RESEARCH ARTICLE

Cancer Prevalence across Vertebrates

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ABSTRACT Cancer is pervasive across multicellular species, but what explains the differences in cancer prevalence across species? Using 16,049 necropsy records for 292 species spanning three clades of tetrapods (amphibians, sauropsids, and mammals), we found that neoplasia and malignancy prevalence increases with adult mass (contrary to Peto's paradox) and somatic mutation rate but decreases with gestation time. The relationship between adult mass and malignancy prevalence was only apparent when we controlled for gestation time. Evolution of cancer susceptibility appears to have undergone sudden shifts followed by stabilizing selection. Outliers for neoplasia prevalence include the common porpoise (<1.3%), the Rodrigues fruit bat (<1.6%), the black-footed penguin (<0.4%), ferrets (63%), and opossums (35%). Discovering why some species have particularly high or low levels of cancer may lead to a better understanding of cancer syndromes and novel strategies for the management and prevention of cancer.

SIGNIFICANCE: Evolution has discovered mechanisms for suppressing cancer in a wide variety of species. By analyzing veterinary necropsy records, we can identify species with exceptionally high or low cancer prevalence. Discovering the mechanisms of cancer susceptibility and resistance may help improve cancer prevention and explain cancer syndromes.

INTRODUCTION

Cancer is a ubiquitous problem for multicellular species (1) and a leading cause of death in humans (2). Every multicellular body is a cooperative cellular system, with cells suppressing replication (3), dividing labor (4), sharing resources (5), regulating cell death (6) and taking care of the extracellular environment (1). However, cooperative systems are susceptible to cheaters, which emerge as cancers in multicellular organisms (7). Because cancer cells can outcompete normal cells with respect to replication, survival, resource use, and other cellular behaviors, natural selection within the body can favor cancer cells via somatic evolution.

Cancer has been a strong selective pressure on multicellular organisms and mechanisms for cancer suppression likely co-evolved along with the evolution of multicellularity (8, 9). Despite this persistent selective pressure of cancer, species vary in their investment in cancer defenses across the tree of life. Sir Richard Peto predicted in 1977 that the risk of cancer should scale with the number of cells in an organism and the length of its lifespan (10, 11). This prediction is based on the fact that tumors evolve from single cells, partially due to the accumulation of somatic mutations over time (10). His observation that cancer risk does not appear to increase with increases in body mass and longevity across species (10), a phenomenon known as "Peto's paradox," launched the field of comparative oncology (12).

Early work in comparative oncology found that birds, and to a lesser extent reptiles, develop fewer neoplasms than mammals (13–15). While single case studies have been reported (16), it has been difficult to estimate true neoplasia prevalence in these taxa. In 2015, we published neoplasia prevalence estimates in 37 mammal species and reported support for Peto's paradox, that is, bigger, longer lived species do not get more cancer (17). Follow up studies have supported Peto's paradox

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and demonstrated the ubiquity of cancer across mammals (18, 19). A recent study of 131 species of mammals with at least five necropsies per species, found no relationship between body mass and neoplasia prevalence, though they did observe an increase in both neoplasia and malignancy prevalence in 25 species of amphibians and 71 species of squamates (bioRxiv 2022.07.12.499088). Our study of 110,148 animals across 191 species of mammals found no relationship between body mass and death due to cancer (18). Other studies have applied the same logic of Peto's paradox to the study of cancer rates in different human organs. Organs with more stem cells and more cell divisions should get more cancer than organs with fewer cell divisions (20–23).

The extensive variation in cancer risk across vertebrates provides a unique opportunity to identify species with exceptional cancer resistance that can lead to new discoveries of cancer resistance mechanisms outside the traditional human and murine studies. Additionally, the discovery of cancer vulnerable species could lead to new insights into cancer syndromes as well as provide spontaneous "natural" animal models of disease that can help us gain a better understanding of various types of cancer and their treatments. Here we present a large, curated database of tetrapod veterinary necropsy records, including 16,049 individual animals across 292 species of animals, encompassing reptiles, birds, amphibians, and mammals. Because necropsies typically are recorded as having "neoplasia," which includes both benign and malignant tumors, we developed a terminology dictionary to distinguish benign from malignant neoplasms in the necropsy reports. We calculate and analyze both neoplasia prevalence as well as malignancy (cancer) prevalence. Only a subset of benign neoplasms evolve into cancers over a lifetime, so neoplasia prevalence is always greater than or equal to malignancy prevalence. We also tested for age bias in the animals that died with neoplasms or cancers. To test for general mechanisms of cancer suppression, we tested for an association with DNA damage response or somatic mutation rate and neoplasia.

We think of the cancer prevalence in a species as the interaction of the species' susceptibility to cancer with its environmental exposures. The cancer prevalence of a species is an aspect of its phenotype, with clear fitness relevance. We investigated how that phenotype evolved across the tree of life. Traditional models of phenotype evolution include a model of neutral evolution [Brownian motion (24, 25)], early diversification followed by neutral evolution [Early Burst (26)], and periods of rapid change on particular branches followed by stabilizing selection around new "optimal" phenotypes [The Ornstein–Uhlenbeck model (27–29)]. We tested these three models to determine that best fit the neoplasm prevalence and malignancy prevalence phenotypes across the tree of life.

RESULTS

Variation in Cancer Prevalence across Clades

We found evidence of neoplastic disease in necropsies across all analyzed taxonomic clades (Fig. 1A–D). For all vertebrates, the median prevalence of neoplasia at death was 4.89% (range = 0%–62.86%) and median malignancy prevalence was 3.20% (range = 0%–40.95%). Mammals had the most neoplasia at death with a median of 12% (range = 0%–63%) and median malignancy prevalence of 7% (range = 0%–41%). Sauropsids, which includes Aves and Squamata, followed with a median neoplasia prevalence of 4% (range = 0%–39%) and median malignancy prevalence of 1.6% (range = 0%–35%). Lastly, amphibians had a median neoplasia prevalence of 1.2% (range = 0%–46%) and median malignancy prevalence of 0% (range = 0%–33%; Fig. 2A and B). The ranking of prevalence by clade is consistent with previous studies (13, 15). In Fig. 2, we have shown Aves and Squamata separately, but because reptiles are not a monophyletic clade, we have grouped reptiles with birds in the Sauropsida clade for the purposes of further analyses. Despite a lower mean prevalence for both benign and malignant tumors, sauropsids and amphibians show a wide range of neoplastic disease burden across species. There is a small but highly statistically significant correlation between the prevalence of benign neoplasms and the prevalence of malignant neoplasms across species ($r = 0.34$; $P < 0.0001$; Supplementary Fig. S1). Supplementary Tables S1 and S2 list the species with the highest and lowest neoplasia and malignancy prevalences, as well as the proportion of neoplasms that are malignant. Among the vertebrates with the highest prevalence of neoplasia, 63% of ferrets (*Mustela putorius*) died with a neoplasm (45% of which was lymphoma), 56% of opossums (*Didelphis marsupialis*) died with a neoplasm (46% of which was in the lung), and 45% of four-toed hedgehogs (*Atelerix albiventris*) died with a neoplasm (42% of which was in the alimentary tract; Supplementary Table S3).

Life History Analyses of Neoplasia and Malignancy Prevalence

Evolutionary life history theory provides a framework for understanding the tradeoffs governing species' survival and reproduction (30, 31). Life history theory can be used to explain how species level traits shape organismal cancer risk based on trade-offs between investment in somatic maintenance (e.g., cancer suppression) and reproduction or growth. Several smaller studies have shown that specific life history traits, such as litter size, can help explain some of the variation in neoplasia prevalence in animals managed under human care (19). In this study, we tested for the relationship between 14 life history traits and two dependent variables: (i) neoplasia prevalence and (ii) malignancy prevalence (Supplementary Figs. S2–S39). We implemented both univariate and multivariate models. To control for phylogenetic relatedness, we used a phylogenetic regression model (pglsSEyPagel, available on GitHub). Because each species had a different number of necropsies, ranging from 20 to 477, we weighted species data points by the number of necropsies in our dataset.

In our univariate analyses, we found a significant positive relationship between body mass and neoplasia prevalence [2.1% neoplasia per Log_{10}(g), $P = 0.007$] across all vertebrates but no significant relationship between body mass and malignancy prevalence (0.65% malignancy per Log_{10}g, $P = 0.287$; Fig. 3A and B). These results are in contrast to three of the four previous studies (bioRxiv 2022.07.12.499088; refs. 17–19) published on body mass and cancer prevalence in animals. We also found a significant positive relationship between neoplasia prevalence and maximum longevity (0.01% neoplasia per

