Population Genomics of Wildlife Cancer

Sarah A. Hendricks, Andrew Storfer, and Paul A. Hohenlohe

Abstract Studies of cancer in wildlife species present unique challenges, but research is beginning to uncover causes of cancer and its impact on wildlife populations. Causes of cancer in wildlife include environmental carcinogens, viruses and other pathogens, hereditary factors, and direct transmission of tumor cells. Here, we review progress and potential for population genomics to address issues such as genetic variation for susceptibility, comparative genomics of tumor suppressor genes, and evolutionary response to cancers. We also address the implications of cancer, and the potential of population genomics research, to inform conservation and management of wildlife populations. As an illustrative case study, we focus on the unique case of a transmissible cancer, devil facial tumor disease (DFTD), which has had a dramatic impact on demography and life history of Tasmanian devils (*Sarcophilus harrisii*). Recent population genomics research has revealed genetic variation underlying DFTD-related phenotypes and signatures of rapid evolution at candidate loci associated with cancer and immune function. The DFTD-devil system illustrates how genomics tools can be applied to an epizootic cancer in a wildlife population, providing insights into basic cancer biology as well as lessons for potential conservation strategies.

Keywords Conservation genomics · Devil facial tumor disease · Tasmanian devils · Transmissible cancer · Tumor evolution · Wildlife disease

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1 Introduction: Cancer in Wildlife

Ongoing research is elucidating the role of cancer in natural populations of wildlife species of ecological, cultural, and conservation importance (McAloose and Newton 2009; Pesavento et al. 2018; Hamede et al. 2020). Cancer, a collection of diseases characterized by abnormal and uncontrolled cell proliferation caused by somatic mutations, affects nearly every known multicellular organism. Rather than an accumulation of genomic aberrations in a single-cell lineage, it is now clear from numerous studies that cancers are heterogeneous collections of cells (Nowell 2002; Maley et al. 2006; Campbell et al. 2008; Merlo and Maley 2010; Park et al. 2010) that evolve in tumor microenvironments with complex ecological interactions (Bissell and Radisky 2001; Ujvari et al. 2019). Cancer can affect wild populations by reducing reproductive success and survival, altering interactions with predators or other species, and directly or indirectly leading to population declines (Dawson et al. 2018; Ujvari et al. 2019). Anthropogenic influences, including direct impacts, such as pollution and the reduction of genetic diversity in natural populations that are fragmented or reduced in size, can increase the prevalence of cancer in wildlife (McAloose and Newton 2009; Giraudeau et al. 2018; Pesavento et al. 2018). Aspects of wildlife behavior, life history, and genetic factors have been shaped by an evolutionary history with cancer as a selective force (Ujvari et al. 2019; Thomas et al. 2018, 2020). Understanding these many impacts of cancer on wildlife populations can help inform management and conservation efforts. Additionally, cancers in wildlife species may provide new biological models for understanding the complex causes of cancer, with the potential for biomedical benefits.

Studying cancer in wildlife species is difficult because of the ethical, logistical, and legal limits on invasive sampling and experimentation. Relatively few studies have estimated cancer prevalence in wild populations. Madsen et al. (2017) found that estimates of cancer prevalence in mammal populations range from 2% (sea otter, *Enhydra lutris*; Williams and Pulley 1981) to 64% (Baltic gray seal, *Halichoerus grypus*; Bäcklin et al. 2016). Low-prevalence cancers may go undetected and are likely to affect many more species than observed. In addition, estimates of prevalence in natural populations may be down-biased due to several factors (Hamede et al. 2020). First, most wildlife cancers lack diagnostic tools, particularly for detection in the absence of obvious clinical signs. Further, the cancer may lead to other conditions, including secondary parasite or pathogen infections, reduced body condition, and an increased level of predation resulting from a compromised ability to avoid predators. Thus, individuals may die from these secondary factors before the cancer exhibits obvious clinical signs (Vittecoq et al. 2013; Ujvari et al. 2019; Perret et al. 2020). Despite these difficulties, genomics research on wildlife cancer is beginning to reveal the underpinnings of cancer in natural populations. Here we review some of these developments and discuss how population genomics tools in cancer research can inform wildlife conservation.

2 Causes of Cancer

The uncontrolled cell proliferation of cancer is caused by somatic mutations and epigenetic alterations in a population of cells (Box 1). The probability of an individual developing cancer is influenced by several fundamental factors that can be considered extrinsic (e.g., environmental conditions) or intrinsic to an individual (e.g., genetic factors). In wildlife species, these factors can be the result of human influence, either on the surrounding environment or on the genetic diversity and evolutionary processes of wildlife populations. Other underlying causes of cancer, such as viruses or direct transmission of tumor cells, can also result in epizootic spread of cancer as an infectious disease across wildlife populations. Factors may interact as well, such as environmental factors or infectious agents interacting with variation in genetic susceptibility.

Box 1 Key Mutation Types in Cancer

Progression to cancer involves somatic mutations or epigenetic changes that remove the constraints to uncontrolled proliferation in a population of cells (Hanahan and Weinberg 2000, 2011). Normal control of cell proliferation involves a number of pathways, and genes whose inactivation can allow tumorigenesis are called tumor suppressor genes (Vogelstein et al. 2013). The "gatekeepers" are the genes directly involved in preventing unregulated cell division by inhibiting growth or promoting death of cells with chromosomal abnormalities, while the "caretakers" are involved in error-free DNA replication, effective DNA repair, and the maintenance of appropriate epigenetic patterning and chromosomal structure (Kinzler and Vogelstein 1997; Stoler et al. 1999; Shields and Harris 2000; Sarkies and Sale 2012). Loss of function at caretaker genes can increase the rate of mutation and chromosomal alteration, and inherited mutations in caretaker genes are often associated with hereditary cancers because they increase the likelihood of cancer from subsequent somatic mutation (Negrini et al. 2010). Oncogenes are those that promote tumorigenesis when increased in activation or expression level by somatic mutation or epigenetic change, and they often exhibit recurrent mutations at the same positions across tumors (Vogelstein et al. 2013).

Because tumor progression is associated with increased mutation rates, tumors typically show large numbers of genetic differences from their respective hosts. Tumors are heterogeneous populations of cells, with selection acting among cellular lineages. Mutations that increase relative fitness of a cell lineage are "driver" mutations, while those that are neutral are "passenger" mutations that increase in frequency solely because of hitchhiking in successful cell lineages (Vogelstein et al. 2013; Cannataro and Townsend 2018). Massive genomic sequencing efforts, including single-cell sequencing and subsampling of tumor cell populations, have revealed a number of genes that

(continued)

Box 1 (continued)
are strongly associated with driver mutations in particular cancer types (Vogelstein et al. 2013; Heng 2017; ICGC/TCGA 2020; Rheinbay et al. 2020). Important types of mutations and genetic factors in cancer progression are:

