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BIOLOGICAL SCIENCES

Catching Cancer

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ABSTRACT

Tasmanian devils, the largest marsupial carnivores, have lived in relative isolation on the island of Tasmania. Consequently, there is limited genetic diversity within the devil population, reducing the population's overall fitness and making them more susceptible to the spread of infectious disease. In the past 30 years one such disease, a contagious cancer, has emerged posing an existential threat to the species. The cancer, devil tumor facial disease, is of non-viral origin and is spread by biting which has enabled it to disseminate throughout the devil population, in-and-between different geographic loci. Under this intense selective pressure an evolutionary arms race emerged between the contagious disease and the genetics of the devil host. Aided by the efforts of conscientious scientists there is now hope for the future of the Tasmanian devil population. Furthermore, the Tasmanian devil facial tumor has served as a case study in the value of interdisciplinary science, bringing together ecologists, immunologists, cell biologists, epidemiologists, and cancer biologist, all with the combined goal of saving the Tasmanian devil species.

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REVIEW

Tasmanian devils (*Sarcophilus harrisii*) are the largest marsupial carnivores [1]. Following the last ice age, the Tasmanian devil population was split between Australia and Tasmania, until 400 years ago when the Australian devils went extinct [3, 4]. In recent times, Tasmanian devils have experienced additional population declines, and as a consequence have experienced a decrease in genetic diversity [5, 6]. The advent of genome sequencing has found evidence of this, with lower diversity in devil genes across the devil genome (e.g., at microsatellite markers), but only in the past few years has it become apparent how this compromise has affected Tasmanian devil immunity and health [7-9].

Currently, Tasmania's apex predator is facing the threat of extinction after the emergence of a contagious cancer, devil facial tumor disease (DFTD) [10, 11]. DFTD was first observed in 1996, in northeastern Tasmania, and has since spread through 85% of Tasmania's total devil population and reduced eastern devil populations by as much as 95% [10, 12] (**Figure 1**). DFTDs cover the face, neck, and often the inside of infected devil's mouths [10, 13]. Once a devil becomes in infected their tumor becomes ulcerative and friable, and frequently metastasizes and proving lethal within six months [13-15]. DFTD affects male and female devils equally, however it remains unclear how the contagious cancer is spread [16, 17].

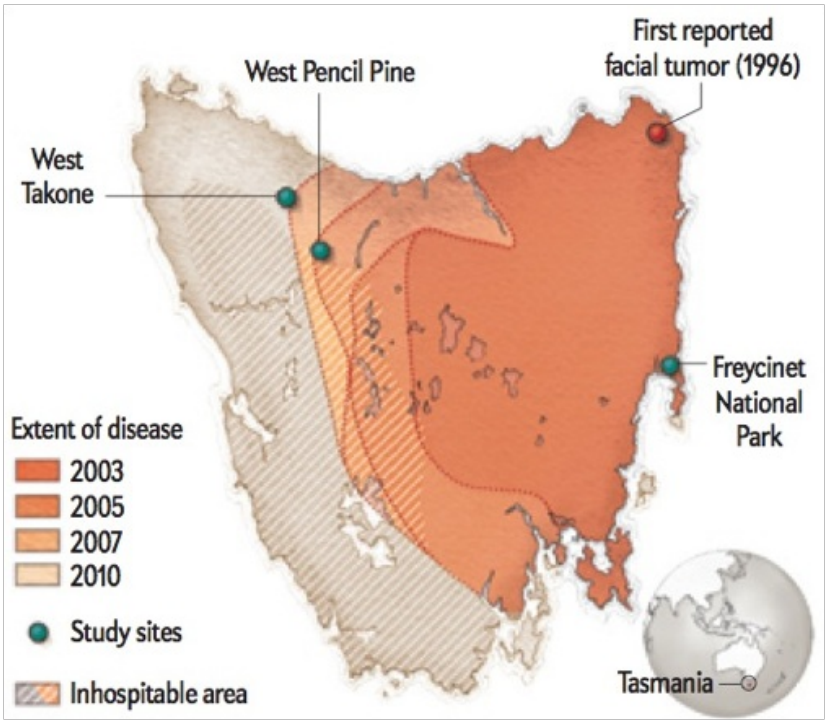

Figure 1. Extent of DFTD outbreak (in orange) since 1996, with healthy Tasmanian devils found in the western Tasmanian areas (grey-checkered)

Credit: Jones, M.E., MC, H. *Scientific American, 2011.*

The pathogenesis of DFTD has many similarities to human cancers[18]. Due to the focal location of the facial tumor, social fighting behavior, and spread of the disease researchers initially suspected DFTD was caused by an infectious agent, similar to the Human papillomavirus (HPV), rather than spread as a contagion. Indeed, there have been several instances reported when DFTD tumors developed from bite lesions [10, 14, 19, 20]. And it has since been accepted that DFTD transmission often occurs as a result of fighting and/or biting during copulation [9, 19-21].

