material: Supplementary subsampling results).

Life-history analyses

In order to test if a life history trait is statistically significantly associated with neoplasia or malignancy prevalence, we used phylogenetically controlled regressions. We performed all phylogenetic analyses using the R packages ape, Phytools, Geiger, Tidyverse, and Caper [55–59] using phylogenetic generalized least squares (PGLS) regressions to take into account the phylogenetic non-independence among species [60] and weighting analyses by 1/(square root of the number of necropsies per species) following Revell [57]. We uploaded our list of species to TimeTree (Timetree.org) and used the resulting phylogenetic tree for our phylogenetic regressions. Given that all life history trait data were not available for all species in the dataset, each phylogenetic regression had a different number of species. Thus, to perform each phylogenetic regression we pruned the above tree using the setdiff and keep.tip/drop.tip functions in R. We performed Grubbs' and Rosner's tests to identify and remove significant outliers in the PGLS analyses. We performed univariate and multivariate PGLS analyses excluding the significant outliers, and separate univariate PGLS analyses including the significant outliers. We ran the former to test if our results were mainly driven by species with extreme values. We also tested all analyses for the presence of any significant heteroscedasticity (Fligner–Killeen test), and if present, we mention this in our results. Because the malignancy and neoplasia prevalence data in our analysis is proportional, we transformed the neoplasia and malignancy prevalence by using an arcsine-square-root prior to running phylogenetic regressions and paired samples statistical tests. We used the 'rr2' package to obtain the R^2 values of the phylogenetic regressions. For visualization purposes, however, we display the data and regression lines in the figures using the non-arcsine-square-root-transformed data.

Sex difference analyses

There may be a trade-off between sexual selection and cancer defenses. To test for this, we quantified the degree of sexual dimorphism, as an indirect measure of sexual selection, in seven biometric variables [plumage brightness, plumage hue, mass (g), and tail size (g)] as the natural log of the male biometric variable divided by the natural log of the female biometric variable. We then used PGLS to test for associations of those sex differences with cancer prevalence. We also compared male malignancy prevalence or neoplasia prevalence versus female malignancy prevalence or neoplasia prevalence. The denominators in the case of the male malignancy prevalence or neoplasia prevalence are the total number of necropsied males, whereas the denominators in the case of the female malignancy prevalence or neoplasia prevalence are the total number of necropsied females. The distribution of the sex differences in cancer (i.e.'female malignancy prevalence minus male malignancy prevalence', 'female neoplasia prevalence minus male neoplasia prevalence') had significant outliers. Therefore, we compared malignancy prevalence and neoplasia prevalence between males and females using the non-parametric paired-samples sign test.

RESULTS

To assess whether selection, rather than other evolutionary forces such as random genetic drift, can explain neoplasia and cancer prevalence across bird species, we reconstructed ancestral state phylogenies using the OU, Brownian, and Early Burst models of phenotype evolution. We found that the OU model best fit our cancer and neoplasia prevalence data and phylogeny (Fig. 1). These results suggest that stabilizing selection has played an important role in the evolution of neoplasia and cancer prevalence in birds (Fig. 1).

To test for trade-offs between life history traits and cancer defenses, we performed correlations between life history traits, such as body mass, lifespan and incubation length, versus neoplasia and cancer prevalence. Because many life history traits are correlated (Supplementary Fig. S11), we tested a series of multivariable regression models to control for those correlations. We performed a PGLS univariate analysis where body mass times lifespan was the independent variable, a PGLS bivariate analysis where both incubation length and body mass were the independent variables, and a PGLS multivariate analysis where incubation length, body mass, and clutch size were the independent variables. We found no significant association between body mass and neoplasia prevalence (75 species; 3124 necropsies) or malignancy prevalence (67 species; an analysis which had a different number of significant outlier species that we removed; 2786 necropsies) (Fig. 2; Supplementary Fig. S1; Supp lementary Table S2A). Second, there was no significant association between lifespan and neoplasia prevalence (51 species; 2665 necropsies) or malignancy prevalence (45 species; 2383 necropsies) (Fig. 3; Supp lementary Fig. 2; Supp lementary Table 2A). Third, there was no significant correlation between body mass times lifespan (an estimate of the total number of cell divisions in an animal) and neoplasia prevalence (36 species; 1829 necropsies) or malignancy prevalence (34 species; 1328 necropsies) (Supp lementary Fig. 3A; Supp lementary Fig. 3B: Supp lementary Table 2A). Fourth, there was no significant