1. *Single nucleotide polymorphisms (SNPs)* make up approximately 95% of mutations from cancer genomes (Vogelstein et al. 2013; Heng 2017). These mutations can result in nonsynonymous changes in proteins, as well as other functional consequences, such as changes in micro-RNA loci or regulatory binding sites that affect gene expression.
2. *Copy number variation (CNV)* is defined as the amplification or deletion of DNA fragments >50bp (Girirajan et al. 2011). Somatic copy number alterations (SCNAs) are common in cancer; however, distinguishing driver SNCAs from numerous SCNAs that randomly accumulate during tumorigenesis is not straightforward (Zack et al. 2013; Heng 2017).
3. *Chromosomal structural abnormalities* such as translocations or aneuploidy are extremely common for many cancer types and can have large effects on gene function and cellular phenotypes (Stephens et al. 2009; Heng et al. 2013). Chromothripsis, which literally means "chromosome shattering," is defined by a single, localized event within genomic regions in one or few chromosomes characterized by thousands of clustered chromosomal rearrangements. Similarly, chromoplexy is characterized by chromosomal rearrangements that involve segments of DNA from multiple chromosomes (e.g., five or more). These abnormalities have been implicated in cancer phenotypes, particularly metastasis and drug resistance (Heng et al. 2013).
4. *Telomere dynamics* are involved in many cancers as well as somatic maintenance, aging, and apoptosis. Progressive shortening of telomeres typically induces cellular senescence, which can provide a defense against cancer. Mutations that affect the function of telomerase or promote telomere lengthening have been associated with multiple types of cancer (Artandi and DePinho 2010; Vogelstein et al. 2013).
5. *Epigenetic factors*, heritable changes in gene expression that are not accompanied by changes in DNA sequence, can contribute to tumorigenesis, for instance, by increasing the expression of oncogenes (Jones and Baylin 2007; Vogelstein et al. 2013). Abnormalities in methylation, histone modification, nuclear topology, and noncoding RNA have been implicated in the silencing of key tumor suppressor, regulatory, and repair genes resulting in cancer (reviewed by Grunau 2017). Epigenetic modifications are not detected by DNA sequencing, but by other approaches such as transcriptomics or methylation profiling.

2.1 Environmental Conditions

Environmental contaminants and other external influences are often associated with human and wildlife cancer incidence. Ultraviolet and other radiation exposure, smoking, environmental pollution, and ingestion of certain foods or toxins influence cancer initiation and progression by increasing somatic mutation rates (Perera 1998; Irigaray et al. 2007; Soto and Sonnenschein 2010). Moreover, there is some evidence that environmental endocrine disruptors, stress, and trauma influence somatic mutation rates and cancer risk (Reiche et al. 2004; Antoni et al. 2006; Aktipis and Nesse 2013; Pesavento et al. 2018). In natural populations of wildlife species, stress and multiple types of environmental pollution may play important roles in cancer incidence (Pesavento et al. 2018). Aquatic and terrestrial wildlife in the Chernobyl area of Ukraine, subject to elevated levels of pollution and radioactivity following the 1986 nuclear disaster, have higher cancer prevalence than populations in less polluted areas (Yablokov 2009; Mousseau and Møller 2015). Similarly, the beluga whale (*Delphinapterus leucas*) population in the St. Laurence River estuary (Canada) has a higher rate of cancer than other populations (Martineau et al. 2002). This population shows evidence of contamination by agricultural and industrial chemicals, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and their metabolites (Letcher et al. 2000; Martineau et al. 2002).

2.2 Viruses and Other Pathogens

Parasite-induced cancers can be due to a variety of subcellular, unicellular, or multicellular parasites and pathogens. Cancer associated with multicellular parasites may be the result of chronic inflammation (Pesavento et al. 2018), such as that caused by infection from ear mites in Santa Catalina Island foxes (*Urocyon littoralis catalinae*; Vickers et al. 2015) or from nematodes in ring-necked pheasants (*Phasianus colchicus*; Himmel and Cianciolo 2017). Many pathogen-induced cancers in wildlife are attributed specifically to viruses, which have direct mutagenic effects on host tissue (McAloose and Newton 2009; McCallum and Jones 2012; Pesavento et al. 2018). Evaluating the effects of pathogens, particularly viruses, on cancer development and establishing a causal link between cancer incidence and infection is challenging partially because of a lag between the presence of the parasite and cancer detection. However, there is growing evidence that viruses may be associated with a substantial proportion of cancers in humans as well as natural populations of wildlife (Ewald and Swain Ewald 2015, 2019). Viruses disrupt a variety of cellular barriers to oncogenesis; for instance, infected cells may lose the ability to control the total number of cellular divisions, apoptosis, adhesive properties to other cells, and/or cellular arrest (Ewald and Swain Ewald 2015). Examples of empirical evidence for virus-induced cancer in wildlife include

otarine herpesvirus-1 and genital carcinoma in California sea lions (*Zalophus californianus*; Lipscomb et al. 2016), deltapapillomavirus associated with fibropapillomas and fibromas in deer (subfamily Capreolinae), giraffe (*Giraffa* spp.), and zebra (*Equus* spp.) species (Pesavento et al. 2018), papillomas and carcinomas in western barred bandicoots (*Perameles bougainville*), and lymphomas in Attwater's prairie chickens (*Tympanuchus cupido attwateri*; Drechsler et al. 2009) (see McAloose and Newton 2009; Ewald and Swain Ewald 2017; Pesavento et al. 2018; Hamede et al. 2020 for further examples).

2.3 Transmissible Cancers

Known from only a handful of animal species, transmissible cancers are much rarer than virus-associated cancers. Transmissible cancers are spread directly by transfer of tumor cells between individuals; in other words, the etiologic agent is the neoplastic cells derived from an original host (Metzger and Goff 2016; Ostrander et al. 2016). The tumor cells are a set of clonal lineages, spreading from the original host to secondary hosts across a population as an infectious disease. Transmission occurs with direct contact during mating, biting, or feeding, or tumor cells may be spread and acquired through the environment in marine systems (Metzger and Goff 2016; Ostrander et al. 2016). A well-studied example is canine transmissible venereal tumor (CTVT), which affects dogs (*Canis lupus domesticus*) and is believed to have originated thousands of years ago, making it perhaps the oldest continuously propagated cell lineage (Murchison et al. 2014; Baez-Ortega et al. 2019). A group of transmissible cancers produces leukemia-like conditions such as disseminated neoplasia or hemic neoplasia, in at least 15 different bivalve species (Metzger et al. 2016). Some lineages within this group of transmissible cancers have spread across species and across wide geographic areas (Yonemitsu et al. 2019), and they are strongly associated with the integration of retrotransposons into the host genomes (Arriagada et al. 2014; Metzger et al. 2018). Devil facial tumor disease (DFTD) and DFT2 are two recent independent origins of transmissible cancer that infect Tasmanian devils (*Sarcophilus harrisii*; Pearse and Swift 2006; Pye et al. 2016); we discuss this case in detail below. In transmissible cancers, the genome of tumor cells descends from the original host, so that the highest degree of genomic similarity is expected among tumors across a host population, rather than between each tumor and its respective, contemporary host. Accordingly, transmissible cancers can be diagnosed by genomic similarities, such as shared chromosomal rearrangements and other mutations, across a set of tumors (Pearse and Swift 2006; Pye et al. 2016; Leathlobhair et al. 2017).