However, after comparing the karyotypes of tumors collected from 11 devils from geographically different areas, Pearse *et al.* reported finding identical karyotypes in all 11 samples, despite a high degree of genomic rearrangement [19]. Tasmanian devils belong to the family Dasyuridae, which are known for their highly conserved diploid karyotype (2n=14), with 6 pairs of autosomes and a pair of sex chromosomes (XX females, and XY males) [22]. Pearse *et al.* reported that all the tumor's had lost both copies of chromosomes 1, 6, and both sex chromosomes; and gained four novel marker chromosomes [19]. Interestingly, one devil had constitutional pericentric inversion of chromosome 5, that was absent from the tumor's karyotype [19].

The presence of identical highly conserved karyotypes with complex rearrangements that were dramatically different compared to their host's normal cells led Pearse *et al.* to propose that the tumors were of clonal origin (**Figure 2**) [9, 15, 19]. Many human cancers appear clonal, sharing specific chromosome aberrations in tissue-specific patterns [23]. For instance, the reciprocal translocation between chromosome 9 and 22, designated t(9;22)(q34;q11), is a clonal aberration found in chronic myelogenous leukemia that forms the cancer's background amidst the emergence of additional genomic rearrangements [24]. Despite the similarity, the overall karyotypes and modal chromosome numbers seen in human cancers varies widely and are not identical between patients [25, 26]. DFTD's, however, share identical aberrations between devils living vast distances apart and are reportedly formed within 6-months of coming into direct physical contact with a diseased devil [11]. Collectively

leading Pearse *et al.* to theorize that the clonal tumor cells were transmitted from donor-to-host by an "allograft" (*intraspecies transfer of tissue that is genetically different between host and recipient*) mechanism [19].

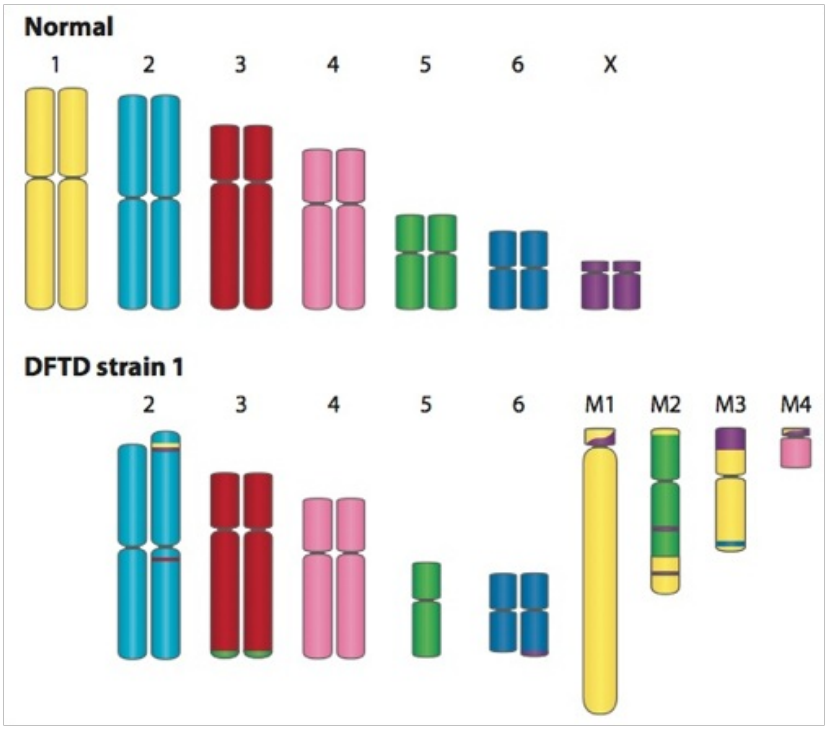

Figure 2. Comparison of normal (top) and devil facial tumor disease (bottom) tumor chromosomes. Tumor chromosomes are color-coded to reflect their homology to normal chromosomes. This figure had been adapted from Deakin *et al.* [2]

The specific gene region effected is known as the Major Histocompatibility Complex (MHC), and plays a critical role in recognizing self vs. non-self. MHC functions analogously to the specific proteins which coat human blood cells and identify that person as belonging to a certain blood group (A, B, AB, or O), meaning they can accept transfusions of blood from person's with the same blood proteins but will reject blood containing cells coated in "foreign" (non-self) protein. The MHC is also responsible for recognizing antigenic peptides and presenting them to cytotoxic (TC) immune cells, targeting the foreign product for destruction.