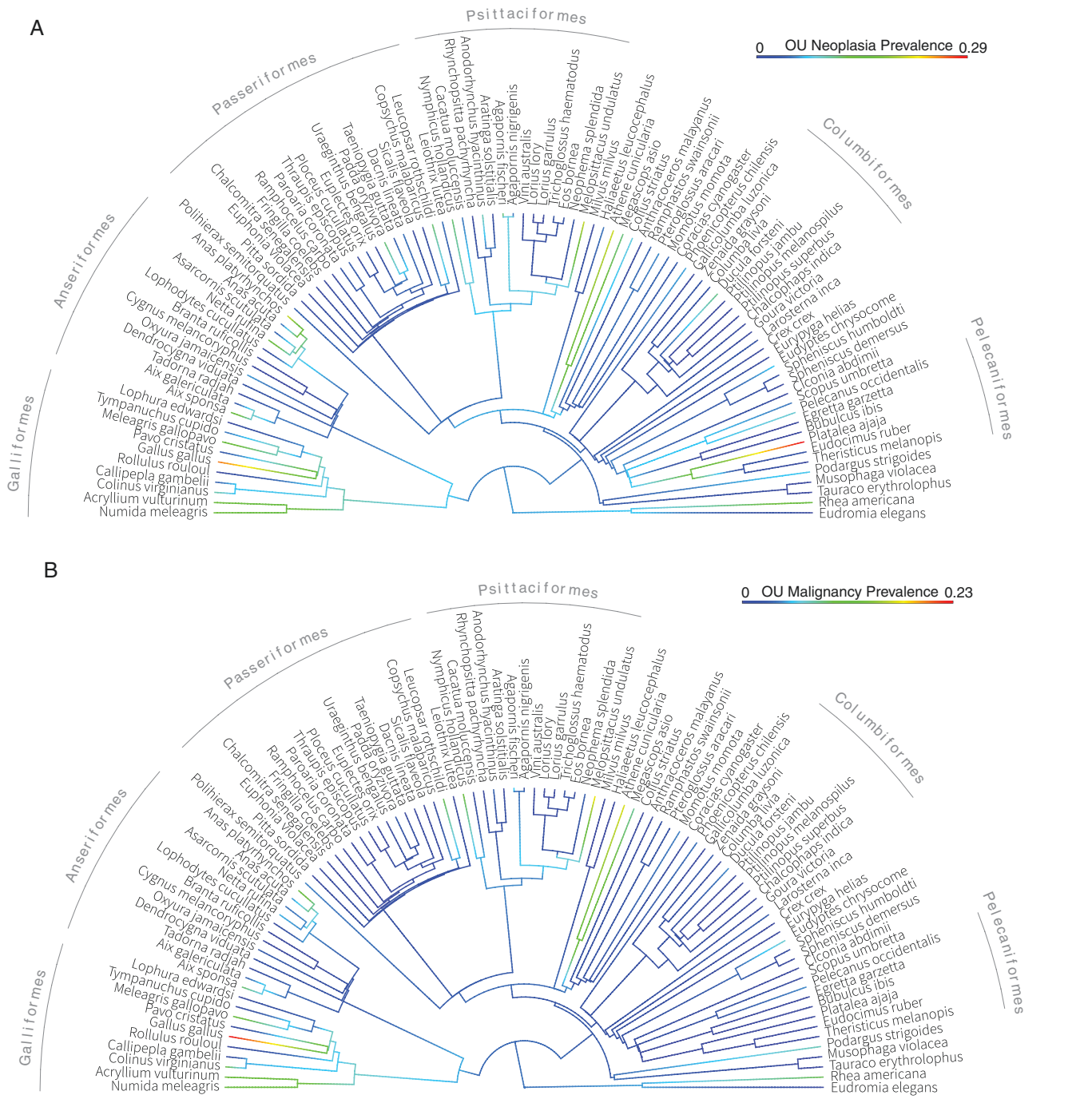

Figure 1. Neoplasia prevalence (**A**) and malignancy prevalence (**B**) across bird species in our dataset. These ancestral state reconstruction phylogenies are presented with the Ornstein-Uhlenbeck (OU) model; the model that best fits our data and phylogeny. The outer labels show the orders that the species belong to. We used a minimum of five species per order to avoid overlap in the labels. The color of the branches indicates the relative neoplasia prevalence (A) and malignancy prevalence (B) in each branch

association between incubation length, when controlling for species body mass, and malignancy prevalence (Supp lementary Table 2A; 31 species and 1699 necropsies). Fifth, there was no significant association between incubation length, when controlling for both body mass and clutch size, and malignancy prevalence (Fig. 4; Supp lementary Table S2A; 30 species and 1665 necropsies). The above PGLS analyses do not have significant outliers. However, even when we included significant outliers in the univariate PGLS analyses, incubation length, body mass, lifespan, and body mass times lifespan were still not significantly correlated with neoplasia or malignancy prevalence (Supp lementary Fig. S12–14, 17–18).