2.4 Hereditary Factors

Hereditary susceptibility to cancer is widely established in humans, with over 200 cases known, most of which are inherited as autosomal dominant alleles (Nagy et al. 2004). The following characteristics designate an inherited cancer susceptibility: "two or more relatives with the same type of cancer on the same side of the family; several generations affected; earlier ages of cancer diagnosis than what is typically seen for that cancer type; individuals with multiple primary cancers; the occurrence of cancers in one family, which are known to be genetically related; and the occurrence of nonmalignant conditions and cancer in the same person and/or family" (Nagy et al. 2004). Hereditary factors include genetic mutations that increase the susceptibility to cancer progression (Box 1). Many of these susceptibility syndromes are rare, but collectively hereditary cancers account for at least 1–10% of all cancers in humans (Fearon 1997; Nagy et al. 2004). Most research on wildlife focuses on a population rather than an individual or family level, so we know little about hereditary cancer in wildlife. In particular, rare variants that increase individual susceptibility are very difficult to detect in wildlife, even if they collectively impose a large cancer burden on the population. Genetic variants that increase cancer susceptibility are expected to behave at a population level much like other deleterious variants, such as those associated with other pathologies. As a result, we can predict that cancer may contribute to reduced population fitness in wildlife populations that are small or fragmented and subject to reduced genetic diversity and inbreeding (Ujvari et al. 2019).

3 Genomics and Evolution of Cancer in Wildlife

3.1 Evolution of Cancer Resistance

An evolutionary perspective is useful for understanding cancer at multiple scales, from the behavior of cellular lineages within a tumor, to understanding the genetics of resistance at the individual level, to population-level susceptibility (Frank 2004; Ujvari et al. 2019; Thomas et al. 2020). Natural selection is predicted to act against cancer susceptibility because of its effect on fitness, but this effect is reduced to the extent that timing of onset is later in an individual's life, after some proportion of reproduction has occurred (Leroi et al. 2003). As a result, early-onset cancers tend to be more attributable to a specific cause, while later onset may reflect multiple causes, such as rare alleles persisting in a population because of weak negative selection (Frank 2004). In general, natural selection favors mechanisms in the genome for resistance and tolerance to cancer (Seluanov et al. 2018; Thomas et al. 2020). These mechanisms in turn suppress the selection acting at the cellular level, which favors cell lineages that proliferate at the expense of the multicellular individual (Michod 2000). A variety of ecological and evolutionary processes also occur within the

population of cells that make up a tumor and its microenvironment. Genetically, there is greater evidence for positive selection than purifying selection in tumors, compared to evolution at the species level, meaning that multiple mutations can increase cell proliferation within a tumor (Martincorena et al. 2017). Nonetheless, the large majority of mutations still appear neutral within tumors, so that neutral theory from population genetics can be fruitfully applied within a tumor cell population as well (Cannataro and Townsend 2018).

Adaptations to prevent cancer may have trade-offs in the capacity for wound healing, growth, reproduction, and aging. This tension is present because somatic maintenance and growth require controlled cell division, while suppressing cell proliferation is central to cancer resistance (Guo and DiPietro 2010; Hofman and Vouret-Craviari 2012). Similarly, there may be trade-offs with reproductive effort, such as the relationships between early menarche or fertility and susceptibility to breast cancer in humans (Smith et al. 2012). There is evidence that wildlife populations in captivity with limited opportunities for reproduction, such as the white rhinoceros (*Ceratotherium simum*), may experience a higher rate of neoplasias because of the proliferative effects of increased estrous cycling (Pesavento et al. 2018). Wildlife species may be frequently exposed to chronic infection by parasites and wounds from predators or other ecological interactions, so the capacity for inflammatory responses and wound healing is important for fitness. However, the inflammatory response can foster neoplastic cell proliferation, cause DNA damage, and create a microenvironment conducive to tumor progression (de Visser et al. 2006; Pesavento et al. 2018; Ujvari et al. 2019).

Balancing among these selective forces in the evolutionary history of wildlife species has left a complex legacy of cancer susceptibility in many populations (Thomas et al. 2018). This balance may help explain Peto's paradox: the lack of correlation between body size, life span, and cancer risk (Abegglen et al. 2015). The general expectation should be that larger body size and life span should require more somatic cell divisions and somatic maintenance, resulting in greater opportunity for somatic mutations leading to cancer. However, the lineages of some long-lived species, such as elephants and naked mole rats, have evolved remarkable resistance to cancer (Seluanov et al. 2018; Tollis et al. 2019). Comparative and other genomics approaches have uncovered some of the mechanisms explaining this resistance, discussed below.

3.2 Genetics of Population Susceptibility

Wildlife species are subject to reduced population size, fragmentation, and inbreeding, which can increase the frequency of slightly deleterious alleles and their presence in the homozygous state. Some of these alleles may increase susceptibility to cancer, thus increasing the overall genetic load and potentially increasing extinction risk of small wildlife populations (Gomulkiewicz and Holt 1995; Frankham 2005). The degree to which cancer susceptibility contributes to genetic load is very difficult to quantify, although population genomics studies could reveal the patterns

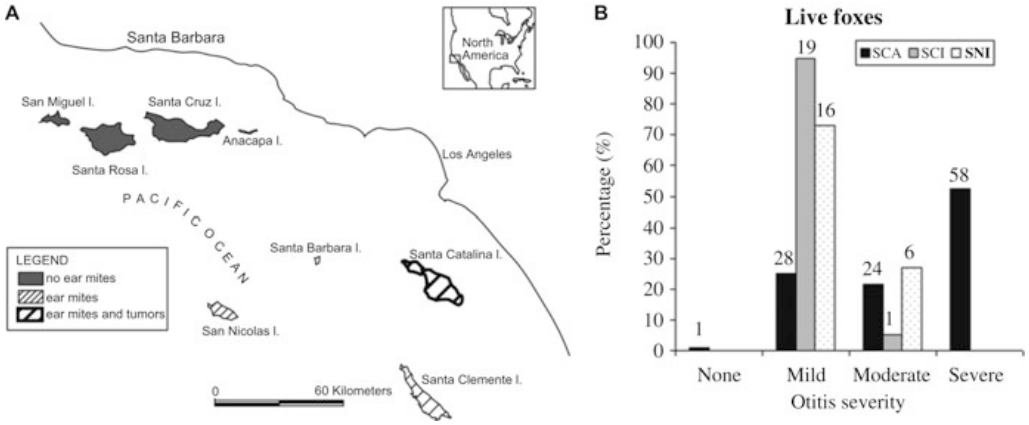

Fig. 1 One population of island foxes on Santa Catalina Island (*Urocyon littoralis catalinae*) exhibits high incidence of inflammation-induced ear canal cancer associated with ear mite infection. (**a**) Map of the Channel Islands off the coast of California, showing the presence of fox populations without ear mites on three northern islands, foxes with ear mites on three southern islands, and high incidence of cancer on just one island. (**b**) Severity of otitis (inflammation of the ear canal) is much higher in foxes on Santa Catalina Island (SCA) compared to the other two islands with ear mites, San Nicolas Island (SNI) and San Clemente Island (SCI). Reproduced from Vickers et al. (2015)

of historic selection on cancer-associated genes and their potential effects on fitness by using functional genetic information from annotated genomes (Oh et al. 2019; Robinson et al. 2018).