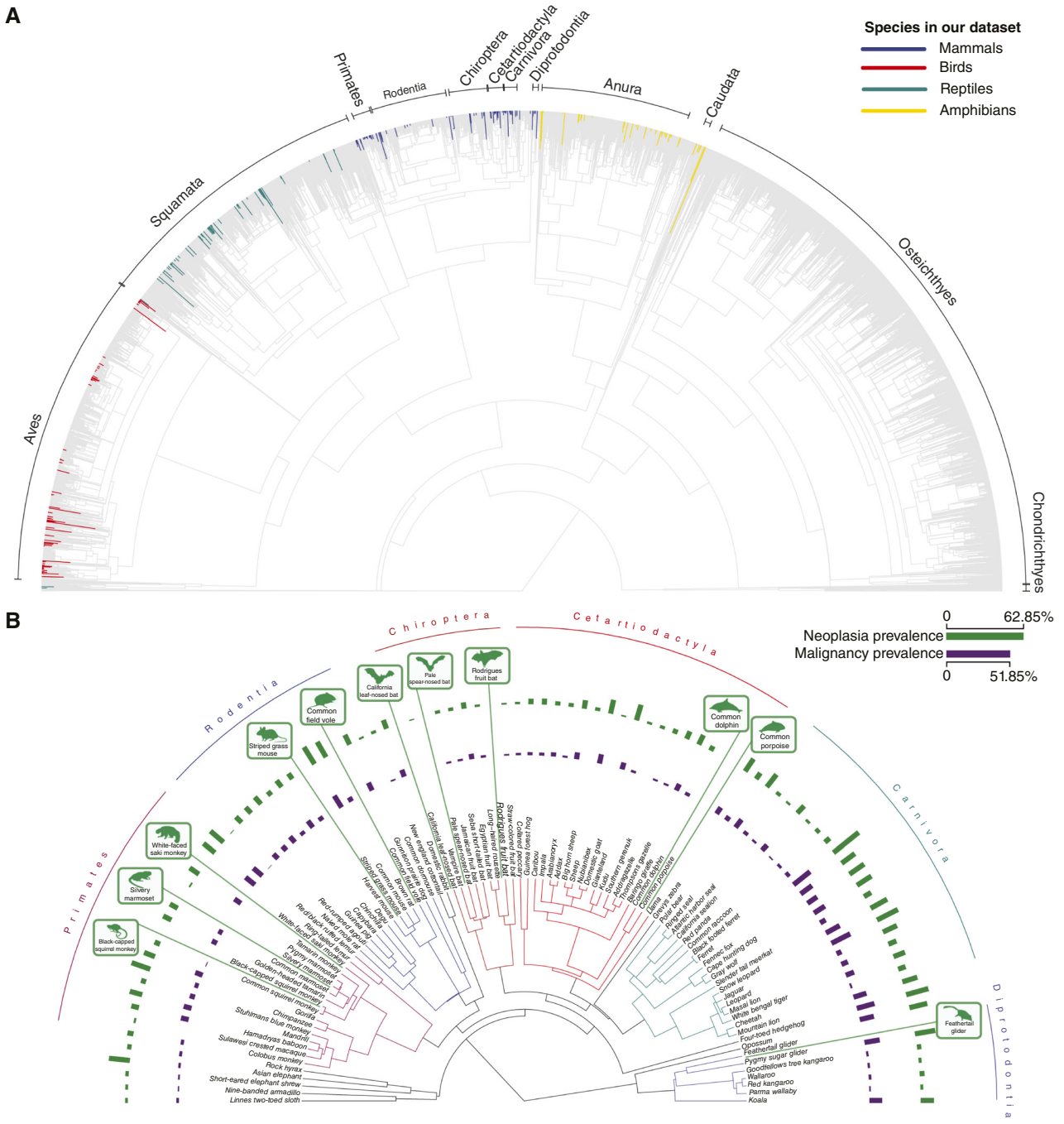

Figure 1. **A,** Vertebrate species represented in our database overlaid upon the entire vertebrate phylogeny. Neoplasia and malignancy prevalence across (**B**) mammals, (*continued on following page*)

Log_{10} months, $P = 0.02$; 0.005% malignancy per Log_{10} months, $P = 0.276$; Fig. 3C and D). Lastly, we found animals with longer gestation times also get fewer malignancies ($N = 151$ species, −5.3% neoplasia per Log_{10} months, $P = 0.1$, −5.65% malignancies per Log_{10} months, $P = 0.02$; Fig. 3E and F).

Given that the life history traits, such as adult body mass, maximum longevity, and gestation time are all positively correlated (Supplementary Fig. S40), we implemented multivariate models that control for multiple life history factors. These multivariate models contained all significant predictors of neoplasia or malignancy prevalence (adult mass, maximum longevity, and gestation time). We found that both adult body mass (2.9% neoplasia per Log_{10}g, $P = 0.01$) and gestation time (−18.6% neoplasia per Log_{10} month $P = 0.0001$) provide independent information for estimating neoplasia prevalence but not longevity ($P = 0.12$; Supplementary Fig. S41).

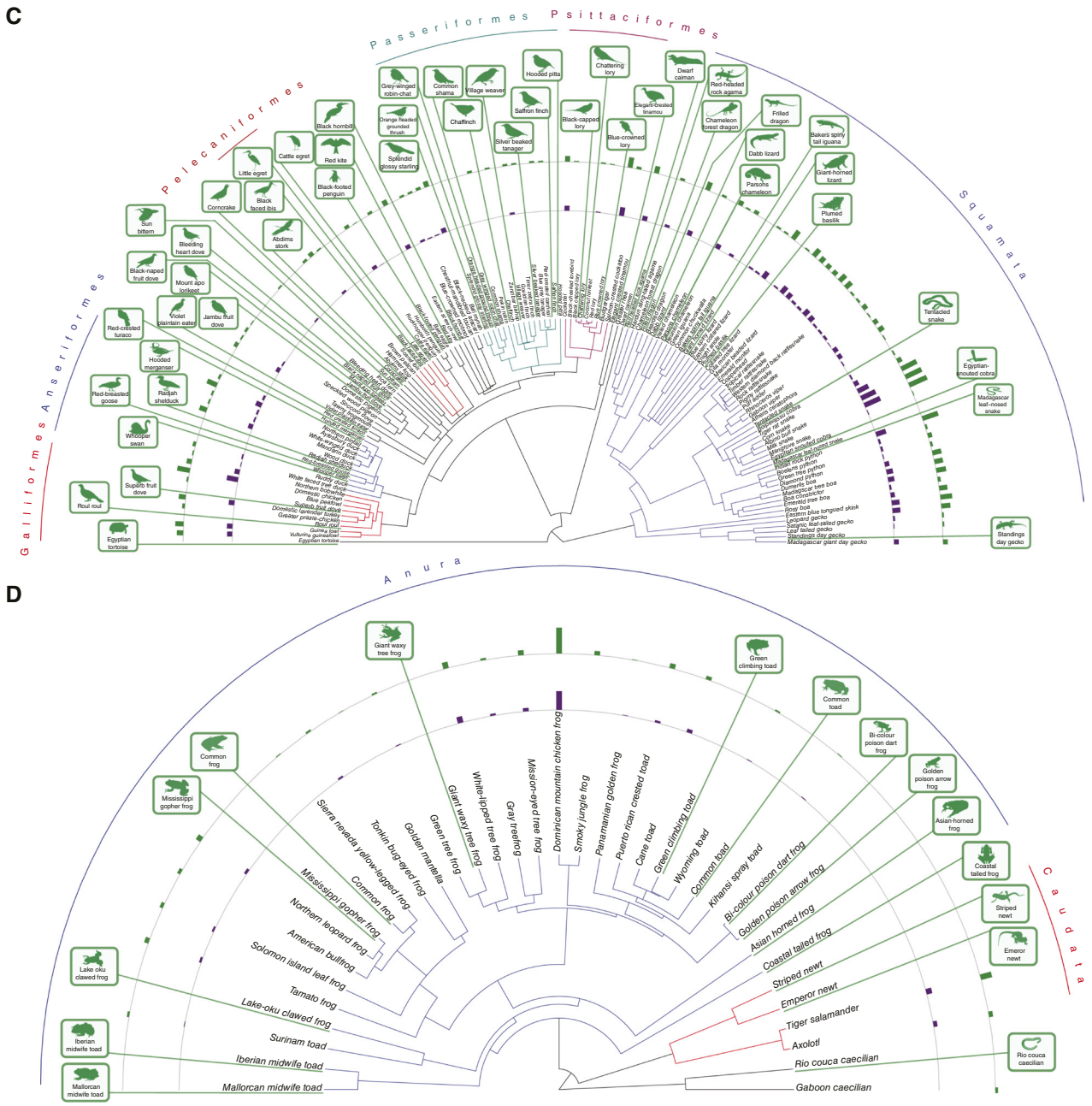

Figure 1. ***(Continued)*** (**C**) sauropsids (Aves, Squamata, Testudines, and Crocodylia), and (**D**) amphibians. Silhouetted species indicate that zero neoplasms were reported in our data.

Because gestation time and body mass are correlated ($r = 0.71$; Supplementary Fig. S40) but have the opposite relationship to neoplasia and malignancy prevalence, we tested a two-variable model and found that when controlling for adult mass [3.8% neoplasia per Log_{10}(g), $P = 0.0006$], gestation time had a significant negative relationship with neoplasia prevalence [−15.8% neoplasia per Log_{10} months, $P = 0.0002$; $R^2 = 0.20$] and *vice versa*. When controlling for gestation time, adult body mass had a significant positive relationship with malignancy prevalence [1.9% malignancies per Log_{10}(g), $P = 0.02$], and when controlling for adult mass, gestation time also predicts malignancy prevalence (−12% malignancies per Log_{10} months of gestation, $P = 0.0002$).Body mass and gestation time were still statistically significant predictors (adjusted $P < 0.05$) of neoplasia and malignancy prevalence after a 5% false discovery rate correction for multiple testing (Supplementary Fig. S41).