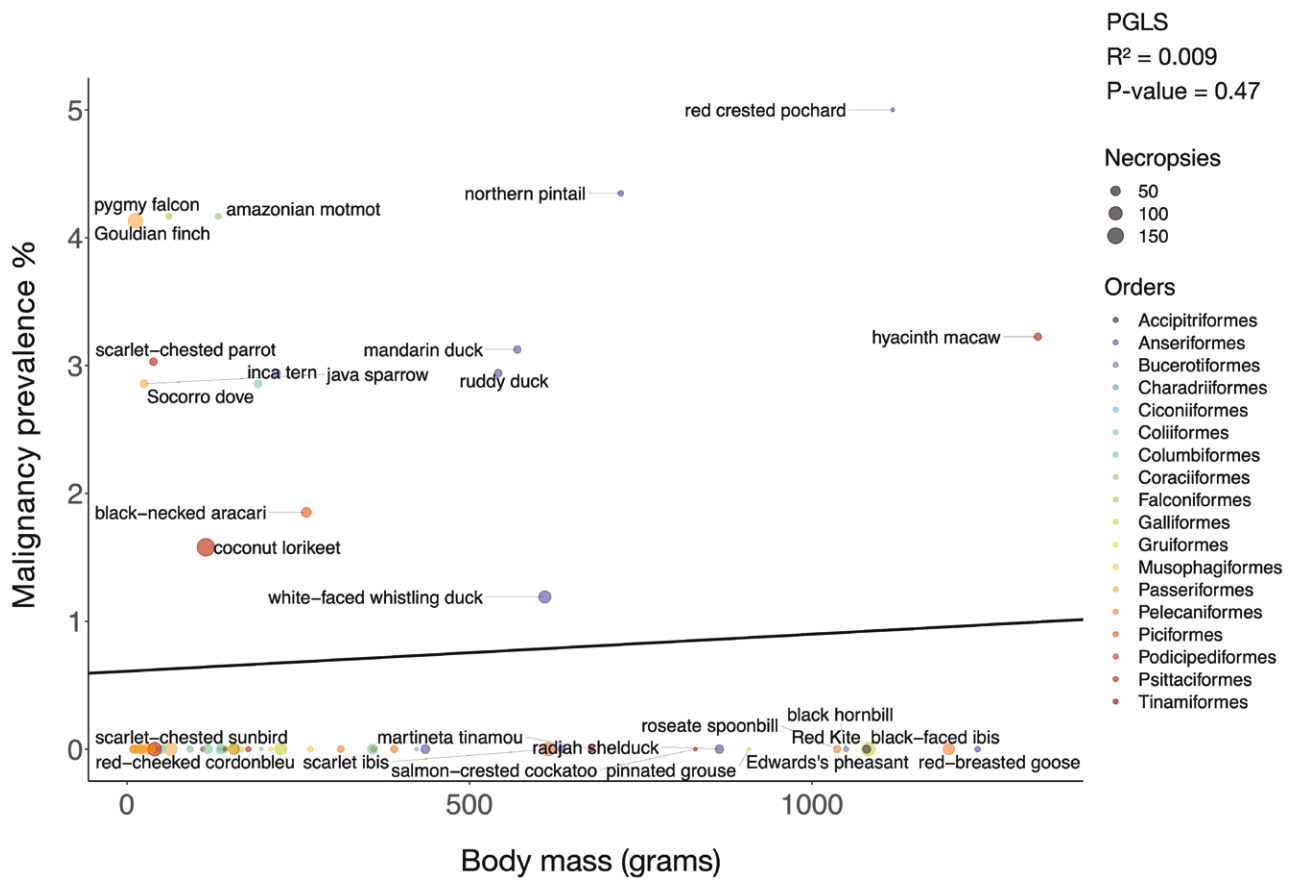

Figure 2. Larger body mass is not correlated with malignancy prevalence across 67 bird species. Dot size indicates the number of necropsies per species. Colors show the taxonomic order of each species, and the black line shows the phylogenetically controlled linear regression of body mass versus malignancy prevalence.