One example of population susceptibility to cancer is found in Channel Island foxes (*Urocyon littoralis*), which are endemic to individual islands off the coast of California (Fig. 1). These populations have undergone severe genetic bottlenecks resulting in strong genetic differentiation among islands and the accumulation of deleterious mutations, although they retain enough genetic variation to facilitate local adaptation to different environmental conditions (Funk et al. 2016; Robinson et al. 2016). Populations among islands differ markedly in the incidence of cancer: on one island (Santa Catalina Island, SCA), foxes have a high prevalence of ear canal (ceruminous gland) carcinoma and adenoma that appear to be associated with inflammation from chronic infection by ear mites (*Otodectes* spp.; Vickers et al. 2015). Ceruminous gland tumors have not been documented on other islands (San Clemente Island and San Nicolas Island) despite similar levels of chronic mite infection, nor in the three island fox populations that do not have ear mites. Treatment of individual foxes with acaricide, which removes ear mite infection, significantly reduced inflammation and hyperplasia, and had a non-significant trend toward reducing tumor progression likely due to low sample size (Moriarty et al. 2015). Ear mite infection also induces changes in the ear canal microbiome (DeCandia et al. 2019). Other factors, such as a virus or environmental differences among islands, could play a role in the strikingly different level of cancer prevalence between SCA and the other populations. Nonetheless, a leading hypothesis is that the severe genetic drift in the SCA population has increased frequencies of alleles that contribute to inflammation-induced cancer susceptibility.

3.3 Population Genomics Studies of Wildlife Cancer

Powerful new genomics approaches in wildlife can be used to estimate the prevalence of cancer, its effects on population fitness and conservation, broad-scale evolutionary patterns, and the specific genetic mechanisms of cancer susceptibility. The large case-control or genome-wide association studies that have been critical to understanding the genetic basis of cancer in humans or other model organisms are often not feasible in wildlife species, but population genomics studies of wildlife cancer are still tractable. For example, in a case-control study of California sea lions (*Zalophus californianus*), urogenital carcinoma was significantly associated with homozygosity of a microsatellite loci within an intron of the heparanase 2 gene (HPSE2; Browning et al. 2014), which has been implicated in several human carcinomas. Two unusual rodents, the naked mole rat (*Heterocephalus glaber*) and the blind mole rat (*Spalax ehrenbergi*), are not closely related to each other but have independently evolved extremely low incidences of cancer, despite their long life spans. Cancer resistance in naked mole rats appears to involve multiple genes that control telomere dynamics (MacRae et al. 2015; Tollis et al. 2017; Seluanov et al. 2018). In blind mole rats, pre-cancerous hyperplasia triggers a strong concerted cell death response through interferon-β, suppressing tumor progression (Seluanov et al. 2018).

Transmissible cancers have been the focus of genomics studies to understand the genetic basis of susceptibility as well as the mechanisms that allow tumors to transmit among individual hosts and evade host immune and tumor suppression responses. In the cluster of transmissible cancers in bivalve molluscs, the *Steamer* retrotransposon exhibits extreme amplification in neoplastic cells as well as evidence of multiple cross-species transfers (Metzger et al. 2018). In the canine transmissible venereal tumor (CTVT), genomics work has dated the origin of the disease, roughly coincident with the domestication of dogs, and characterized the mutational signature of CTVT (Baez-Ortega et al. 2019). One particular mutational type, a C->T transition in the context of the five-nucleotide motif GTCCA, was prevalent until ~1,000 years ago but then subsided. Additionally, most driver mutations appear to have occurred relatively early in CTVT history, and more recent genetic evolution appears to be neutral (Baez-Ortega et al. 2019).

The ability to produce whole-genome sequence data and reference genome assemblies in wildlife species allows for comparative genomics studies of cancer, examining the genomes of a group of related taxa in a phylogenetic framework to reveal evolutionary history (Gorbunova et al. 2014; MacRae et al. 2015; Tollis et al. 2017; Seluanov et al. 2018). Comparative genomics has revealed evolutionary relationships among body size, life span, and cancer susceptibility and the genetic mechanisms that relate these factors (Tollis et al. 2017; Seluanov et al. 2018). Studies of genetic adaptations to reduce cancer susceptibility in long-lived and large-bodied animals have revealed cancer-related genomic evolution such as copy number variants of specific tumor suppressor and genome maintenance genes, and they help to resolve Peto's paradox (Caulin et al. 2015; Seluanov et al. 2018). Elephants, which have low cancer mortality, possess more copies of the TP53

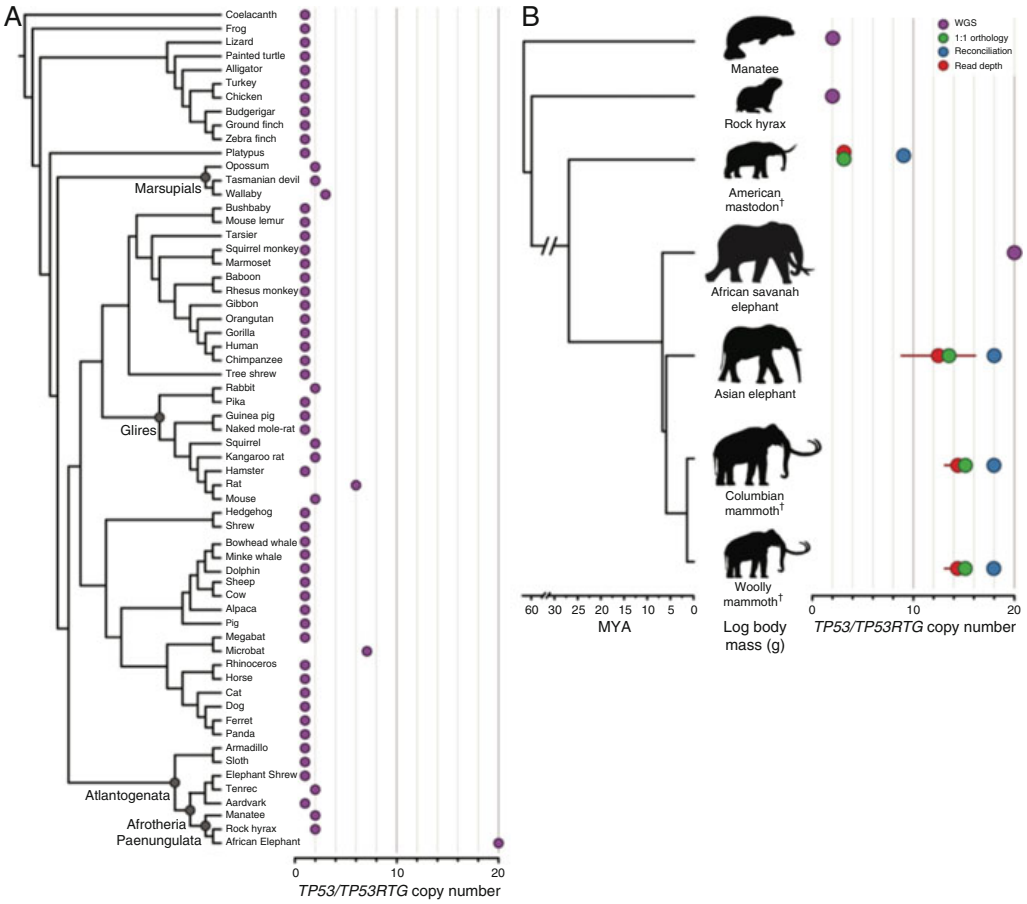

Fig. 2 Elephants, despite large body sizes and long life spans, have evolved resistance to cancer by increasing copy number of the tumor suppressor gene TP53 as revealed by comparative genomics at two different evolutionary scales: (**a**) across a phylogeny of tetrapod vertebrates, and (**b**) among elephants and two closely related mammalian orders. Reproduced from Sulak et al. (2016)

(p53) tumor suppressor gene than 61 other vertebrate species (Fig. 2; Sulak et al. 2016). Elephant cells, as compared to human cells, demonstrate an increased p53-mediated apoptotic response following DNA damage (Abegglen et al. 2015), which may be due to the transcription and likely translation of several of the TP53 retrogenes (Sulak et al. 2016). Sulak et al. (2016) also found a positive association between body size and copy number of TP53 retrogenes (Fig. 2).