We validated our results by implementing phylogenetic binomial regressions (bioRxiv 2022.07.12.499088) on records that include the age of the animal ($N = 3{,}022$ mammals). All the above statistically significant relationships remain significant at $P < 0.05$ level using binomial regressions

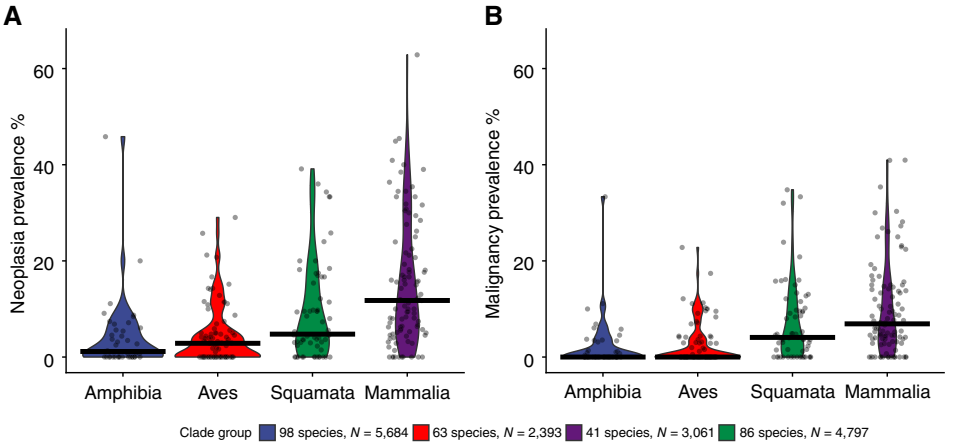

Figure 2. Distributions of (**A**) neoplasia (Kruskal–Wallis test: $P = 2.906 \times 10^{-12}$) and (**B**) malignancy (Kruskal–Wallis test: $P = 6.519 \times 10^{-11}$) prevalences are different across four clades, Amphibia, Mammalia, Aves, and Squamata. Dots show the estimated species neoplasia prevalence and bars show the median for the clade. Neoplasia and malignancy prevalence for species were calculated by the proportion of the reported lesions among the total number of necropsies for that species. *N* indicates the number of necropsies in each clade.

(Supplementary Table S4). In addition, binomial regressions reveal a significant positive relationship in mammals between body mass and neoplasia [OR = 9.5% neoplasia per Log_{10}(g), $P = 0.012$ but $P = 0.06$ with PGLS], as well as malignancy prevalence [OR = 1.5% malignancy per Log_{10}(g), $P = 0.015$ but $P = 0.458$ with PGLS; Supplementary Figs. S16 and S17]. Within mammals the relationship between longevity and neoplasia prevalence (OR = −0.50 neoplasia per Log_{10} month lifespan, $P = 0.14$) or malignancy prevalence (OR = 0.21 malignancy per Log_{10} month lifespan, $P = 0.57$) were not statistically significant. Within mammals, analyses of adult body mass and gestation time together showed the same relationships as all vertebrates [OR = −18% neoplasia and −15% malignancy per Log_{10} months of gestation, $P = 0.036$ and $P < 10^{-10}$; 3.4% neoplasia and 2.6% malignancy per Log_{10}(g) of adult body mass, $P = 0.017$ and 0.0066, respectively, with PGLS; Supplementary Figs. S38 and S39]. The positive relationship between body mass and neoplasia/malignancy prevalence were even stronger with binomial regressions [OR = 14% neoplasia and 14% malignancy per Log_{10}(g) adult body mass, $P = 0.002$ and $P = 0.0035$]. Similarly, the binomial regression revealed gestation time had a significant negative relationship with neoplasia/malignancy prevalence (OR = −44% neoplasia and −48% malignancy per Log_{10} months of gestation, $P = 0.0009$ and 0.001).

Overall, we found no evidence of a relationship between litter or clutch size and neoplasia prevalence (Supplementary Figs. S2 and S3). However, when we restrict the analysis to mammals, litter size is positively associated with both neoplasia and malignancy prevalence (neoplasia: $P = 0.02$, $R^2 = 0.07$; malignancy: $P = 0.03$, $R^2 = 0.11$; Supplementary Figs. S19 and S20), supporting our earlier analysis of 37 mammals from the San Diego Zoo (19). In birds, larger clutch size is also associated with higher neoplasia and malignancy prevalence (32). We also found that time to sexual maturity, growth rate, and basal metabolic rates (which were only available for mammals) were not significant predictors of neoplasia or malignancy prevalence (Supplementary Figs. S7–S10, S13, S14, S23, and S24). In addition to calculating the prevalence of neoplasms and malignancies, we also calculated the proportion of neoplasms that were malignant, which for some neoplasms is a measure of the likelihood that a benign neoplasm transforms into a malignant one. We found no statistically significant relationships between any of those life history factors and the proportion of neoplasms that were malignant (Supplementary Figs. S42–S53).

DNA Damage Response and Somatic Mutation Rates

DNA damage response is an important anticancer mechanism. Accurate repair of damaged DNA or clearance of damaged cells through apoptosis ensures that mutations capable of driving tumorigenesis do not persist or accumulate. For example, we previously reported that increased apoptosis in response to DNA damage in elephant cells was associated with low cancer mortality (17). To determine if DNA damage response is a general mechanism of cancer defense across species, we measured the ability of primary fibroblasts from 15 species to respond to DNA damage (Fig. 4A; Supplementary Figs. S54–S68). DNA damage response was assessed by measuring cell cycle arrest, which halts cell division until damage is repaired, and apoptosis, which kills cells that cannot be repaired. (17, 33). We hypothesized that animals with less neoplasia and malignancy would respond more robustly to DNA damage. Cell cycle arrest in response to increasing doses of ionizing radiation and apoptosis in response to increasing doses of a chemotherapeutic drug (doxorubicin) was measured over time. We observed variability in DNA damage response across the species that we tested and this variability was not associated with neoplasia or malignancy (Fig. 4A; Supplementary Figs S54–S68), although cell cycle arrest in response to 10 Gy radiation trended toward an association with neoplasia prevalence (Fig. 4A).

Perhaps the most important role of DNA damage response is the suppression of somatic mutation accumulation, because mutations drive tumor formation and cancer progression (22, 34, 35). Therefore, we hypothesized that lower somatic

mutation rates across species would correlate with lower cancer prevalence. We obtained previously reported somatic mutation rates from nine species in our dataset and tested for a correlation with neoplasia prevalence (36). Mutation rates had been estimated based on dividing the number of mutations detected in DNA sequencing of single colonic crypts by the age of the animal (36). We discovered a positive association between somatic mutation rates and neoplasia prevalence and that species with fewer somatic mutations also developed less neoplasia (Fig. 4B; PGLS $P = 0.0059$). We also observed a similar trend for somatic mutation rates and malignancy prevalence but it was not statistically significant (Supplementary Fig. S69, PGLS $P = 0.087$).

Age at Death with Cancer in Animals

Age is the single biggest risk factor for the development of cancer in humans (37). Most mechanisms of somatic maintenance, including immune cell surveillance, DNA damage response, and telomere shortening, decrease in efficacy as we age (38–42). To test if observed neoplasms in animals under human care may be due to the animals living beyond their natural lifespans, we plotted the age of the animals with neoplasia at death, compared to the animals that died without neoplasms, scaled by their average lifespan (Fig. 5A–F). The vast majority of animal deaths with neoplasia diagnoses occur before the average lifespan in most animals. Only amphibians seem to be developing more neoplasms as they live past their normal lifespan under human care (Fig. 5C). The distribution of tumor diagnoses across lifespan in these three clades also demonstrates that cancer is not limited to a disease solely of extended lifespan, and in sauropsids, neoplasia is not particularly a disease of old age (Fig. 5B).

Evolution of Cancer Suppression and Susceptibility

Comparative phylogenetics provides a wealth of computational tools to model species' trait evolution across a phylogeny (24). To explore how cancer susceptibility evolved across the tree of life (Fig. 6A–C), we fit three of the most common phenotype evolution models (Ornstein–Uhlenbeck, Brownian Motion, and Early Burst) to neoplasia prevalence as a continuous trait. We found that a model of stabilizing selection on neoplasia prevalence (Ornstein–Uhlenbeck) fits the distribution of neoplasia prevalence the best (Supplementary Table S5). Malignancy prevalence evolution is also best explained by the Ornstein–Uhlenbeck model of sudden shifts followed by stasis in the phenotype.

DISCUSSION

We estimated cancer prevalence across a wide range of tetrapod species that includes mammals, amphibians, reptiles, and birds. Importantly and contrary to previous studies, our analyses highlight limitations to Peto's paradox, by showing that large animals do tend to get somewhat more neoplasms and malignancies when compared with smaller animals. The observation that larger animals tend to get more cancer was only apparent when we used a binomial regression or controlled for the fact that animals with longer gestation times tend to get both fewer neoplasms and fewer cancers. Previous studies, including ours, likely missed this relationship because it has a small effect; most previous studies involved fewer species and they did not control for gestation time (bioRxiv 2022.07.12.499088; refs. 18, 19). Large animals only get slightly more cancer than small animals. Whether or not they get as much cancer as one would expect from their body size and longevity depends on the model one uses to predict cancer prevalence as a function of body mass (43–45).