Species with larger clutch sizes had significantly higher malignancy prevalence (PGLS: *P*-value = 0.003; $R^2 = 0.16$, Fig. 5; 56 species and 2454 necropsies). This correlation was not significant after applying FDR corrections for multiple testing (FDR-corrected *P*-value = 0.08), nor after controlling for species body mass (FDR-corrected *P*-value = 0.11) (Supp lementary Table S2A). The correlation between clutch size and malignancy prevalence was not significant after removing domesticated and semi-domesticated species (PGLS: *P*-value = 0.38; 41 species and 1765 necropsies; Supp lementary Table S2A; Supp lementary Fig. S6B). Clutch size was not significantly correlated with neoplasia prevalence Supp lementary Table S2A; Supp lementary Fig. S5; Supp lementary Fig. S6A; 47–58 species, 1979–2955 necropsies). The above clutch size PGLS analyses did not include significant outliers. When including significant outliers in the analyses (Supp lementary Fig. S12–18), larger clutch size is still correlated with malignancy prevalence (Supp lementary Fig. S16B). This is true when including domesticated and semi-domesticated species (Supp lementary Fig. S16B; PGLS, *P*-value = 0.001) as well as when excluding domesticated and semi-domesticated species (Supp lementary Fig. S17B; PGLS *P*-value = 0.004). When including significant outliers in the analyses, neoplasia prevalence positively correlated with clutch size (Supp lementary Fig. S16A; PGLS *P*-value = 0.03).

To test whether older animals had more cancer than younger animals, we compared the age of animals that had or did not have cancer when they died. We found that animals with a diagnosis of cancer at death were not older on average than animals with a diagnosis of no cancer at death (in 1287 individuals from 51 species; for which we had age data) (Supp lementary Fig. S10).

To test for trade-offs between sexual dimorphism or dichromatism and cancer defenses, we looked for correlations between sexually dimorphic and dichromatic traits versus neoplasia and malignancy prevalence. Sexually dimorphic or dichromatic species with extreme phenotypes, such as large and colorful ornaments or weapons, may have an increased risk of cancer We found no significant associations between neoplasia or malignancy prevalence and several sexually dimorphic and dichromatic traits (Supp lementary Fig. S7; Supp lementary Table 2A). Also, in the 31 species for which we had at least 10 female and 10 male necropsies, there was no

