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# Telomere-driven karyotypic and molecular convergence mimics the transmissibility of cancer in the Tasmanian devil

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The currently prevailing theory of a transmissible cancer cell lineage in Tasmanian devils was based on the discovery of apparently identical chromosomal aberrations in facial tumors of several animals. New findings of facial tumors that have no detectable cytogenetic similarities to previously published cancer karyotypes and the recent detection of varying portions of chromosome Y in all tumor cell lines of male devils (but none in tumors of females) cast doubt on the theory of a cancer transplant. Thus, I propose an alternative scenario in which similar chromosomal and genetic aberrations in individual cancers are a consequence of the low genetic diversity in populations of the Tasmanian devil resulting in a unique telomere length profile. Critically short telomeres on certain chromosome ends lead to chromosome-specific fusions and the activation of species-specific transposable elements that cause the observed karyotypic and molecular convergence. This new concept can explain the existence of genetic signs of tumor clonality within a population despite the independent origin of each facial cancer in these cancer-prone animals.

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**DATE RECEIVED:**
August 21, 2016

**DOI:**
10.15200/winn.147180.05742

**ARCHIVED:**
August 21, 2016

**KEYWORDS:**
telomere, transmissible cancer, Tasmanian devil, devil facial tumor disease, molecular convergence

**CITATION:**
Reinhard Stindl, Telomere-driven karyotypic and molecular convergence mimics the transmissibility of cancer in the Tasmanian devil, *The Winnower* 3:e147180.05742 , 2016 , DOI: 10.15200/winn.147180.05742

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## INTRODUCTION

The current mainstream theory of a transmissible cancer cell lineage in Tasmanian devils was originally based on the discovery of similarly rearranged chromosomes in facial cancers of unrelated individuals (devil facial tumor type 1, DFT1) (Pearse and Swift 2006). Several karyotypic variants of this unique transmissible cancer strain have been described in the literature since the original report (Pearse et al. 2012), mostly based on the analysis of Giemsa (G)-banded chromosomes. Nevertheless, recent evidence published by Pye and colleagues of a karyotypically distinct cancer cell line (DFT2) in a devil population in southeast Tasmania (Pye et al. 2016) make the scenario of a transmissible cancer lineage very unlikely. According to this study, the facial cancers of five male individuals bore no detectable cytogenetic similarities to DFT1, yet in all five tumors similarly rearranged chromosomes were present. Furthermore, DFT2 cells harbored a Y chromosome, which has been reportedly missing in DFT1 samples. Among all the DFT2 cases, no evidence for shared DNA markers with DFT1 could be found, and the authors concluded that based on microsatellite analysis DFT2 tumors were no more similar to DFT1 than they were to their hosts, or to other devils in the population (Pye et al. 2016).

Accordingly, Pye and colleagues proposed that at least two transmissible cancer cell lines must be causing the widespread facial tumor disease in Tasmanian devils and suggested that transmissible cancers may arise more frequently in nature than previously considered. In other words, the authors suggested the independent origins of two highly contagious cancer cell lineages in wild solitary animals within two decades with identical routes of inter-individual cell transmission, i.e. bite-related transplantation of cancer tissue clumps, resulting in macroscopically identical facial tumors with a fatality rate of 100% (Pye et al. 2016). However, the disease patterns observed do not support the