Tollis et al. (2019) compared the genomes of ten cetacean species – the largest-bodied animals – and found substantial evidence for selection for multiple mechanisms of cancer resistance that differ from elephants. These included segmental duplications of regions containing genes associated with apoptosis and evidence for positive selection in other loci linked to cell cycle checkpoints, cell signaling, and proliferation (Tollis et al. 2019). In another recent study on rodents, telomere maintenance strategies were found to differ depending on body mass and differential cancer risks (Tian et al. 2018). Larger species evolved repression of somatic telomerase activity and replicative senescence while longer-lived smaller species

evolved telomere-independent anticancer mechanisms that act to slow down cell proliferation and prevent premalignant hyperplasia. Patterns are similar across mammals: body size is related to telomere length and telomerase activity as a result of trade-offs among selection for cancer resistance and selection for protection against DNA damage and replicative senescence (Tollis et al. 2017; Risques and Promislow 2018). In contrast, animals with longer life spans tend to reduce cell proliferation rates and evolve toward early-acting tumor suppressor genes (Seluanov et al. 2018).

4 Tasmanian Devils and DFTD

4.1 An Epidemic Transmissible Cancer

Devil facial tumor disease (DFTD) was first observed in 1996 by a wildlife photographer who documented ulcerative neoplasias on the face of Tasmanian devils (*Sarcophilus harrisii*; Hawkins et al. 2006). The disease has since spread across most of the island of Tasmania with only a few devil populations yet unaffected in the far western and northwestern parts of the species' range. The census population size has been reduced by ~80% due to these metastatic tumors that typically result in mortality within 6 months to 1 year of transmission (Hamede et al. 2012, 2015; Lazenby et al. 2018; Jones et al. 2019). DFTD cells are undifferentiated neoplasms with highly pleomorphic and anaplastic cells (Pyecroft et al. 2007). Tumors result in ulcerating proliferative masses that tend to occur around the face and jaw, and masses within the oral cavity can prevent feeding and are prone to secondary infection (Hawkins et al. 2006). Live cancer cells are the infectious agent and are transmitted to new hosts by biting during social interactions (Pearse and Swift 2006; Pyecroft et al. 2007; Hamilton et al. 2019). Uninfected, aggressive biters become infected after biting the tumors of infected, less aggressive bite recipients; therefore, more socially dominant devils appear more likely to get DFTD (Wells et al. 2017). Thus far, there is no evidence of vertical transmission from mothers to their offspring, and low levels of prevalence in juveniles could be associated with dramatic changes in immune capacity at sexual maturity (Cheng et al. 2017, 2019). The pattern of infection and mortality has effects on population age structure, with substantial shifts toward younger animals (Lachish et al. 2009; Hamede et al. 2012). Changes in life history strategies have also been observed, and age at first breeding has shifted from 2+ years to 14 months in some areas (Jones et al. 2008; Lachish et al. 2009). Early models predicted extinction of the species in the wild (McCallum et al. 2009), but growing evidence from multiple sources suggests extinction is unlikely (Hohenlohe et al. 2019; Wells et al. 2019), and local populations have not gone extinct in the wild (Lazenby et al. 2018; Storfer et al. 2018).

The etiology of DFTD and characterization of the cell of origin were largely determined through molecular cytogenetic, immunogenetic, and genomics methods. Clonality of DFTD was initially established by karyotypic data, which showed that tumors from different individuals contain the same complex chromosomal rearrangements (Pearse and Swift 2006; Deakin et al. 2012). Microsatellite and MHC analysis indicating a lack of diversity across tumors, consistent with clonal transmissibility (Siddle et al. 2007), and further genomic sequencing and genotyping of somatic mutations revealed details of the pattern of spread and mutational process in the DFTD tumor cell population (Murchison et al. 2012). Tumors were found to express diagnostic neuron-specific markers indicating that the ancestral cell type of DFTD was Schwann cell origin (Murchison et al. 2010; Loh et al. 2016). Antibody staining indicated that tumor cells produce a Schwann cell-specific protein, periaxin (Murchison et al. 2010), which is now considered a sensitive and specific diagnostic for DFTD tumors (Tovar et al. 2011).

A few hypotheses have been offered regarding host evasion leading to the rapid spread and near-universal susceptibility to DFTD. First, irregular tumor MHC expression and downregulation of host MHC by DFTD may help the tumor to escape host surveillance (Siddle et al. 2013). During the initial neoplastic transformation, epigenetic downregulation of multiple aspects within the antigen-presenting system occurs (Siddle et al. 2013). This leads to the inability of DFTD to display functional MHC class I molecules, in vivo or in vitro, thereby avoiding recognition by T cells. Additionally, devils may lack enough MHC diversity to recognize and destroy aberrant tumor cells (Siddle et al. 2007). Siddle et al. (2007) did not detect lymphocyte response when lymphocytes from devils were tested against each other as well as lymphocytes isolated from other parts of the island. However, MHC diversity is not linked to variation in disease susceptibility among individuals, and devils can reject tissue allografts (Kreiss et al. 2011; Lane et al. 2012). More recent work has implicated the ERBB-STAT3 signaling pathway in MHC expression and tumor transmissibility (Kosack et al. 2019). Rather than alternative explanations, it may be that all of these factors – reduced species-wide diversity in MHC, downregulation of MHC expression in tumor cells, tumor suppression of the host immune response, and alteration of other genetic pathways in tumor cells – act in combination to facilitate DFTD transmission.

Remarkably, a second transmissible cancer has arisen recently in Tasmanian devils, called DFT2, with multiple lines of evidence supporting an independent origin from the first DFTD (Pye et al. 2016). DFT2 appeared in a geographically distinct area (southern Tasmania, as opposed to northeast Tasmania for DFTD), and cytogenetic evidence suggests that DFT2 originated in a male devil, in contrast to a female devil for DFTD. While similar in cell type origin, mode of transmission, and gross appearance, these two transmissible cancers differ in histology, in the specific mutations characteristic to each, and in the way in which changes in MHC expression facilitate evasion of the host immune system (Pye et al. 2016; Caldwell et al. 2018; Stammnitz et al. 2018; James et al. 2019; Patchett et al. 2020). Nonetheless, DFTD and DFT2 appear to share some broad-scale chromosomal rearrangements that may point to genetic changes that play a role in transmissibility in both cancers

(Deakin et al. 2012; Taylor et al. 2017; Storfer et al. 2018). Both diseases spread in an epidemic fashion across the devil population, although DFT2 exhibits sex bias with males appearing to be more susceptible, perhaps due to rejection of tumor cells by females based on Y-chromosome-associated factors (James et al. 2019). The independent origin of two transmissible cancers in Tasmanian devils within just two decades raises the hypothesis that devils are uniquely susceptible to this type of disease, and similarities among them may point toward the specific mechanisms that allow transmissible cancers in this species (Stammnitz et al. 2018; Patchett et al. 2020). It may also be the case that transmissible cancers are more widespread across the animal kingdom than previously recognized.