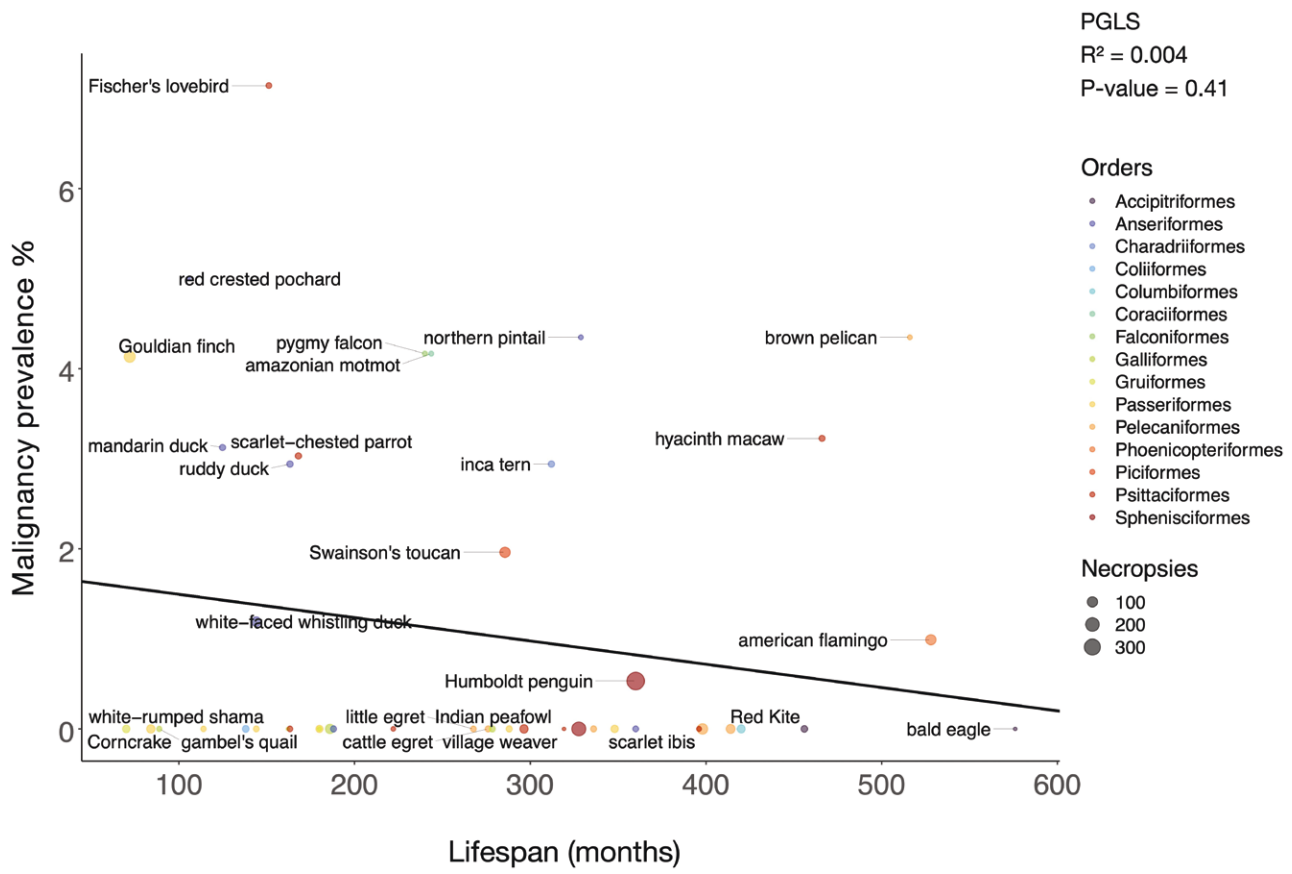

Figure 3. Longer lifespan is not correlated with malignancy prevalence across 45 bird species. Dot size indicates the number of necropsies per species. Colors show the taxonomic order of each species. The black line shows the phylogenetically controlled linear regression of lifespan versus malignancy prevalence

significant difference in neoplasia or malignancy prevalence between females and males (Supp lementary Fig. S8; Supp lementary Fig. S9; Supp lementary Table S2A).

DISCUSSION

We hypothesized that the variation in species trade-offs between investment in reproduction versus somatic maintenance can explain some of the variation in cancer prevalence across bird species. We found that species with larger clutch sizes had more cancer in our dataset. The discovery adds to a growing evidence that links reproductive strategies to disease susceptibility in animals. However, other life-history traits that we tested, such as body mass, incubation length, lifespan, sexual size dimorphism, or sexual dichromatism, were not correlated with avian cancer prevalence, nor was there a significant difference in cancer or neoplasia prevalence between male and female birds.

We also found that cancer susceptibility in birds appears to have evolved under stabilizing selection with occasional shifts to different stable values. These results suggest that cancer prevalence and clutch size are correlated due to similar underlying selective pressures that are relatively stable, though they may shift with sudden changes in the ecologies of the birds. What those pressures were remains an open question for future research.

Significant relationship between clutch size and cancer prevalence

Our results are consistent with previous findings in mammals that larger litter size is associated with cancer prevalence [12, 17]. Larger clutch size is correlated with malignancy prevalence across 56 bird species; a result that persisted in the majority (≥86%) of repetitions of these analyses using 40 randomly chosen bird species from our dataset (Supplementary material: Supplementary subsampling results). Many of the life-history traits described in this article, such as body mass, number of offspring produced per brood, incubation time, and longevity, are tightly linked with each other [61–65] (Supplementary Fig. S11). We found that clutch size explained a statistically significant portion (14–17%) of the variation in cancer prevalence when significant outliers were included in the analyses regardless of whether domesticated or semi-domesticated species were excluded. When significant outliers were excluded, clutch size also explained a statistically significant portion (16%) of the