A previous study on 191 mammal species found that longer gestation lengths protect against cancer mortality (46). Our findings also show that longer gestation periods predict neoplasia and cancer prevalence across animals, with species that have longer gestation periods exhibiting lower rates of malignancy. There are multiple hypotheses to explain the link between gestation length and cancer risk. First, from a life history perspective, animals with longer gestation lengths may be "growing slow" to invest more resources toward somatic maintenance during fetal development, including cell cycle regulation and differentiation, thereby reducing their vulnerability to cancer. Notably, some of the species reported to get very little cancer, such as Cetacea (e.g., dolphins and whales) and Chiroptera (e.g., bats), are also exceptions to the typical relationship between gestation length and body size (bioRxiv 2023.10.22.563491). This uncoupling of life history traits could help explain the observed link between cancer risk and gestation length in our study. Additionally, this "slow growth" during fetal development may also prevent "jackpot" somatic mutations in gestation, which expand to large clones through the process of development and can significantly contribute to the risk of progressing to cancer later in life (47, 48). Another consideration is that species with longer gestation lengths may have reduced genomic conflict. Additionally, mammals with long gestations typically have singleton births (bioRxiv 2023.10.22.563491). Long gestation periods with one offspring allow for more effective maternal–fetal communication and regulation, reducing conflicts between maternal and paternal alleles that can lead to cellular dysregulation and cancer (49). Lastly, it may be the case that hormonal exposures during pregnancy help protect against cancer in the mothers, as we see for some forms of cancer in humans (50, 51). This last hypothesis could be tested by examining the relationship between gestation time and cancer in females versus males, across species.

Cancer prevalence across species varies greatly. Here we have used a large collection of species, and expanded our analyses beyond mammals (17–19), to test for patterns in cancer prevalence. We only include species with at least 20 necropsies (median 35), compared with 10 individuals per species in our original study (17), and weighted species more in our regression analyses if their cancer prevalence estimate is more accurate because it is based on more necropsies. In a similar study by Bulls and colleagues, they also found a positive relationship between body size with both neoplasia and malignancy prevalence in amphibians and squamates when restricting their data to species with at least five necropsies (bioRxiv 2022.07.12.499088). Further, a reanalysis of *cancer mortality* data from Vincze and colleagues (18)

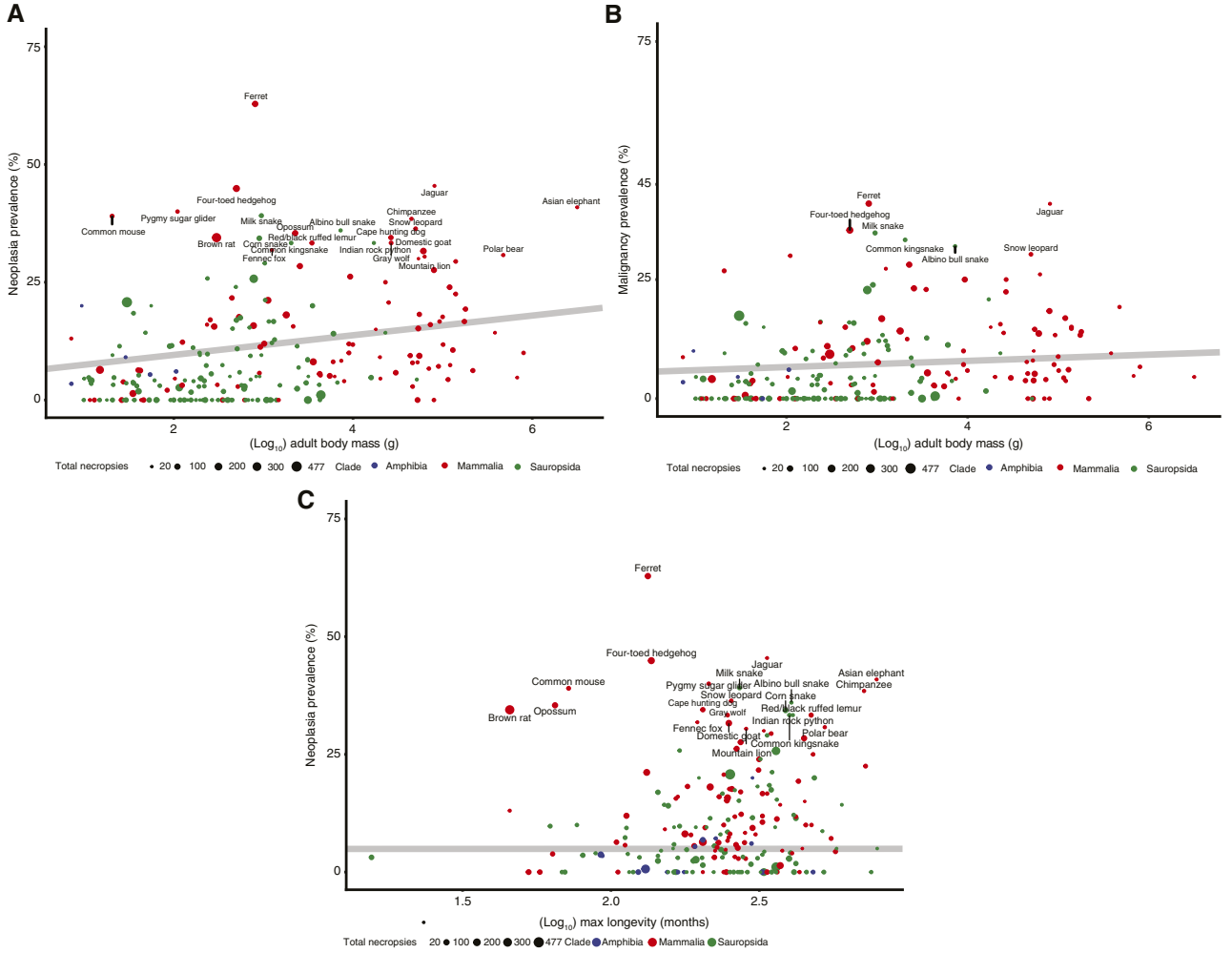

Figure 3. Significant life history factors associated with neoplasia and malignancy prevalence. **A,** Larger organisms have a higher neoplasia prevalence than smaller organisms (2.1% neoplasia per Log_{10}g adult body mass, $P = 0.007$, $R^2 = 0.26$, $\lambda = 0.46$). **B,** Larger organisms may have a higher malignancy prevalence than smaller organisms but it is not statistically significant when we do not control for gestation time (0.65% malignancies per Log_{10}g adult body mass, $P = 0.29$, $R^2 = 0.28$, $\lambda = 0.61$). **C,** Longer lived organisms have more neoplasia (0.01% neoplasia per Log_{10} month lifespan, $P = 0.02$, $R^2 = 0.19$, $\lambda = 0.34$). (*continued on following page*)

across 189 species found that larger animals were more likely to die due to cancer than smaller mammals [3% cancer mortality per Log_{10}(g), $P < 0.001$]. Using a binomial regression, both our data and from Bulls and colleagues found a negative relationship between neoplasia and longevity, but this relationship is nonsignificant in our dataset (bioRxiv 2022.07.12.499088).

The fact that neoplasia prevalence seems to evolve by sudden shifts followed by stabilizing selection (the Ornstein–Uhlenbeck model of phenotypic evolution) is consistent with life history theory predictions that investment in somatic maintenance should be under selection in specific ecological conditions (30), rather than drifting neutrally consistent with random Brownian motion. We hypothesize from these results that changes in cancer suppression may be due to large–if rare–changes in genomic architecture and/or their ecology that changes the tradeoffs with cancer suppression. These apparent changes in the investment in –cancer suppression may help pinpoint the mechanisms of cancer suppression adaptations in those lineages. Some of the variation in cancer prevalence may be noise, due to estimating cancer prevalence from tens of individuals. However, much of that variation comes from the vast diversity of species across amphibians, reptiles, birds, and mammals. We have explained only a small portion (~20%) of the variation in species vulnerability and suppression of cancer. There is clearly more to be discovered.

Peto's paradox is based on the expectation that large, long-lived animals should get more cancer because they have more cells that divide for a longer amount of time, increasing the likelihood that cancer will arise (10, 12). Comparative oncology studies have used body mass as a proxy for the number of cells in an animal (bioRxiv 2022.07.12.499088; refs. 18, 19, 44). This is reasonable because cell sizes vary little across animals, with the exception of adipocytes (52, 53). Future studies might benefit from more rigorous measures and modeling of cell numbers (as well as turnover rates and