4.2 Devil Genomics

Population genomics tools have been used in Tasmanian devils to understand responses to the DFTD epidemic, as well as inform multiple aspects of management and conservation priorities in natural populations and management of the captive insurance population. First, we have an emerging view of the demographic history of devils and their current levels of genetic diversity and phylogeographic relationships among populations. These factors will strongly influence the ability of devils to adapt to DFTD, as well as other threats such as environmental change and anthropogenic disturbances (Hendricks et al. 2017). Previous studies have revealed that devils have low genetic diversity, based on data from microsatellite loci (Jones et al. 2004; Brüniche-Olsen et al. 2014; Storfer et al. 2017), MHC loci (Siddle et al. 2010; Cheng et al. 2012), SNPs (Hendricks et al. 2017; Fraik et al. 2020), and whole-genome sequencing (Miller et al. 2011; Murchison et al. 2012; Patton et al. 2019). Low genetic diversity in Tasmanian devils is potentially the result of historical fluctuations in population size and extinction of the species on mainland Australia and its restriction to the island of Tasmania (Guiler 1978; Hawkins et al. 2006; Brüniche-Olsen et al. 2018; Patton et al. 2019). The quality of the reference genome has been improved, and re-sequencing of 12 individuals robustly supports demographic reconstructions of a historic bottleneck using multiple genomic analyses (Patton et al. 2019). Nonetheless, devils show consistent evidence of population structure, particularly a large genetic cluster covering the eastern half of the island, another cluster in the northwest, and a broad zone of admixture between them (Hendricks et al. 2017).

Despite the overall low genetic diversity of the species, several lines of evidence suggest a rapid evolutionary response to the strong selection imposed by DFTD (Hohenlohe et al. 2019). First, three independent populations were found to show a parallel, rapid (4–6 generations) evolutionary response to the disease (Fig. 3; Epstein et al. 2016). This study scanned across 90K SNP loci, generated by high-density RAD sequencing, for signatures of selection and found two genomic regions, which contained genes with immunological and oncogenic functions. Second, using the data from Epstein et al. (2016), another study used a maximum likelihood approach

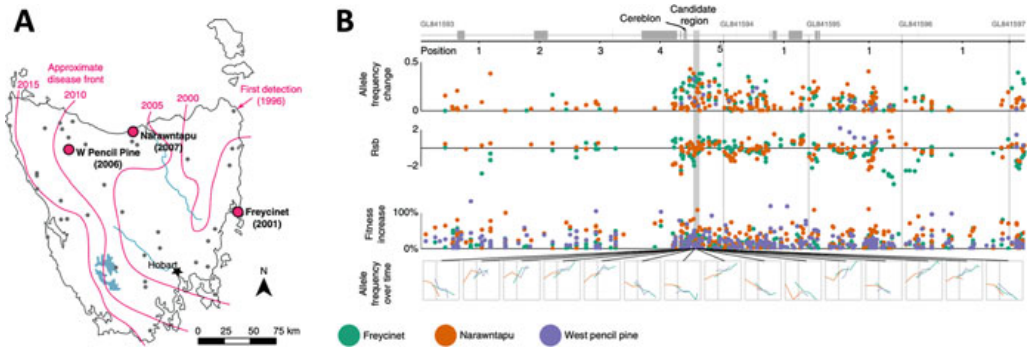

Fig. 3 Genomic evidence of rapid evolution in response to transmissible cancer in Tasmanian devils. (**a**) Map of Tasmania showing spread of devil facial tumor disease (DFTD) across the species range, with three focal populations for which genomic samples were collected before and after the disease appeared. (**b**) A region of chromosome II showing multiple concordant signatures of selection based on SNPs derived from RAD sequencing. The gray bar highlights the candidate selected region based on three signatures of selection: allele frequency change, a metric of linkage disequilibrium, and estimated fitness effect of the increasing allele. SNP loci are colored by population, and allele frequency changes over time at individual SNPs are shown across the bottom; note the concordance in the direction of allele frequency change across the three populations. Reproduced from Epstein et al. (2016)

and improved functional annotations to find more signatures of selection in the devil genome (Hubert et al. 2018). In total, 97 genomic regions showed evidence of selection, most of which were population-specific with one region common to all three populations. These regions harbored 148 protein-coding genes (or human orthologues), nearly all of which have a link with cancer. Third, a genome-wide association study (GWAS) of ~600 individuals found that phenotypic variation in female survivorship (length of time after infection) could be explained by a few loci of large effect (~5 SNPs explained about >61% of the total variance; Margres et al. 2018a). Further, Margres et al. (2018a) found that female infection rates (female case-control) could be explained by more SNPs of smaller effect (~56 SNPs explained about >23% of the total variance). Given that DFTD has spread across multiple genetic clusters in the devil population, any allelic variation for resistance to DFTD may be able to spread across the devil population and increase in frequency because of selection (Hendricks et al. 2017).

Given early predictions of extinction of devils in the wild, a captive insurance metapopulation distributed across a number of locations was established in 2006 with the goal of maintaining a disease-free population that is "genetically representative of the species" (CBSG/DPIPWE/ARAZPA 2009). The insurance population has been managed using a combination of molecular and pedigree information geared to maximize genetic diversity across the genome (Hogg et al. 2015; Grueber et al. 2018). A panel of microsatellite markers (Wright et al. 2015) has been used to monitor genetic diversity in the insurance population, and genomic information can be more informative than pedigree relationships for assessing diversity and inbreeding (Kardos et al. 2015; Hogg et al. 2018; Brandies et al. 2019). Disease-free wild populations have been established on Maria Island and Forestier Peninsula, but long-

term genetic conservation using these isolated populations would require continued supplementation (McLennan et al. 2018). While genomics tools have been used, none of the captive or reintroduced populations are currently managed for variation at any specific cancer-related loci. As our understanding of the genetic basis of DFTD susceptibility continues to improve, it would be possible for management of the insurance populations to consider maintaining overall diversity at the growing list of genes associated with DFTD (Hohenlohe et al. 2019).

4.3 Tumor Genomics

A number of different karyotypic strains of DFTD have been discovered (Pearse et al. 2012). These strains resemble the original DFTD karyotype reported by Pearse and Swift (2006), designated strain 1, but are characterized by additional cytogenetic rearrangements consistent with ongoing tumor evolution as the disease continues to spread through the population (Deakin et al. 2012). It appears from both cytogenetic and sequencing analysis that DFTD strains are continuing to accumulate karyotypic, copy number, and sequence variants, but compared with most human cancers, DFTD strains are remarkably stable (Deakin et al. 2012; Murchison et al. 2012; Taylor et al. 2017). Selection may be working to maintain the tumorigenic properties of the DFTD genome while permitting genomic instability and sequence substitutions in regions not critical for the survival of the DFTD cell (Deakin et al. 2012; Taylor et al. 2017). The number of somatic point mutations varies widely in humans, yet the mutation rate in DFTD is likely to be less than some human cancers, such as lung or skin cancer (Martincorena and Campbell 2015). As compared to the reference devil genome, two sequenced DFTD genomes identified approximately 17,000 somatic mutations that are present in the tumor (Murchison et al. 2012).