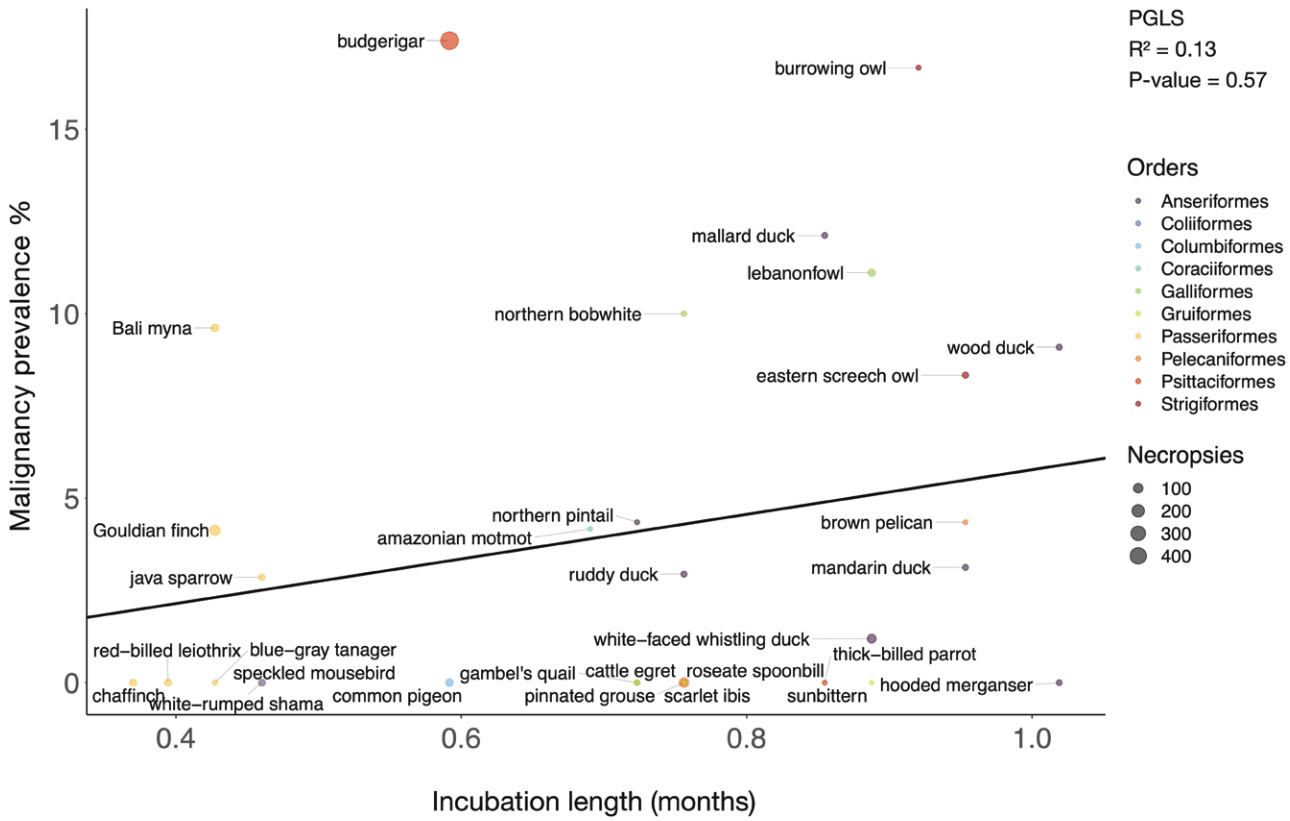

Figure 4. Incubation length is not correlated with malignancy prevalence when controlling for body mass and litter size across 30 species. Different colors indicate the order in which each species belongs and the size of the dot indicates the number of necropsies per species. The black line shows the phylogenetically controlled linear regression of incubation length versus malignancy prevalence

variation in cancer prevalence, though when excluding domesticated species from the analysis malignancy prevalence was not correlated with clutch size.

Incubation length is not associated with cancer in birds

Incubation length is not correlated with cancer prevalence in birds even after controlling for body mass and clutch size. However, gestation or incubation length is negatively correlated with malignancy prevalence when controlling for variation in body mass across vertebrates [14].

Evidence for Peto's paradox in birds

Our findings show no significant correlation between neoplasia or malignancy prevalence and body mass and lifespan in birds, supporting Peto's paradox [66]; the lack of relationship between body mass and neoplasia prevalence is in contrast to the observation of a positive correlation between body size and tumor prevalence in free-living birds [19]. Bulls et al. [20] found no significant correlation between neoplasia prevalence and body mass or lifespan in birds but found a significant negative correlation between cancer prevalence and lifespan in birds when using a threshold of ≥10 necropsies per species. The discrepancies between our study, that of Bulls et al. [20] and Møller et al. [19], may be due to the different number of individuals sampled per species (≥20 necropsies per species in our study, ≥5 and ≥10 necropsies in the correlations between life history traits and neoplasia or cancer prevalence in Bulls et al. [20] versus ≥3 records per species in Møller et al. [19]), the different species of birds analyzed (108 managed bird species from multiple institutions, 204 species [20] versus 238 free-living bird species in Denmark [19]), or body mass collected from the literature [20] (this study) versus mostly measured with a precision balance [19]. Unfortunately, only six species of birds are common in Møller et al'.s [19] and this study's dataset, limiting our ability to compare cancer prevalence in wild versus managed birds. In general, patterns of tumor incidence or neoplasia prevalence were consistent between these free-living birds and populations managed under human care (Supplementary Table S3).