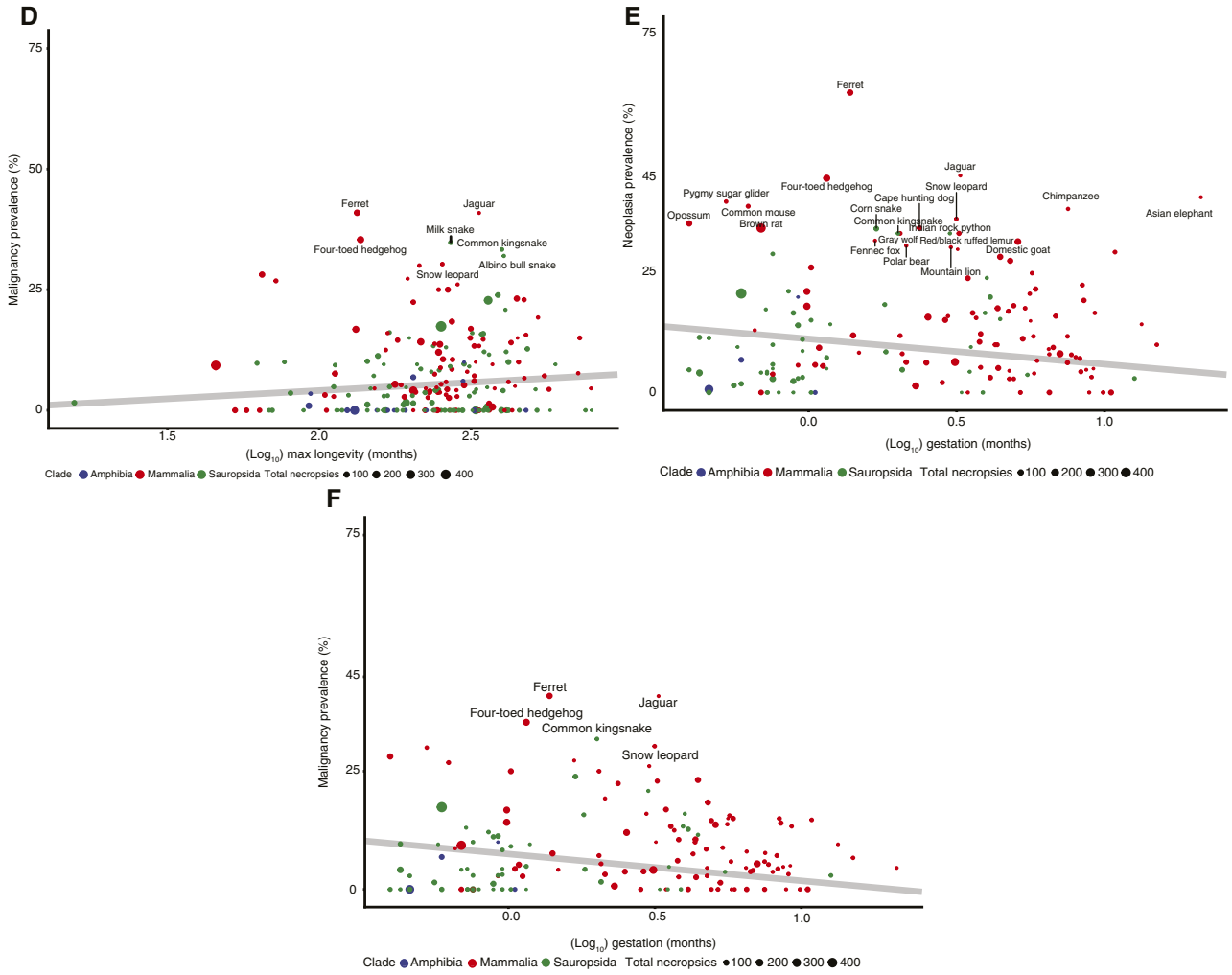

Figure 3. ***(Continued)*** **D,** Longer lived organisms also have more malignancies (3.3% malignancies per Log_{10} month lifespan, $P = 0.17$, $R^2 = 0.21$, $\lambda = 0.47$). **E,** Organisms with longer gestation times have a lower neoplasia prevalence (−5.30% neoplasia per Log_{10} months, $P = 0.10$, $R^2 = 0.11$, $\lambda = 0.34$). **F,** Organisms with longer gestation times have a lower malignancy prevalence (−5.65% malignancies per Log_{10} months, $P = 0.02$, $R^2 = 0.16$, $\lambda = 0.41$). When controlling for adult body mass, organisms with longer gestation times also have fewer neoplasms at death (−15.8% neoplasia per Log_{10} months gestation, $P = 0.0002$).

stem cell numbers) across species. Although adult body mass is positively correlated with both neoplasia and malignancy prevalence, partially resolving Peto's paradox, the effect size is larger for neoplasia (2.1% neoplasia per Log_{10}g) than for malignancies (1.9% malignancies per Log_{10}g), when controlling for gestation time. Without controlling for gestation time, the association between adult body mass and malignancy prevalence is not strong enough to be statistically significant. To be precise, Peto's paradox only relates to cancers, not benign neoplasms, so the stronger relationship between body mass and neoplasia prevalence does not resolve the paradox.

There may be several explanations for the stronger association of body mass with neoplasia prevalence than malignancy prevalence. The simplest is that malignancies are less common than neoplasms, which include both benign and malignant neoplasms. This reduces the statistical power and the expected size of the effects. However, the blunted relationship between body mass and malignancy prevalence may also be due to natural selection acting to evolve mechanisms to suppress malignant transformation. Cancer suppression mechanisms are likely under stronger selection among these larger and longer lived organisms because it was critical to suppress cancer for longer in order for these organisms to successfully reproduce. Thus, we might expect a relatively constant cancer rate across species with more cancer suppression mechanisms in large, long-lived organisms (7, 17, 54–58) and fewer in small, short-lived organisms.

Further, there are at least four transitions in neoplastic progression that natural selection might alter to increase the survivability of cancer in a species: (i) initiation of a neoplasm, (ii) transformation of that neoplasm into malignancy (i.e., invasion through the basement membrane), (iii) metastasis, and (iv) death caused by the cancer. Our data relate to the first two transitions. Specifically, we quantify the prevalence of neoplasms in a species, the prevalence of malignant neoplasms, and the proportion of neoplasms detected that are malignant.

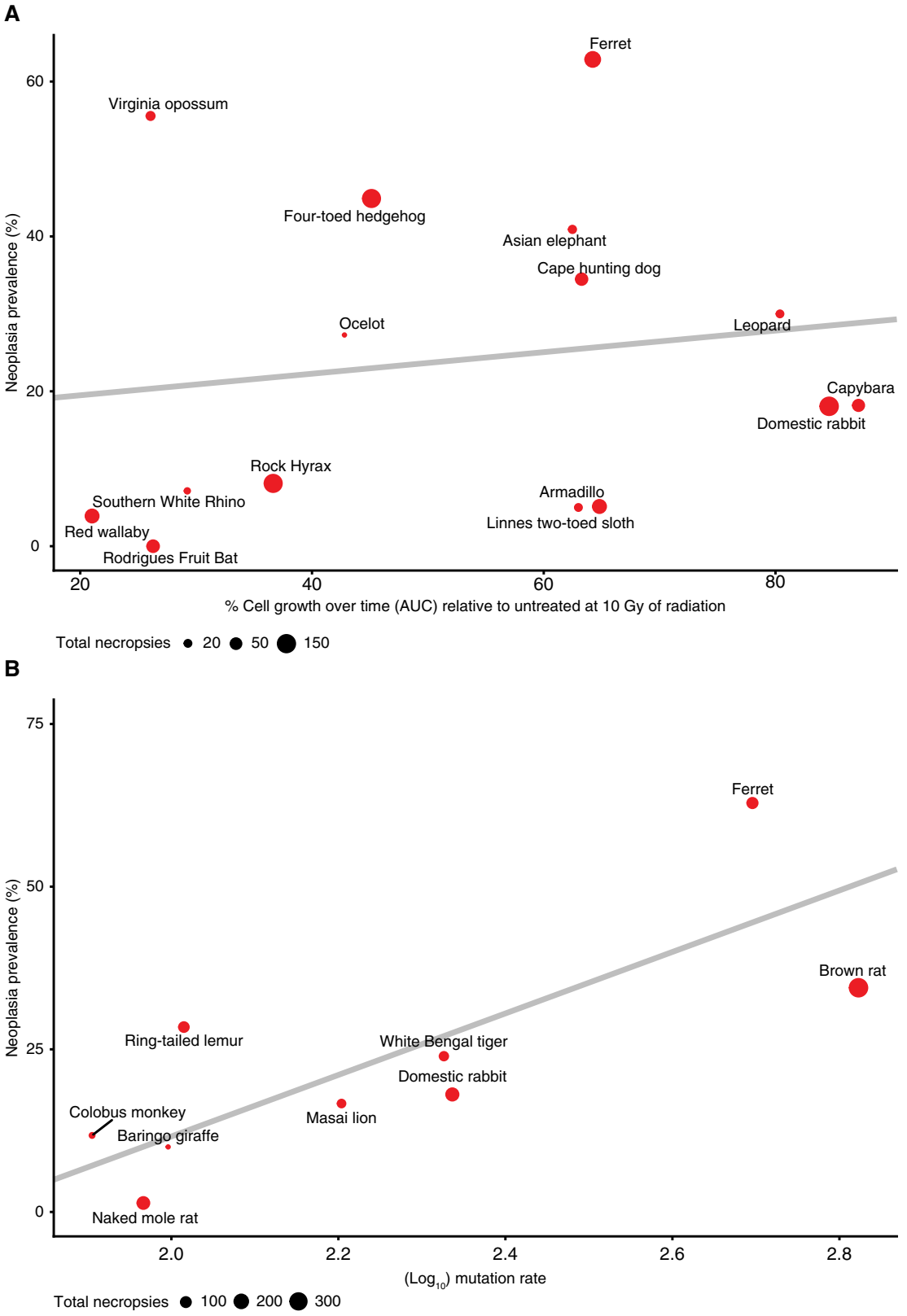

Figure 4. **A,** Cell cycle arrest as measured by % cell growth over time (AUC) relative to untreated at 10 Gy of radiation (plotted and analyzed on a Log_{10} scale) as a predictor of neoplasia prevalence in species' fibroblast cell lines. (0.31% neoplasia per % cell growth over time, $P = 0.19$, $R^2 = 0.32$, $\lambda = 0.86$). **B,** Log_{10} mean mutation rate as a predictor of neoplasia prevalence (47.26% per single base substitution per genome per year, $P = 0.0059$, $R^2 = 0.63$, $\lambda = 1.00$).