A candidate gene approach has identified rearrangements in tumor genomes at several genes known to be associated with cancer in other species, providing a possible list of driver mutations for transmissibility of DFTD (Taylor et al. 2017). While evolution in the tumor cell population of DFT2 has had much less time to proceed, genomic comparisons of DFT2 and DFTD are already revealing similarities between the two, for instance, in frequencies of single-nucleotide mutations (Stammnitz et al. 2018). DFT2 still appears to express MHC class I molecules, demonstrating that complete suppression is not necessary for transmissibility, but the most highly expressed molecules are either common or non-polymorphic among hosts, and MHC expression in DFT2 appears to be evolving (Caldwell et al. 2018). In both DFTD and DFT2, distinguishing somatic mutations from those found in the original or transient host is important for understanding what drives tumor growth and how the tumor evades immune detection by accumulating mutations in pathways related to recognition of self versus non-self. Genomics approaches, such as those used in canids (Decker et al. 2015; Baez-Ortega et al. 2019), would involve including large catalogs of variation found in modern devils, which are critical for identifying these somatic mutations.

Genomics is beginning to reveal mechanisms leading to spontaneous tumor regression or even complete recovery from the disease in some devils (fewer than 20), and it appears that features of both the host and tumor genomes may play a role (Pye et al. 2016; Wright et al. 2017; Margres et al. 2018b). Using a comparative case-control approach, two key genomic regions in the tumor were identified to putatively be associated with tumor regression and, therefore, the ability of the host to survive DFTD (Wright et al. 2017). Using targeted genotyping in additional samples, the authors were able to confirm that three genes may be involved in slowing tumor growth and allowing additional time for the effected individual to mount an immune response (Wright et al. 2017). Another study compared the genomes of devils that showed evidence of tumor regression to those that succumbed to DFTD and found a different set of three highly differentiated regions containing several genes with immunological or oncogenetic functions (Margres et al. 2018b). Putative regulatory variation in candidate genes suggests that changes in gene expression may drive natural tumor regression. Despite the small number of animals that have recovered from the disease, strong selection pressure from the disease may cause the frequency of these variants to increase over time in the devil population.

4.4 Conservation of Tasmanian Devils

One application of population genomics tools for devil conservation in the face of DFTD is the development of vaccines or other intervention techniques to promote population-level resistance (Owen and Siddle 2019; Patchett and Woods 2019). Several studies have explored immune-stimulatory agents and vaccines against DFTD (Tovar et al. 2017, 2018; Patchett et al. 2017; Pye et al. 2018). For example, heat shock proteins (HSPs) derived from tumor cells have been used as a source of antigens for cancer immunotherapy in humans (see review by Murshid et al. 2008). A recent study by (Tovar et al. 2018) found that DFTD cancer cells express inducible HSP, which supports that a HSP-based vaccine against DFTD could be developed. A promising target could be the ERBB-STAT3 pathway, with therapies that could potentially recover MHC expression and arrest tumor growth (Kosack et al. 2019; Patchett and Woods 2019). Despite this progress, work remains to be done to show that an immune stimulation or vaccine protocol could confer sufficient immunity or resistance to treated individuals for a sufficient length of time to be effective in natural populations and to confirm that it would not have unintended consequences for DFTD epizootic behavior.

A population genomics understanding of variation in devil susceptibility to DFTD is also important for conservation and management of both captive and wild populations. As described above, multiple studies have established that devils have genetic variation for disease-related traits, even including tumor regression, and that populations are responding to selection by DFTD. Demographic modeling also predicts devil persistence under most scenarios, allowing time for an evolutionary response in nature (Wells et al. 2017, 2019). In contrast, captive populations have

not been exposed to the disease and are not managed for any disease-related variation. As a result, supplementing wild populations with devils from captive populations that have not been exposed to the disease could increase the severity of the disease by increasing transmission rates and population-level susceptibility (Hohenlohe et al. 2019). In other words, attempts at demographic rescue – increasing population size with supplementation in areas where the disease has greatly reduced devil density – could be counterproductive by impeding evolutionary rescue, the ability of populations to evolve higher fitness in the face of the disease. Additionally, the discovery of DFT2 favors the view that conservation strategies for devils consider not just genetic variation relevant to DFTD but also genetic variation relevant to immune function and cancer in general that could provide adaptive potential for the future (Hohenlohe et al. 2019). Genetic monitoring of both captive and wild populations should target allelic variation at both DFTD-specific and broader functional categories of loci associated with both transmissible cancers. The devil-DFTD system illustrates how population genomics tools can allow detection of adaptive and functionally significant loci associated with threats to species persistence, and this knowledge can guide conservation efforts.

5 Future Directions in Population Genomics of Wildlife Cancer

Many wildlife species are the focus of conservation efforts because of historic population declines, fragmentation and loss of genetic diversity, and social and economic importance. Population genomics tools have wide applications to management of natural and ex situ wildlife populations (Walters and Schwartz 2020), and cancer may be an important challenge that some wildlife species face and that can be incorporated into population genomics-based conservation strategies (Box 2; Hamede et al. 2020).

Box 2 Management and Conservation of Wildlife Using Population Genomic Data

Cancer may be one of many factors creating concern for conservation of wildlife populations, and genomics can provide powerful tools for assessing its impact. High-throughput genomic technologies have increased our ability to assess inbreeding coefficients, gene flow, demography including effective population size, epidemiology, adaptive potential, and population viability (Kardos et al. 2016; Flanagan et al. 2018; Hoelzel et al. 2019; Hohenlohe et al. 2020; Storfer et al. 2020). These sources of information have been used to guide wildlife management efforts in natural and captive populations (Walters and Schwartz 2020). When populations are small, both inbreeding

(continued)

Box 2 (continued)
and genetic drift can increase homozygosity at loci with deleterious alleles, reducing fitness (i.e., increasing "genetic load") and contributing significantly to extinction risk (Frankham 2005; Díez-del-Molino et al. 2018). Hereditary cancer susceptibility due to the accumulation of oncogenic mutations could be a source of genetic load in wildlife populations.

If a population suffers from genetic load or inbreeding, genetic rescue through mediated migration, translocation, and reintroduction via captive breeding programs can increase population fitness due to an increase in heterozygosity, which can mask deleterious mutations, and facilitate adaptive evolution (Bell et al. 2019). Population genomics tools can be used to inform genetic rescue, for instance, by identifying source populations or assessing the risk of outbreeding depression (Fitzpatrick and Funk 2019). Alternatively, evolutionary rescue, evolution from standing genetic variation without migration (Hufbauer et al. 2015), may be possible particularly when there is evidence that a population has adaptive genetic variation. In wildlife populations, hereditary cancer may be caused by relatively rare, deleterious variants. If so, genetic rescue or evolutionary rescue may be highly effective in reducing cancer susceptibility, although there may be trade-offs between the two (Hohenlohe et al. 2019).

5.1 Monitoring and Population Management

With the expanding set of tools for designing and genotyping panels of genetic variants in wildlife species, cancer-related marker panels could be informative for monitoring. Particularly in wildlife populations with high prevalence of a specific hereditary or environmental contaminant-caused cancer, or in the case of transmissible cancers, the disease may have a substantial impact on population fitness and viability. Genetic marker panels targeting the host genome could be used to predict population-level susceptibility and disease impacts, screen individuals or family groups with particularly high cancer susceptibility, or track evolution of genetic variation at loci associated with cancer incidence (Leroy et al. 2018). Genetics tools can also be used to detect the disease itself, for instance, in transmissible, pathogen-driven, or environmental cancers, where it can be important to detect cancer in individuals before obvious clinical signs (McAloose and Newton 2009; Kwon et al. 2018). For instance, assessing population-level cancer incidence would provide information on overall population health, consequences of reduced genetic diversity or inbreeding, and the effects of exposure to environmental contaminants or viral pathogens (Leroy et al. 2018; Pesavento et al. 2018). Finally, genetic marker panels designed for tumor samples could also be used to track tumor evolution.