By analyzing the distribution of the age at which birds died, we found that birds with a diagnosis of cancer at death were not older than birds with no cancer found at death. This was also found in a larger taxonomic group (the sauropsids) that included birds [14]. This may be explained by the observation that long-lived birds have coevolved pathways that increase longevity in

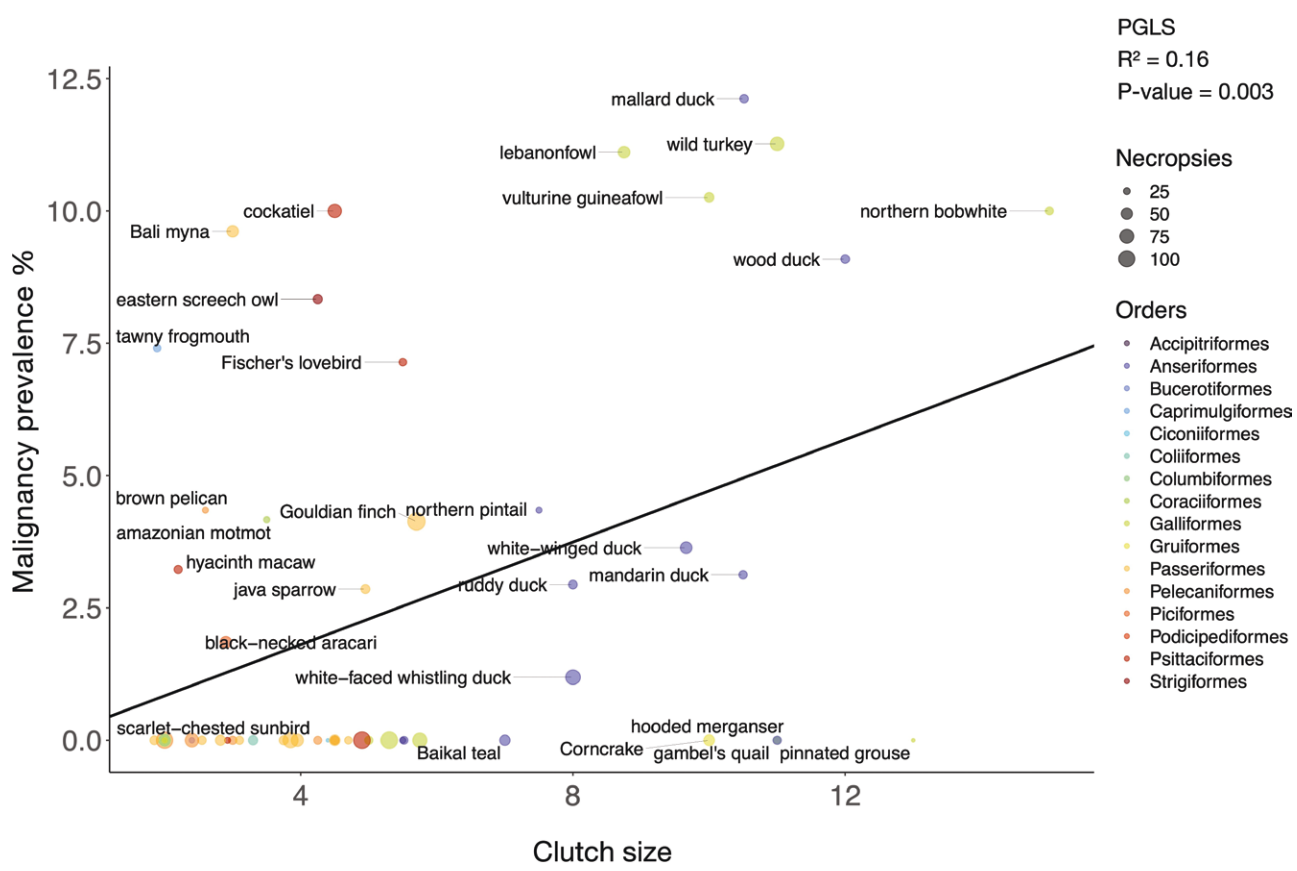

Figure 5. Clutch size is positively correlated with malignancy prevalence across 56 bird species. Dot size indicates the number of necropsies per species. Colors show the taxonomic order of each species. The black line shows the phylogenetically controlled linear regression of clutch size versus malignancy prevalence.

part through decreasing cancer rates [67, 68]. The fact that erythrocyte telomeres of long-lived birds shorten at a slower pace than erythrocyte telomeres of shorter-lived birds [69] may provide an additional mechanistic explanation for the lower than expected cancer prevalence in long-lived birds.