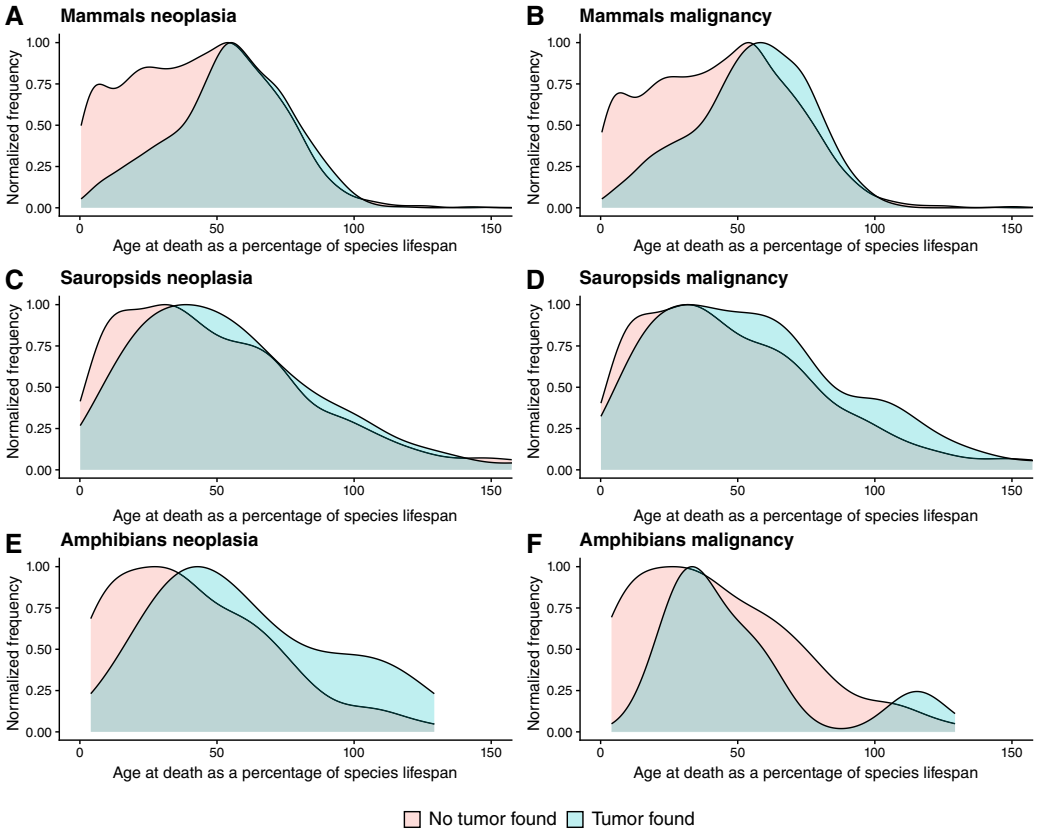

Figure 5. The density distribution of ages at death in animals with neoplasia vs. non-neoplasia, adjusted for each species' lifespan as specified in PanTHERIA. While the distributions of ages at death are different between necropsies showing neoplasia vs. those that do not (two-sample Kolmogorov–Smirnov test: (**A** and **B**) Mammals: $D = 0.11$, $P = 1.81 \times 10^{-6}$; (**C** and **D**) Sauropsids: $D = 0.18$; $P = 4.48 \times 10^{-8}$; (**E** and **F**) Amphibians: $D = 0.5$, $P = 0.011$); we found few neoplasms that could be explained by an organism living an extraordinarily long time in captivity, except in amphibians. We only had seven amphibians with a malignancy at death, one of which lived past its normal lifespan, so the shape of the distribution in **F** is noisy.

However, the selective pressure of cancer is ultimately due to its effects on mortality and so quantifying the prevalence of cancer as a cause of death is also important for evolutionary studies of comparative oncology (18). The discrepancy between our earlier analysis of a different data set of 191 mammal species, which found no relationship between body mass and cancer mortality, and our current results may be attributed to at least three differences: (i) here we analyze cancer prevalence, not cancer mortality, (ii) our current results are based on all vertebrates, not just mammals, and (iii) in our previous analyses, we separately analyzed the chance that a species had non-zero cancer mortality and then tested for a relationship between log body mass and cancer mortality in species with non-zero cancer mortality, so that the species with very low cancer mortality (zero in that sample) were excluded from the second analysis.

To test for general mechanisms of cancer defense across species, we included cross-species functional assays highlighted in Fig. 4A. Our results demonstrate variation in the fibroblast response to radiation and chemotherapy induced DNA damage. Here we provide the first test of association between DNA damage response and cancer prevalence across species. While response to DNA damage was not a significant predictor of neoplasia or malignancy prevalence after 4 or 10 grays radiation, the trend follows our hypothesis that sensitivity to DNA damage may be one mechanism of cancer suppression (17). As we continue to accumulate DNA damage response data across more species and more individuals from each species, future studies may reveal a clear relationship. On the other hand, the variation in DNA damage response may suggest that different species evolved unique mechanisms of cancer suppression and, in some cases, they do not require enhanced DNA damage response. For example, some species may rely more on enhanced mechanisms of immune surveillance, thereby eliminating a relationship between DNA damage response measurements and neoplasia or malignancy prevalence.

However, related to DNA damage response, we did find a connection between somatic mutation rates (36) and neoplasia prevalence (Fig. 4B). This result is consistent with the known importance of somatic mutations in carcinogenesis (21–23, 35, 59–61). Even with only nine species in our analysis, a strong relationship between somatic mutation rate and neoplasia prevalence was apparent. This relationship should be validated with the addition of both more somatic mutation rate data and more cancer prevalence data. Furthermore, future research should determine what mechanisms evolved in some species to suppress the accumulation of somatic mutations.

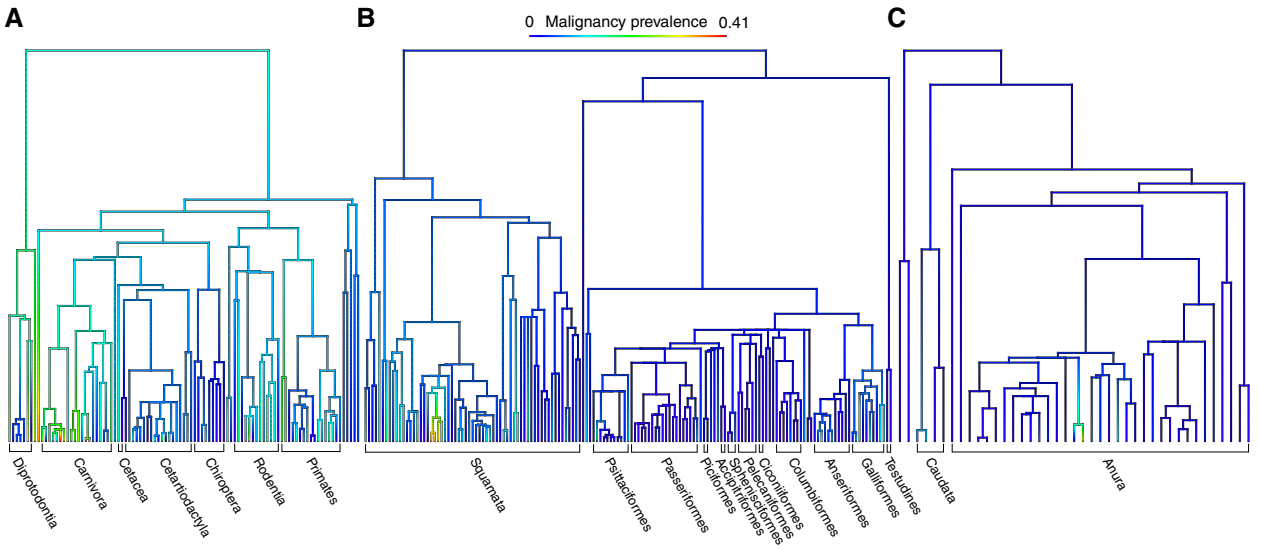

Figure 6. Cladogram depiction of cancer prevalence within (**A**) Mammals, (**B**) Sauropsids, and (**C**) Amphibians. Cladograms with the species labels at each tip can be found in Supplementary Fig. S72. Heat map coloration indicates relative prevalence of cancer within each branch, illustrating the diversity of neoplastic disease among closely related species. The scale is the same for each panel so that the differences between the clades are apparent.

Potential mechanisms include prevention of DNA damage, accurate repair of DNA damage, death of damaged cells, or use of high fidelity DNA polymerases (17, 62–64).

Insights from Comparative Oncology for Human Cancers

Species with a high prevalence of particular cancers may help to generate targeted studies to elucidate the biological basis of those cancers, help draw informative parallels to particular cancer syndromes in humans, and serve as more realistic models for studying those cancers (65). For instance, 46% of the malignant tumors diagnosed in the opossum (*Didelphis marsupialis*) were lung adenocarcinomas (Supplemental Table S7), which is a leading cause of human cancer mortality in the United States (66). Forty-five percent of the cancers in four-toed hedgehogs (*Atelerix albiventris*) were gastrointestinal cancers, mostly oral squamous cell carcinomas (67). They may hold insights for oral cancer. Forty-two percent of cancers in ferrets (*Mustela putorius*) were lymphomas, which may make them a good animal model for that disease. These spontaneous cancers may be more similar to human cancers compared to those that develop in genetically engineered mouse models, though that remains to be tested.

There is an exciting opportunity to discover cancer suppression mechanisms in species with few to no observed neoplasms and in species that seem to prevent benign neoplasms from progressing to malignancy (Fig. 1). For example, the paucity of neoplasms in dolphins and porpoises may result from having large, long-lived cetacean ancestors that were under strong selection to suppress cancer (54). Our earlier analysis of cancer gene evolution in cetaceans found evidence of positive selection in a large number of tumor suppressor genes and proto-oncogenes (54).