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hypothesis of bite-related disease transmission, since no seasonal changes in the prevalence of devil facial tumors could be found despite the fact that most biting injuries occur in autumn, during the reproduction period of these solitary animals (McCallum et al. 2009). Furthermore, cell transmission through bites is rather unlikely, since animals with a large number of bites have been shown to have a comparably lower disease risk (Hamede et al. 2013). Despite the "highly infectious" nature of these tumors, the proportion of diseased animals does not correlate with population densities (McCallum et al. 2009). Even in areas where devil populations were depleted by 90%, over a 12-year period, more than half of adult animals were still affected (McCallum et al. 2009). Similarly, a large culling project of diseased devils did not have any impact on the disease progress in one population (Lachish et al. 2010). Why young devils never develop facial tumors and cancers are not passed from mother to offspring, has remained an enigma (Belov 2012). In contrast to highly infectious agents like viruses and bacteria, devil tumor cells cannot survive outside of the body for more than a few minutes ((Hamede et al. 2013), p.185). Contrary to popular belief, Tasmanian devils do not tolerate allogenic skin grafts. Transplantations of normal skin between devils resulted in all allografts being infiltrated with T cells and a complete rejection within a few weeks (Kreiss et al. 2011). This contrasts with what is seen in devil facial tumors, which show no signs of a host immune response (Belov 2012). One study published the DNA sequence of a complete devil genome as well as that of its tumor, and the authors estimated that about 30% of the tumor's nuclear DNA originated from the diseased animal (Miller et al. 2011), which is unusual for a tumor transplant, since mammalian red blood cells do not contain DNA and white blood cells represent only a minor fraction of blood cells. During completion of this paper, a highly interesting sequencing study was published (Cui et al. 2016). The authors searched for the devil sex-determining region Y (SRY) in 12 devil facial tumor cell lines. In all six tumors isolated from male devils they found SRY, in some cell lines even different portions of the gene, in contrast to the tumors isolated from females, where they could not detect any SRY (Cui et al. 2016). This is another major blow to the currently believed DFT allograft transmission theory. Based on the accumulation of conflicting results, the following basic questions must be asked: How can cancer cells spread between such solitary animals (Hamede et al. 2009) if they reproduce only once a year during autumn (the biting season) and if all diseased animals reportedly die within 6 months (McCallum et al. 2009)? Even if one postulates a latency period of 6 months, how can the cancer cell lineage survive in the majority of wild-living solitary adults until the next breeding/biting season if an apparent tumor in the oral region is the requirement for cancer cell transmission and if the “infectious window” is limited to only a few months (McCallum et al. 2009)? This leads us to further questions: Is the transmissible cancer story a myth? Can the remarkably slow spread of DFT disease from east to west – less than 200 miles in two decades, despite devils reportedly traveling up to 30 miles per night (Department of the Environment Australian Government 2016) – be explained by any other disease-causing agent? Have all cancers in affected Tasmanian devils developed independently? Is there any possible scenario that might result in similar chromosomal and genetic aberrations in individual cancers within a population?

## DEVELOPING AN ALTERNATIVE THEORETICAL SCENARIO

In a Danish human study (consisting mainly of twins), a unique telomere length profile was found. That is, certain chromosome ends tended to have the shortest telomeres in all individuals. The shortest telomeres were found on the short arms of chromosomes 13, 21 and 15 (acrocentrics), followed by two metacentric chromosomes 20 and 19 and the acrocentrics 22 and 14 (Graakjaer et al. 2003). Such chromosomes with critical short telomeres are known to form telomere associations and fusions and subsequently to be involved in structural and numerical chromosomal aberrations in vitro (Der-Sarkissian et al. 2004) and in vivo (Martens et al. 1998). Short telomeres on the short arms of acrocentric chromosomes are associated with the occurrence of Robertsonian translocations in wild house mice (Sánchez-Guillén et al. 2015). In humans too, Robertsonian translocations are the most common chromosomal rearrangements (Bandyopadhyay et al. 2002), involving the acrocentric chromosomes with very short telomeres mentioned above. Furthermore, the loss of a sex chromosome (X or Y), is a frequent event in normal somatic cells of older humans and in cancer cells (Stone and Sandberg 1995).

By 1979, an unusually high rate of spontaneous neoplasia had already been reported for captive Tasmanian devils (Griner 1979), long before the discovery of devil facial tumor disease. Tasmanian devils have been shown to have exceptionally low levels of genetic diversity (Pye et al. 2016), and this might also be true for their chromosome-specific telomere length profile. In accordance with the evolutionary theory of telomere-driven aging of the germline (Stindl 2004), Tasmanian devils may be characterized by critically short telomeres and by an identical telomere length profile in most individuals in a local population, which eventually results in similar chromosomal rearrangements in their facial tumors (karyotypic convergence).