No relationship between sexual dimorphism or dichromatism and cancer prevalence

We found no significant difference in cancer or neoplasia prevalence in relation to sexual dimorphism and dichromatism. This means that sexually dimorphic birds who spend time and energy in creating colorful plumage or larger body parts do not seem to pay a cost in terms of cancer susceptibility. It is possible that the birds in our study did not experience such trade-offs because under human care they may have high energy budgets that allow them to invest both in sexually selected traits as well as in somatic maintenance in the form of cancer suppression.

Do female birds have higher cancer prevalence than male birds?

Cancer rates in most other species, including humans, are biased toward males [33]. Current theory hypothesizes that males, with a hemizygous sex chromosome, may be vulnerable to recessive cancer risk alleles on the X chromosome and that the double X chromosome found in females may offer some cancer protection against those recessive alleles [33]. In alignment with the two-X chromosome theory of cancer protection, previous work has shown that female birds (ZW) have more neoplasms than male birds (ZZ), but this was not validated statistically with sex-specific neoplasia prevalence [2]. We found that females do not have significantly different neoplasia prevalence or malignancy prevalence than male birds. This suggests that the sex chromosome hemizygosity of female birds does not increase their cancer risk. It is possible that female birds may get more cancer than males, but the effect size of hemizygosity is too small to be detected by a study of our size. So the lack of a statistically significant difference in our study may not be conclusive. It just puts in doubt the hemizygosity hypothesis for sex bias in cancer risk.

Future directions

Future work investigating both the ultimate and proximate causes of cancer in birds would help us both understand cancer better and protect birds. What are the links between the number of oncogenes and tumor-suppressors across bird species

and the Ornstein–Uhlenbeck model of stabilizing selection and random changes in bird cancer prevalence? Why are large clutch sizes a risk factor for cancer in birds? Several ecological factors may also be driving many of the cancers in birds in our dataset. Future studies would also benefit from knowledge of the relationships between distinct cancer types and life history in birds. Hormonal variation, the mechanisms that protect birds from radiation-induced DNA damage [70], as well as the molecular associations between unpredictable environments and fast life history strategies (e.g. production of more offspring) explaining cancer susceptibility across species would need to be found.

SUPPLEMENTARY DATA

Supplementary data is available at *EMPH* online.

ACKNOWLEDGEMENTS

Thanks to all of the pathologists, veterinarians, and staff at the zoos, aquariums, and private veterinary centers for contributing to the data collection by diagnosing malignancy prevalence and neoplasia prevalence. Specifically, we would like to acknowledge the following institutions: Akron Zoo, Atlanta Zoo, Audubon Nature Institute, Bergen County Zoo, Buffalo Zoo, Capron Park Zoo, Central Florida Zoo, Dallas Zoo, El Paso Zoo, Elmwood Park Zoo, Fort Worth Zoo, Gladys Porter Zoo, Greensboro Science Center, Greenville Zoo, Henry Doorly Zoo, Utah's Hogle Zoo, Jacksonville Zoo, John Ball Zoo, Los Angeles Zoo, Louisville Zoo, Miami Zoo, Northwest ZooPath, Oakland Zoo, Oklahoma City Zoo, Philadelphia Zoo, Phoenix Zoo, Point Defiance Zoo, Pueblo Zoo, San Antonio Zoo, Santa Ana Zoo, Santa Barbara Zoo, Sedgwick County Zoo, Seneca Park Zoo, The Brevard Zoo, The Detroit Zoo, The Oregon Zoo, and Toledo Zoo. Thanks to Diego Mallo and Ping-Han Huang for help with the statistical analyses, and two anonymous reviewers for helpful comments.

AUTHOR CONTRIBUTIONS

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FUNDING

This work was supported in part by National Institutes of Health grants U54 CA217376, U2C CA233254, P01 CA91955, R01 CA140657, and T32CA272303, as well as Congressionally Directed Medical Research Programs' Breast Cancer Research Program Award BC132057 and the Arizona Biomedical Research

Commission grant ADHS18-198847. The findings, opinions, and recommendations expressed here are those of the authors and not necessarily those of the universities where the research was performed or the National Institutes of Health.

CONFLICT OF INTEREST

We declare we do not have any conflicts of interest.

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