We previously found that animals that live longer than would be expected for their body size, like bats, tend to have more copies of tumor suppressor genes (68). In support of these observations, we found nine bats, with an average lifespan of 16 years, have low neoplasia prevalence. We had hoped to discover species that are able to prevent malignant transformation by finding species that get a fair number of benign neoplasms but few to no malignant neoplasms. The common paradigm in understanding the evolution of cancer suppression emphasizes the importance of protecting against tumor initiation. However, mechanisms that suppress malignant transformation could be similarly important in maintaining an organism's fitness. Unfortunately, only a few species in our data set fit that description. The species with the lowest proportion of malignant to benign neoplasms was the common squirrel monkey (*Saimiri sciureus*), with only 12% of their tumors being malignant.

Challenges for Comparative Oncology

There are a number of potential sources of bias in comparative oncology data. Similar to humans, there are likely both intrinsic genetic predispositions and extrinsic environmental exposures that play a role in cancer initiation and development in animals in our study. The diverse range of species in our data poses challenges in generalizing explanations for cancer trends. Understanding the etiology for the cancer-prone species is a major next step and can help reduce some of these biases.

Some species could live longer in zoological institutions, thereby unmasking a vulnerability to cancer at an age that they would be unlikely to attain in the wild. For example, an extended lifespan in managed care may be an explanation for the high prevalence in some species, especially for short-lived species that tend to benefit more from living in zoos (69). For two species that had some of the highest reported neoplasms in our study, Virginia opossums and domestic hedgehogs, the etiology of their cancers is unknown (70, 71). However, if extended lifespan were the only factor, we would

not expect to see such a high prevalence of the same diagnoses (e.g., squamous cell carcinoma of the oral cavity in hedgehogs and pulmonary adenocarcinoma in opossums), suggesting there could be other components such as a genetic predisposition, viruses, or submission bias. Further, we would expect the effects of extended lifespan to only impact a small subset of animals with high predation in the wild. Supporting our conclusions, Fig. 5 shows that the neoplasms were diagnosed prior to average lifespan in most cases, suggesting that extended lifespan due to managed care is not a large factor in these data. In fact, one unexpected finding was that neoplasms appear in birds and reptiles at relatively young ages, although some birds such as chickens are known to be prone to virally induced cancers when they are young (72). In our data, sauropsids and amphibians, but not mammals, sometimes substantially out-live their estimated lifespans (Fig. 5A–F). These results may be due to differences in how lifespans have been estimated for mammals versus non-mammals.

We hypothesized the highest cancer rates in our dataset might be due to a particular viral etiology or environmental mismatch, especially if it is a single specific cancer type. Our data comes from species living in artificial conditions of managed populations, sometimes called an evolutionary mismatch (73). These animals were generally protected from predators, provided with veterinary care, had different diets and exercise from their wild counterparts, many lived in an urban environment, and interacted with different species and microbes than a free-ranging animal. Novel conditions could contribute to cancer risk for some species that would normally not be exposed to such conditions in the wild. However, when we excluded species with the highest neoplasia or malignancy prevalences, the statistical significance of our results did not change (Supplementary Fig. S70). It is striking to us that four of the species with the lowest prevalence of neoplasms in our data set, the gray squirrel, the common dormouse, the striped grass mouse, and the common field vole are all from wild, urban populations. These necropsies come from the London Zoo that has a policy of performing a necropsy on any animal they recover that dies on its grounds, not just the animals under its care. This is a hint that cancer may well be less common in the wild, although this observation may be dependent on the species and their wildlife habitat (74).

Infections or exposures to viruses can explain the high cancer prevalence among certain species. For example, lymphoma in koalas has been linked to viral infections (75). In our study, lymphomas were common in ferrets, however an infectious etiology was not documented. There is some speculation that retroviruses are linked to lymphoma in ferrets (76). However, infections are often identified or suspected by veterinarians during physical examinations and postmortem analyses. Histopathology would identify lesions that are suggestive of viral origin and infections would be confirmed through PCR analyses, so it is unlikely that infections alone are driving these high rates of cancer in ferrets. We cannot completely dismiss the possibility of a genetic predisposition contributing to the high prevalence of cancer in these cases. Future research should focus on species with both a high cancer incidence and a significant proportion of cases stemming from a single diagnosis to investigate potential genetic predispositions.

To reduce potential sampling bias, we restricted our data to Association of Zoos and Aquariums (AZA)–accredited institutions, which are encouraged to necropsy all animals that pass away under their care. However, if the "gross" cause of death was obvious for an animal, an institution may not have submitted the animal's samples for histopathology and would not be included in our data collection. Similarly, if a particular type of neoplasia is difficult to detect in a necropsy (including some leukemias and intracerebral tumors) or was only present at a microscopic level, it may have been undercounted. Ninety-four percent of our necropsies were evaluated by a single pathology laboratory, limiting the potential for bias in diagnosis due to using different laboratories.

If cancers regularly regress prior to death, we would underestimate cancer prevalence in those species. However, cancer regression without treatment is exceedingly rare in veterinary experience and has only been consistently reported in a few species: melanoma in pigs (77) and benign histiocytoma in dogs (78, 79).

The functional assays also had some limitations. Currently, the most available cell type from animals is fibroblasts, and as more cell lines become available in the future, it will be important to test DNA damage response in other cell types. Additionally, with more sample availability in the future, important biological factors can be controlled for, like age and sex. The data analyzed here represent the first attempts to determine if mechanisms of cancer suppression are shared across species, and our data suggests that suppression of somatic mutations may be a common defense mechanism.

The Future of Comparative Oncology

In the future, it will be important to collect additional data to validate our discoveries of species with particularly low and high cancer prevalence, such as those highlighted in Fig. 1. Several life history traits, such as basal metabolic rate (BMR), may explain cancer risk but with BMR measured in only a few species, we lacked statistical power to detect a relationship with neoplasia or malignancy prevalence. Here we have dramatically expanded the amount of data on cancer prevalence in non-human animals, but the accumulation of data must continue if we are to match the robustness seen in human cancer epidemiology. In particular, much could be learned from analyzing the age-incidence curves of cancer (18, 80) but that would require significantly more individuals for each species.

There is a large gap in comparative oncology data on wild animals. Gathering data from free-ranging populations is challenging, as it is difficult to detect cancer due to the animals decomposing or being eaten before they can be necropsied. Additionally, accurate age estimates are much more difficult to obtain in wild populations compared to those managed by humans. However, wild animal populations would greatly enhance the field of comparative oncology by validating species that have low cancer prevalence and testing for evolutionary mismatches for animals in captivity.

Conclusion

Cancer is a problem of multicellularity (1). While we found a relationship between both body mass and gestation time with cancer prevalence, we are just beginning to discover

patterns of cancer susceptibility and cancer defenses across species. It is likely species evolved a variety of mechanisms to suppress cancer. The discovery of particular species with extremely low neoplasia prevalence provides opportunities to elucidate cancer suppression mechanisms that are compatible with life and reproductive success. Investigation of species with extreme vulnerability to a particular cancer may also help us understand those cancers as well as human syndromes that predispose to those cancers. We hope to learn from nature how to better prevent cancer in both humans and non-human animals.

METHODS

Analysis of Veterinary Necropsy Records

We collected necropsy records with permission from 99 zoological institutions, aquariums, and other facilities that house animals under managed care. Gross necropsies had all been conducted by veterinarians who specialize in nondomestic species and neoplasia was identified histologically by board-certified veterinary pathologists. Ninety-four percent of the histopathology from the necropsies in our dataset was evaluated by a single pathology laboratory, the others were evaluated by just two other pathology laboratories. Cases where suspect neoplasms were not examined histologically were excluded from the dataset. We used a terminology dictionary (Supplementary Table S6) to distinguish benign from malignant neoplasms based on the diagnoses in the necropsy reports. We excluded neonatal records to reduce bias from high levels of neonate and infant mortality that is common in many species. Because only common names were recorded for most records, we developed a tool, kestrel, to translate common names into scientific names which is available at https://pkg.go.dev/github.com/icwells/kestrel.

All of the institutions that provided prior approval for the use of their data in these analyses are AZA accredited. AZA accreditation encourages the institution to perform a necropsy on all animals that die under their care to determine cause of death and to monitor morbidity and mortality of each species. Furthermore, each institution had Institutional Animal Care and Use Committee approval with the Exotic Species Cancer Research Alliance and the Arizona Cancer and Evolution Center for the use of their deceased animal's records of animals with neoplasia for use in this study. Previous analyses included both necropsies for animals diagnosed with neoplasia and animals that were still alive (18). In this study, we restricted our analyses to only necropsies—for both cancer and non-cancer diagnosed animals—because alive animals may have undetected cancer or might be eventually diagnosed with cancer, thus skewing estimates in cancer prevalence.

Comparative Phylogenetic Methods in Comparative Oncology

Interspecies comparisons must account for the shared ancestry and the constraint of natural selection on species' traits before a determinant of any correlations can be made. For the life history models of neoplasia and malignancy prevalence, the R programming packages (RRID:SCR_001905) "phytools" (81), "ape" (82), and "caper" (83) were all used for phylogenetic comparisons and the handling of phylogenetic data. To accomplish this, we wrote the function *pglsSEyPagel* which is built upon phytool's *pglsSEy* (phylogenetic generalized least squares for uncertainty in Y). pglsSEyPagel expands upon the pglsSEy function by adding the estimate of Pagel's lambda (84) to the regression, rather than assuming it is fixed at 1 (i.e., Brownian motion). Because malignancy and neoplasia prevalences are constrained to the unit interval and many are close to zero, we transformed the prevalence with the standard arcsine-square root but that did not change the statistical significance for any of our analyses. We have reported the untransformed regressions for ease of interpretation (Supplementary Fig. S71). Compar.gee is another method from the "ape" package that utilizes phylogenetic input but uses a Generalized Estimating Equation to carry out binomial regressions (bioRxiv 2022.07.12.499088) that take into account the sample size for each species. The *P* values for malignancy and neoplasia prevalence varied minimally when univariate tests for body mass, longevity, and gestation length were conducted. We did see larger coefficients with the results from this model (see Supplementary Table S4).