A surprisingly high number of reversal and convergence events seem to have occurred during chromosomal evolution in marsupials, and the authors concluded that chromosomal aberrations are a disappointingly imprecise indicator for phylogenetic relationships and further that all the diversity of karyotypes in the studied marsupials can be accounted for by the rearrangement of just 19 evolutionary blocks (Rens et al. 2003). It was further speculated that the frequent evolutionary reuse of chromosomal breakpoints might be a consequence of the low numbers of chromosomes in marsupials (Rens et al. 2003). In any case, the unique patterns of chromosomal evolution in this lineage lends further support to the theory of karyotypic convergence of independently arising tumors. In regard to the partial overlap of genetic markers between the tumors of a population, an evolutionary concept in which telomere-driven transposon activation can lead to molecular convergence has been introduced previously (Stindl 2014). Transposable elements, DNA transposons and retrotransposons, were originally thought to be ubiquitous controlling elements of gene action (McClintock 1956), and Barbara McClintock proposed that their activation by damaged telomeres could lead to a restructuring of the genome (McClintock 1984). The current knowledge on the dominant role of transposable elements in genotypic and phenotypic change during evolution is reviewed in (Warren et al. 2015). Even the independent originations of nearly identical pericentric inversions (with slightly different breakpoints) in the gorilla and chimpanzee chromosome 16 is thought to be a consequence of the activity of transposable elements (Goidts et al. 2005).

An unusual telomere length dimorphism in the somatic tissues of Tasmanian devils with one set of homologous chromosomes displaying very short telomeres has been reported by Bender and colleagues (Bender et al. 2012) and requires further investigation. Consequently, it is proposed that the transmissible cancer in Tasmanian devils is a myth and further that all those cases of facial tumor disease arose independently. If telomeres progressively erode in the germline of Tasmanian devils between generations (Stindl 2014), and if telomeres are already very short, chronic tissue damage and repetitive wound healing (e.g. due to biting) may be responsible for the epidemic of facial cancers in adult animals. An additional risk factor could be ultraviolet (UV) radiation exposure, a factor that has sharply increased in Australia since the 1990s (Lemus-Deschamps and Makin 2012). Sunshine in Tasmania is highly differentiated and has an east-west gradient, with annual quotients ranging from up to 7 hours a day in the northeast of the island, to around 5 hours daily in the west (Figure 1).

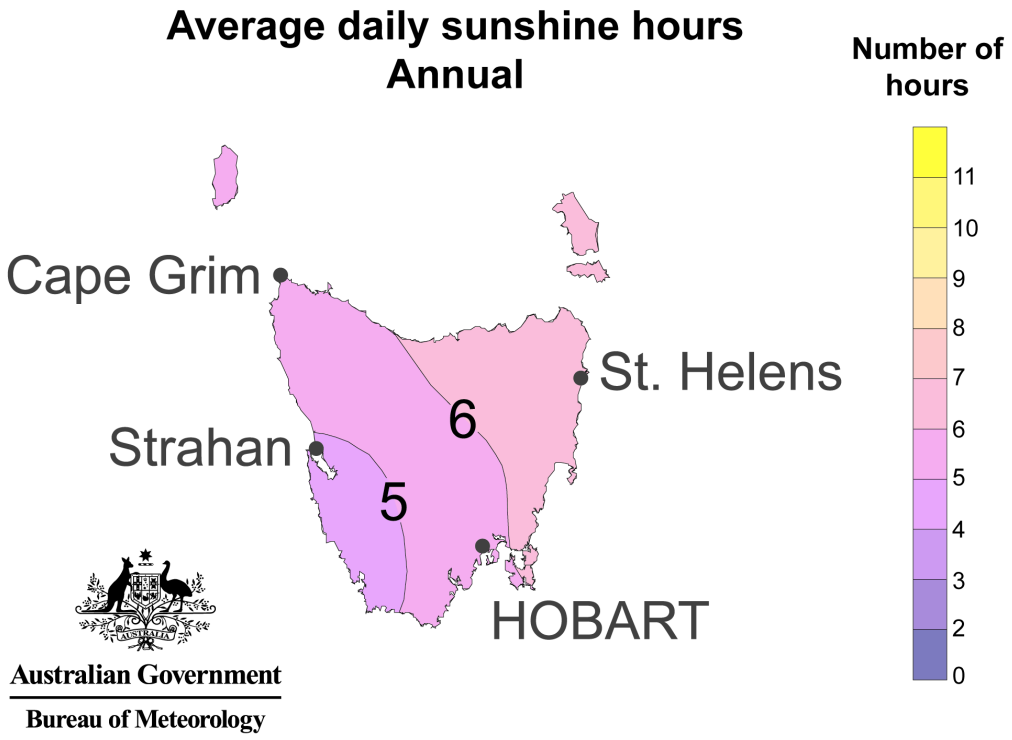


Figure 1: The east-west gradient of average daily sunshine hours in Tasmania based on at least 15 years of record (Bureau of Meteorology Australian Government 2016). Combined with the increase in UV radiation over the last two decades (Lemus-Deschamps and Makin 2012), it can explain the east-west spread of devil facial tumors since 1996.