In the 1920s Snell *et al.* explored histocompatibility by transplanting tumors between inbred mice and their offspring. Snell *et al.* found that tumors could be transplanted successfully within the inbred and F1 hybrid mice strains, but not other strains suggesting selection for MHC-compatible tissue [27]. MHC-incompatible tumor cells transplanted from a parental strain onto an F1 hybrid showed the recipient's immune system responded against tumor cells that had not lost their MHC molecules [27]. Interestingly, Tasmanian devils maintain effective immune system and yet do not display an immune response to DFTD [28, 29]. However, nude (immunodeficient) mice injected subcutaneously with DFTD cells rapidly developed tumors, indicating a lack of immunoresponsiveness enabled tumor progression [30].

Indeed, the theory was not without additional support from cancers seen in other species. Although rare, in humans, the risk of malignancy following organ transplantation is well recognized [31-33]. However, the critical factor enabling this mechanism results from a medically induced immunosuppression that prevents the recipient from rejecting the transplanted organ. The ability to

functionally identify and target foreign cells for destruction is known as immunosurveillance, and it typically prevents organ transplantation between most genetically dissimilar individuals; however, this is not true for a type of canine cancer.

Canine transmissible venereal tumor (CTVT) is the oldest malignant cell line, estimated to have evolved 10,000 years ago [34, 35]. Like DFTD, CTVT is clonal in origin and spread by direct contact, but in stark contrast to DFTD, CTVT is not lethal [35, 36]. CTVT rapidly progresses and evades initial detection by the host's immune system through MHC down-regulation, which may favor immune inactivation [35-39]. Based off these observations, a similar mechanism has been suggested for DFTD. In the presence of a competent immune system, low levels of genetic diversity (devils share genetically similar MHC antigens with the tumor) the recipient devil's immune system fails to see foreign cells as "non-self", thereby affectively 'accepting' DFTD as an allograft.

Support for this came when Siddle *et al*. compared genomic makeups of MHC alleles from different DTFD samples and found they were identical [8, 9, 15]. Further evidence came from sequencing of over 100 DFTD genomes, that found they were identical, or derived from a single devil haplotype [40].

Despite no sex chromosomes being in any sample being analyzed, duplicates of 11/14 X-chromosome genes were found scattered amongst the marker chromosomes, suggesting positive selection for these X-chromosome genes [40, 41]. Fluorescence hybridization confirmed these findings, and in addition found traces of male genes, suggesting the ancestral donor was female. This was supported by both the absence of Y chromosome hybridization and the lack of SRY sequence in DFTD tumors [40, 41].

Interestingly, despite being passed to new individuals for nearly twenty-years, genomic sequencing and staining with immunofluorescence demonstrate that DFTD's karyotype is remarkably stable [19, 42]. Additionally, examination by eye reveals poorly differentiated tissue, and immunohistological staining is positive for periaxin (a Schwann cell-specific myelin protein), suggesting DFTD is of neuroendocrine origin [13, 15]. Moreover, comparisons between DFTD's transcriptome with that of unaffected tissue, showed heightened expression of myelination pathway associated genes [15, 40, 43]. Thus, these observations collectively suggest that DFTD is of clonal origin, comprised of Schwann cells, and transmitted via an autograft mechanism [13, 15, 44].

In the twenty years since DFTD's discovery cancer research has experienced a boom in technological growth that has enabled wildlife conservationists, ecologists, and geneticists to decipher this deadly cancer's etiology and design strategies to ensure the survival of this unique species. DFTD is intriguing to researchers because it offers a unique opportunity to study the evolutionary process underlying cancer's progression in a system that appears to behave like human cancer. Understanding how DFTD evolved the ability to avoid immune detection, and more importantly determining how DFTD selected for its stable complex karyotype will have huge implications for the entire field of cancer research.

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