In order to validate our *pglsSEyPagel* findings, we carried out a bootstrap analysis. For each species, we randomly selected half of its individual records to be aggregated at the species level. Each re-sample would then be regressed using *pglsSEyPagel*, testing for the relationship between malignancy prevalence or neoplasia prevalence and the chosen life history variable. This iterative process was repeated 100 times each for body mass, longevity, and gestation length. Additionally, we controlled our *pglsSEyPagel* results by individual age for those which age records available, instead of depending upon longevity records from life history databases. Values did change but conclusions remained the same (see Supplementary Table S7).

Testing for Relationships with Life History Factors

We extracted data for maximum lifespan, adult body mass, basal metabolic rate, gestation length, litter size, time to sexual maturity, and growth rate from PanTHERIA, AnAge, and Amniote (85–87). We used a weighted phylogenetic regression to control for non-independence of phenotypes (e.g., neoplasia prevalence) in closely related species. We report the phylogenetic signal, lambda, for each analysis, along with the *P* value and R^2. Due to the nature of the PGLS model, R^2 is not a standard output. To report the fit of the model, we employed a pseudo R^2 approach using the "rr2" R package. The function "R^2" from the package can utilize phylogenetic relationships within the R^2 calculation. A single phylogenetic tree encompassing the three clades was collected from timetree.org. We pruned the tree to the 292 species in our data set using the setdiff and keep.tip/drop.tip functions in the APE R package. Estimates for neoplasia and malignancy prevalence are more accurate in species with more necropsies. To address the differences in number of necropsies and to limit the noise from prevalence estimates based on few individuals, we weighted the species data points by the square-root of the number of necropsies records we have. Our R code for all analyses and figures included in this manuscript is freely available at https://github.com/zacharycompton/cancerAcrossVertebrates.git or at https://codeocean.com/capsule/7079513/tree/v1. The key files are species-cancer-prevalence-data.csv along with the data-description-min20.txt file that provides the documentation for the meaning of each column in the data file. In addition, we only analyzed species for which we had at least 20 necropsy results [previous studies had used 10 (17) or 20 (18, 19) for the lower bound number of individuals]. The main *pglsSEyPagel* analyses were done with all species together, including mammals, sauropsids, and amphibians. In the analyses of litter size and gestation time, we also tested for a relationship with neoplasia prevalence in mammals alone. We carried out a total of 28 *pglsSEyPagel* analyses. To control for multiple testing, we used a false discovery rate of 5%.

DNA Damage Response Assays

Established, primary cells from mammals were obtained from San Diego Zoo Wildlife Alliance Frozen Zoo [capybara, Linnaeus's (also called Linne's) two-toed sloth, red-necked wallaby, rock hyrax, Rodrigues fruit bat, six-banded armadillo, southern white rhinoceros, and Virginia opossum] or generated at Huntsman Cancer Institute from tissues collected from four-toed hedgehog (also known as African pygmy hedgehog), ferret, domestic rabbit, leopard, Asian

elephant, and Cape hunting dog. Brown rat (Cell Applications) fibroblasts were obtained from the indicated commercial source. Experiments were performed on cells passaged 20 or fewer times from initial establishment. San Diego Zoo Wildlife Alliance Frozen Zoo karyotypes all established primary cells to confirm species source. Experiments were initiated within 3 months of obtaining or generating cells and completed within 1 to 5 years. Cells routinely tested negative for mycoplasma as measured by MycoAlert Mycoplasma Detection Kit (Lonza). In order to include more species in our analysis of DNA damage response, we included species with a minimum of 10 necropsies. Detailed information on primary donor demographics, passage numbers, and dates obtained can be found in Supplementary Table S8. Cells were seeded in 96-well plates at 2,000 cells per well in cell growth media and allowed to adhere overnight. The following day, doxorubicin was added at one of four concentrations [0 μmol/L (DMSO vehicle control), 0.11 μmol/L, 0.33 μmol/L, and 1 μmol/L]. Each condition was tested in triplicate in three separate experiments. Cell proliferation and apoptosis were measured by real-time fluorescence microscopy (IncuCyte, Sartorius) at 2-hour intervals for 3 days. Apoptosis was measured using a fluorescent cell death marker, Annexin V dye (Sartorius). Images were processed and analyzed using IncuCyte software. The number of dead or dying cells was identified by counting Annexin V positive cells. In addition, cell count overtime was calculated using IncuCyte cell-by-cell software. To measure response to radiation-induced DNA damage, cells were irradiated with one of four doses: 0, 0.4, 2, and 10 Gy. Radiation dose was delivered using an RS-2000 X-Ray Irradiator (Radsource). Cells were then seeded in 96-well plates in cell growth media containing Annexin V dye (Sartorius). Cells were imaged by real-time fluorescence microscopy (IncuCyte, Sartorius) at 2-hour intervals for 5 days. We estimated cell cycle arrest by normalizing the cell count of irradiated cells to untreated cells by dividing the area under the curve (AUC) of cell count over time for treated cells by the AUC of cell count over time for the untreated cells. We converted that number into a percentage that represents the percent of cell proliferation relative to untreated cells. We then tested if this normalized amount of cell growth was predictive of neoplasia prevalence using the phylogenetically controlled *pglsSEyPagel* regression (Fig. 4A).

Somatic Mutation Rates

Cagan and colleagues (36) published somatic mutation rates (single base substitution per genome per year) for 16 species based on sequencing 208 individual colonic crypts from 56 animals from the London Zoo. They divided the number of somatic mutations, detected by whole-genome sequencing, by age (in years) of the individual at the time that the tissue was taken, to estimate the mutation rate per year. Nine of those species are in our dataset, allowing us to use a pgls regression to test for an association between mean somatic mutation rates and neoplasia prevalence.

Data Availability

All data and code are available at zacharycompton/cancerAcrossVertebrates (gEithub.com) and https://codeocean.com/capsule/7079513/tree/v1, with the exception of the ages of individual animals and the locations of their tumors, which are restricted due to privacy agreements with the contributing zoos. Access to that data may be granted with permissions of the zoos.

Authors' Disclosures

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Authors' Contributions

Z.T. Compton: Conceptualization, data curation, software, formal analysis, investigation, methodology, writing–original draft, project administration, writing–review and editing. **W. Mellon:** Data curation, software, formal analysis, investigation, visualization, methodology, writing–original draft. **V.K. Harris:** Data curation, software, formal analysis, writing–original draft, writing–review and editing. **S. Rupp:** Resources, data curation, software. **D. Mallo:** Data curation, software, formal analysis, writing–review and editing. **S.E. Kapsetaki:** Data curation, software, formal analysis, writing–review and editing. **M. Wilmot:** Resources, data curation, formal analysis, methodology. **R. Kennington:** Resources, formal analysis, investigation. **K. Noble:** Resources, data curation, software, investigation. **C. Baciu:** Supervision, project administration, writing–review and editing. **L.N. Ramirez:** Resources, data curation, software, formal analysis, visualization, methodology. **A. Peraza:** Data curation, software, validation. **B. Martins:** Data curation, software, visualization, methodology. **S. Sudhakar:** Data curation, software, visualization, methodology. **S. Aksoy:** Data curation, software, formal analysis, visualization. **G. Furukawa:** Data curation, investigation, methodology. **O. Vincze:** Formal analysis, validation, writing–review and editing. **M. Giraudeau:** Supervision, validation, writing–review and editing. **E.G. Duke:** Resources, data curation, writing–original draft, writing–review and editing. **S. Spiro:** Resources, data curation, supervision, writing–review and editing. **E. Flach:** Resources, data curation, validation, writing–review and editing. **H. Davidson:** Resources, validation, writing–review and editing. **C.I. Li:** Validation, methodology. **A. Zehnder:** Conceptualization, resources, validation, investigation, writing–original draft, project administration, writing–review and editing. **T.A. Graham:** Supervision, validation, investigation, writing–review and editing. **B.V. Troan:** Resources, validation, methodology. **T.M. Harrison:** Conceptualization, resources, data curation, formal analysis, supervision, validation, writing–original draft, writing–review and editing. **M. Tollis:** Conceptualization, supervision, funding acquisition, investigation, methodology, writing–original draft, writing–review and editing. **J.D. Schiffman:** Conceptualization, resources, funding acquisition, validation, investigation, writing–original draft, project administration, writing–review and editing. **C.A. Aktipis:** Conceptualization, supervision, funding acquisition, validation, investigation, writing–original draft, project administration, writing–review and editing. **L.M. Abegglen:** Conceptualization, resources, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing. **C.C. Maley:** Conceptualization, funding acquisition,

validation, investigation, writing–original draft, project administration, writing–review and editing. **A.M. Boddy:** Conceptualization, data curation, formal analysis, supervision, funding acquisition, investigation, writing–original draft, project administration, writing–review and editing.

Disclaimer

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 NIH

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