The correlation with the observed east-west spread of facial cancer occurrences during the last two decades is intriguing (see Figure 1 in (Bender et al. 2014)). It has been known that, although Tasmanian devils are nocturnal, they like to rest in the sun (San Diego Zoo 2016). Tasmanian devils may be abnormally prone to UV radiation-induced skin cancers in some facial areas that are less protected by hair, i.e. the skin around the mouth and eyes as well as at bite scars. The reported Schwann cell origin of devil facial tumors (Murchison et al. 2010) was partly based on antibody stainings against some antigens (e.g. S100 protein), which can also be found in skin cancers and thus would support a possible UV radiation-related carcinogenesis. Similarly, what was first thought of as a disease-specific antibody against myelin (periaxin) turned out to not stain the newly discovered DFT2 tumors (Pye et al. 2016). In mice and humans, multipotent neural crest cells in the hair follicles are capable of differentiating into either Schwann cells or melanocytes (Bender et al. 2014). Similar stem cells in the skin of Tasmanian devils could be the target of malignant transformation and the reason for the varying histology of these tumors. Independent of the kind of skin damage – biting and/or UV radiation – the modus operandi is suggested to be as follows: Lifelong tissue regeneration depends on stem cell proliferation. Replicative telomere erosion in tissue-specific stem cells causes chromosomal instability and subsequent aneuploidy, which finally leads to malignant transformation of chronically damaged and aged tissues (Li et al. 2000). The proposed causal mechanism, which is telomere-driven aging of the germline (Stindl 2014), will result in disease anticipation in subsequent generations (Vulliamy et al. 2004) and probably the extinction of the Tasmanian devil.

## CANCER TRANSPLANT OR GENETIC CONVERGENCE – HOW TO FIND OUT?

When Pearse and Swift introduced the hypothesis of a transmissible cancer cell line in 2006, they described a diseased devil with a constitutional inversion of one copy of its chromosome 5 that could

not be found in its cancer cells, which supposedly harbored two normal homologous chromosomes (Pearse and Swift 2006). However, loss of heterozygosity is a ubiquitous phenomenon in human carcinomas that involves the loss of one homologous chromosome and the eventual duplication of the other (see Figure 3 in (Thiagalingam et al. 2001)), also known as uniparental disomy (Tuna et al. 2009).

Based on a literature review, the published cytogenetic studies on Tasmanian devil tumor chromosomes seem to lack the quality standards usually applied to human cytogenetics. Cytogenetic analysis of G-banded animal tumor chromosomes is not sufficient to show clonal origin, and the few published cases of fluorescence-in-situ-hybridization (FISH) seem to be of poor quality (Deakin et al. 2012). Therefore, it is suggested that a chromosome-arm specific 15-color-FISH probe set should be developed for the Tasmanian devil, which can be done in cooperation with MetaSystems (Altlussheim, Germany), a company that provides high-quality multicolor probe kits for several species. Equipped with the required hardware (Isis-mFISH-workstation, MetaSystems), this would enable cytogenetic labs to standardize and compare their findings from DFT disease across Tasmania. Based on the procedure outlined above, it is hypothesized that the disease will turn out to be less "clonal" than it currently appears. In addition, telomere length studies in the Tasmanian devil may provide answers to the following questions: Does the telomere length progressively change in the germline between devil generations, as has been suggested many years ago (Stindl 2004) and recently shown for humans (Holohan et al. 2015)? Which chromosome ends have the shortest telomeres in the white blood cells of Tasmanian devils? Is there a negative correlation between the telomere length of a chromosome arm and its later involvement in chromosomal aberrations in DFT disease? The telomere lengths of each homologous chromosome have to be measured separately. Lastly, the DNA of tumor samples and, if possible, the corresponding animals, must be sequenced and the locations of transposable elements mapped. Ideally, these investigations should be performed by research groups that have not been involved in the genesis of the transmissible cancer theory to prevent any biases in an already very challenging situation due to the low genetic diversity in the Tasmanian devil, where not even animal parentage can be properly resolved by microsatellite analysis ((Grueber et al. 2015), p.533) despite its widespread use in studies addressing the question of a single clonal origin of the tumors (Pearse et al. 2012).