All of these sources of information could inform the targeting of conservation efforts toward natural populations in which cancer may have strong effects on population persistence (Box 2). For example, small or isolated populations in

which hereditary cancer contributes to genetic load could be targets for genetic rescue (Bell et al. 2019). Conversely, evidence of evolution in natural populations in response to disease could argue against translocations for genetic rescue (Hohenlohe et al. 2019). Individual-level metrics for cancer susceptibility, as well as genetic diagnostic tools for preclinical cancer screening, could be applied in selecting individuals for reintroduction or translocation (Fitzpatrick and Funk 2019).

5.2 Captive Breeding Programs

Genomics can support the identification of candidate loci responsible for heritable disorders, which can inform breeding decisions in captive populations of wildlife species. Genome-wide association studies (GWAS) have found large numbers of variants associated with complex human traits and diseases such as cancer, leading to genetic panels for preventive and personalized medicine (Vazquez et al. 2012; Vogelstein et al. 2013). Despite the limits on statistical power from feasible sample sizes in wildlife studies, GWAS, along with a range of other population genomics tools, is increasingly being applied in wildlife species, including studies of cancer (Leathlobhair et al. 2017; Margres et al. 2018a; Baez-Ortega et al. 2019). In a captive wildlife population, genetic information on cancer-associated loci could be combined with pedigree information and used for strategic breeding. For example, a similar method was used in the case of the critically endangered California condor (*Gymnogyps californianus*), which suffered from high incidence of the lethal disease chondrodystrophy (Romanov et al. 2009; Grueber 2015). Through the pedigrees obtained in the captive breeding program, researchers found this disease to show Mendelian segregation (Ralls et al. 2000). Genomic resources were developed to identify causal polymorphisms linked to the disease, with the aim of informing the captive breeding protocols to reduce the frequency of chondrodystrophy while maintaining genetic diversity at other loci (Romanov et al. 2009; Walters et al. 2010). Overall, this approach would help to safeguard against inbreeding depression to avoid further decreases in individual fitness (Frankham 2010). Finding a single locus of major effect on inbreeding depression may be unusual, and cancer susceptibility may be more often highly polygenic. However, the ability of genomics tools to screen many thousands of loci and lead to genotyping marker panels of hundreds to thousands of markers opens the door to managing captive populations with genetic metrics targeting multi-locus traits.

5.3 Interventions in Wildlife Cancer

As our understanding of cancer treatment options in humans improves, these advances may be translated to wildlife in the context of direct interventions at the individual level. For example, the recognition of tumor cell populations as

heterogeneous, evolving systems can be used in designing treatment strategies (Gatenby et al. 2009; Hanahan and Weinberg 2011), and this is particularly relevant in the case of transmissible cancers (Caldwell et al. 2018; Stammnitz et al. 2018). In natural wildlife populations, ongoing invasive treatment of individuals is typically not possible. However, advancements in oral vaccine development and delivery for infectious diseases have led to successful infectious disease control as seen in the case of sylvatic plague affecting prairie dogs (*Cynomys* spp.) and the endangered black-footed ferret (*Mustela nigripes*; Salkeld 2017). These approaches to reduce infectious pathogens could reduce cancer impacts, for instance, in the case of virus-associated cancers or the ear mites and island foxes discussed above. It may also be possible to develop vaccines against transmissible cancers that could suppress epidemic spread (Owen and Siddle 2019; Patchett and Woods 2019).

5.4 Advances in Wildlife Cancer Genomics Research

As the taxonomic scope of genomic data continues to increase, comparative genomics approaches will continue to increase our understanding of the genetic basis of cancer susceptibility and mechanisms of cancer suppression in wildlife (Caulin and Maley 2011; Tollis et al. 2017). In addition to the mammal studies discussed above, the long lives, slow developmental rates, probable low cancer rates, and the rapid development of genomic resources for large reptiles will provide ample opportunity to study genomic mechanisms of cancer suppression in these ectothermic amniotes (Tollis et al. 2015). Birds may also have relatively low incidence of cancer in most species (Madsen et al. 2017), which suggests that the numerous avian genomes available (Zhang et al. 2014) could provide more comparative genomics information about cancer suppression.

Many general types of cancer are shared across species (Schiffman and Breen 2015; Madsen et al. 2017; Pesavento et al. 2018), as evidenced by the widespread use of non-human mammal species used as models for human cancer. Investigation of shared cancers using a multi-species approach will highlight genes associated with carcinogenesis in the context of both genetics and environmental exposure. Important insights can also be gained from studying lineages that have a high prevalence for cancer, such as the marine mammals, Santa Catalina Island foxes, and Tasmanian devils discussed above. The wide diversity of causes associated with cancer in these taxa means that conclusions may not be applicable across systems, but wildlife can provide a broad view of multiple types of cancer susceptibility.

Technical advances in sequencing and bioinformatics will benefit the study of cancer in wildlife. For instance, the high levels of diversity and gene duplication (Nei et al. 1997; Temperley et al. 2008) that make immunity highly adaptable also make immune-gene regions challenging to assemble. Therefore, it is difficult to determine how many copy number variants of genes exist in a species or individual genome (Cheng et al. 2012; Alcaide et al. 2014). However, technological advances, such as Oxford NanoPore and Pacific Biosciences (PacBio) sequencing, continue to increase

the length of single DNA molecules that can be directly sequenced. Additionally, continued development and assessment of computational approaches (e.g., Putnam et al. 2016) may aid in resolving the challenges presented by gene duplications and repetitive regions. Long sequence reads can also help identify runs of homozygosity, a measure of inbreeding and critical for identifying deleterious loci in small populations (Hohenlohe et al. 2020). These advances will help identify candidate loci associated with disease susceptibility and inbreeding depression in wildlife populations.

6 Conclusions

Cancer affects nearly all multicellular organisms, yet our understanding of the role of cancer in wildlife populations remains limited. In reduced or fragmented wildlife populations with reduced genetic diversity or inbreeding, cancer may contribute to genetic load and reduced population fitness (McAloose and Newton 2009; Pesavento et al. 2018). Additionally, this may have impacts on interacting species and ecosystem function (Vittecoq et al. 2013; Ujvari et al. 2019). Population genomics approaches can inform multiple aspects of wildlife cancer. As genomic data continues to accumulate across taxa, our understanding of how evolutionary forces have shaped cancer suppression mechanisms will improve, providing new models for biomedical cancer research and a clearer view of the genetic susceptibility of wildlife populations to cancer (Seluanov et al. 2018). Intensive genomic studies of wildlife populations can reveal the specific genetic mechanisms of cancer susceptibility. The ability to rapidly identify putative functional loci and design marker genotyping panels opens the door to high-throughput genetic monitoring and management tools for wildlife populations (Leroy et al. 2018). As population genomics tools continue to develop across wildlife applications, as detailed throughout the chapters of this volume, cancer and other diseases will be an important component of wildlife genomics.

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