As has been pointed out by two previous reviewers, a highly cited study, published in the prestigious journal Cell, seems to have shown beyond doubt that devil facial tumors are clonal and consequently that devil facial cancer cells are transmitted by biting (Murchison et al. 2012). Because much of the current scientific literature – perhaps half – may simply be untrue (Horton 2015), it may be worthwhile to investigate this paper further and test it against the new theory provided here. The performed analysis of mitochondrial DNA (mtDNA) of several tumors and animals in the Murchison et al study just confirms that devil facial tumors contain mtDNA variants, which are widespread in current populations of devils in Tasmania. Similarly, because of low genetic diversity in populations of Tasmanian devils, microsatellite analysis cannot even resolve animal parentage let alone clonality of tumors, as has been already discussed above. The cytogenetic and multicolor-FISH results are poorly documented in the Murchison et al paper: number of metaphases analyzed, number of cells with the representative karyotypes shown in figure 2, any information on the clonality of the chromosomal aberrations within a cell line or detailed descriptions of the chromosomal aberrations are not provided (Murchison et al. 2012). The main part of the paper focuses on sequencing results of nuclear DNA from two DFT cell lines and one supposedly normal fibroblast cell line. Yet, the immortal fibroblast cell line – used as a control – was aneuploid (one additional chromosome 6) (Murchison et al. 2012). Since the diploid chromosome number of Tasmanian devils is 14, it is surprising that the DFT cell line 87T with 13 chromosomes was described as pseudodiploid and the 53T cells with 32 chromosomes as pseudotetraploid in the main text ((Murchison et al. 2012), p.782). Based on the international cytogenetic nomenclature the correct terms are near-diploid and near-tetraploid, whereas "pseudo-" stands for the multiple of the haploid chromosome number with an abnormal chromosomal composition. In this context, it would be interesting to know what the modal chromosome number of

these cell lines was and if chromosome numbers varied between cells of a lineage. Both cell lines were established in 2007 and the initial cells were isolated from a primary tumor (87T) and a lung metastasis (53T), supposedly from different animals at different locations (Murchison et al. 2012). Yet, I asked the corresponding author and she did not know, who established the 53T and 87T cell lines and where. The only information she could provide was that they were established by researchers (unknown to her) working as part of the Save-the-Tasmanian-Devil program. Consequently, it is tempting to suggest that 53T is a near-tetraploid variant of 87T, which acquired a few additional chromosomal aberrations. Both clones either stem from the same individual (primary tumor and corresponding lung metastasis) or they are the result of a cell contamination during propagation of these cell lines in several labs over the years, which has been, and still is, a common source of error in numerous scientific studies worldwide (Nelson-Rees et al. 1981).

## CONCLUSIONS AND IMPLICATIONS

In humans, every second male and every third female develops cancer during their lifetime (Stindl 2008), but no naturally transmissible cancer cell line has been found, in more than 100 years of extensive medical research. Modern biological science must be based on logic, probability and reproducible experimental data, and must not be influenced by dominant global trends. The proposed theory of independent occurrences of facial cancers in Tasmanian devils, which can be masked by telomere-driven karyotypic convergence and subsequently by transposon-mediated molecular convergence (Stindl 2014), honors these classic scientific standards. In contrast to a 2015 Cell report of a transmissible cancer in soft-shell clams, with “infectious” blood cancer cells supposedly traveling long distances in sea water (Metzger et al. 2015); a warning example of the implications that a false theory can have on modern biology. Clearly, similar patterns of transposon-mediated mutagenesis in tumors of unrelated individuals can develop independently, if synchronized by species-specific telomere length profiles (Stindl 2014).

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## APPENDIX

Submitted to BioEssays 03/11/2016, rejected 03/18/2016, submitted to Molecular Cytogenetics 03/23/2016, rejected 04/18/2016, again submitted to BioEssays 04/27/2016, rejected 06/07/2016, submitted to JEZ Part B: Molecular and Developmental Evolution 06/12/2016, rejected 08/21/2016.